Therapeutic Uses of Pulsed Magnetic-Field Exposure: A Review

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1. Introduction

Bioelectromagnetics is the study of the interaction between non-ionizing electromagnetic fields and biological systems. In the extremely low frequency (ELF, $\leq$ 300 Hz, [1, 2, 3]) part of the electromagnetic spectrum, experimental therapies have been emerging for a variety of medical conditions, such as non-union bone fractures, skin ulcers, migraines, and degenerative nerves. Pulsed electromagnetic fields have been used as therapeutic agents over the last 40 years, following convincing evidence that electric currents can accelerate bone formation [4]. Specifically, electromagnetic-field stimulation gained credibility as a therapy following observations that the application of physical stress on bones promoted the formation of very small electric currents that are related to bone formation. A similar mechanism has been observed for cartilage, whereby electrical stimulation of chondrocytes increased the synthesis of the major component of cartilage matrix, known as proteogylcans [5].

A subset of ELF electromagnetic fields, i.e., pulsed electromagnetic fields (PEMF), displays frequencies at the low end of the electromagnetic spectrum [6], from 6 Hz up to 500 Hz. Another characteristic of PEMF waveforms is their rate of change. High rates of change (e.g., Teslas/second) are able to induce significant biological currents in tissues, thereby enabling them to have greater biological effects than waveforms of lower rates of change, if the biological effect is dependent on the magnitude of the induced current [1].

Extremely low frequency fields are non-ionizing and athermal (defined as either inducing no significant heating of the tissue, or thermal heating below the naturally occurring thermal fluctuations in tissue [2]). The waveforms associated with PEMFs can be asymmetric, biphasic, and quasi-rectangular or quasi-triangular in shape [6]. However, most ELF sources of electromagnetic-field stimulation produce a sinusoidal waveform [1]. In 1979, the United States Food and Drug Administration (FDA) approved both quasi-rectangular and quasi-triangular waveforms as safe and efficacious forms of treatment of disorders associated with fractures [6]. Specific types of low-level EMFs have the ability to produce specific biological responses, depending on the parameters (e.g., magnitude, frequency, waveform) of the field [2]. Intermittent use of PEMF stimulation has been shown to produce superior outcome responses to continuous use [7].

There are two methods in which PEMF stimulation can be non-invasively applied to biological systems: capacitive or inductive coupling. Capacitive coupling does not involve any contact with the body. In contrast, direct coupling requires the placement of opposing electrodes in direct contact with the skin surface surrounding the tissue of interest [7]. For example, if PEMF therapy is desired for the long bone of one's right arm, the opposing electrodes would be placed on the skin on either side of the right arm, surrounding the bone of interest.

Inductive coupling does not require the electrodes to be in direct contact with the skin. Rather, the time-changing magnetic field of the PEMF induces an electric field (Faraday's Law of Induction), which, in turn, produces a current in the body's conductive tissue [7, 8, 9, 10].

Pulsed electromagnetic-field stimulation – used as a treatment for conditions such as non-union bone fractures, failed joint fusions, and congenital pseudarthroses – has yielded success rates of 70% to 95% in prospective and double-blind studies. Treatment times range from 20 minutes to 8-10 hours per day, depending on the condition to be treated and the field parameters used [11]. There is no

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Study	Parameters	Effect of MF
Bassett et al. [18] (Beagle dogs)	2 mV/cm, 1.5 msec, 1 Hz, biphasic; 20 mV/cm, 0.15 msec, 65 Hz, biphasic	Accelerated bone repair
Bassett et al. [19] (Beagle dogs)	2 mV/cm, 1.5 msec, 1 Hz, biphasic; 20 mV/cm, 0.15 msec, 65 Hz, biphasic	Accelerated bone repair
Wilson & Jagadeesh [41] (Rats)	Diapulse; 65 μsec bursts, 80-600 pulses/sec	Increased speed of nerve regeneration
Bassett et al. [20]	ElectroBiology Inc.; quasi-rectangular, assymetrical, 300 μs pulse width; 75 Hz 12-16 hrs daily; 3-6 months	Promoted osteogenesis
De Haas et al. [23] (Rabbits)	0.1 Hz, 0.015 T; 1 Hz, 0.015 T; 4 Hz, 0.025 T 6 hrs/day; 5 days/week; 2 weeks	Not effective; healing initiated at 1 Hz but effect not maintained
Heckman et al. [13]	Electro-biology Inc., Fairfield, N.J. Min. 12 hrs/day; min 3-4 months	Healed 64.4% of ununited fractures
Barker et al. [22]	0.3 T/s, 15 Hz 12-16 hrs/day; 24 weeks	Established tibial unions (questionable effect)
Binder et al. [32]	73 $\pm$ 2 Hz; 2.7 mT (peak) 5-9 hrs daily; 4 weeks	Reduced pain, improved active range
Raji [42] (Rats)	Diapulse; 400 pulses/sec 15 min daily; 3.5 days, 1, 2, 3, 4, or 8 weeks	Acclerated rate of recovery of injured nerve; enhanced regeneration of damaged nerves
Kavaliers et al. [89] (Mice)	Rotating magnetic field, 1.5 G – 90.0 G Several exposure periods	Abolished morphine-induced analgesia
Devereaux et al. [33]	Single pulse of 200 μs, 15 Hz 8+ hrs daily; 1-2 days	No effect on lateral humeral epicondylitis
Kavaliers & Ossenkopp [97]	0.2 mT - 3.5 mT, 30min, 10 consecutive days	Reduced tolerance to morphine
Ossenkopp et al. [83] (Mice)	static: 1470 $\pm$ 0.2 G; radiofrequency: 6.25 MHz, 2 bursts (gaussian, square) every 100 msec pulsed: 8 x 10^3 G/sec (z), 10 x 10^3 G/sec (x, y) 22.5 min pre- and post-morphine injection	Attenuated morphine-induced analgesia
Frykman et al. [12]	Bi-osteogen, System Electro-biology Inc., Fairfield, N.J. 8-10 hrs daily; mean 4.3 months	Healed non-union scaphoid fractures
Prato et al. [86] (Mice)	static: 0.15 T; pulsed: 0.4 mT_{pk} - 0.9 mT_{pk} radiofrequency: Gaussian pulse, 2 and 4 ms widths 23.2 min, 62 MHz	Static component had no effect, radiofrequency component reduced, and pulsed component abolished morphine- induced analgesia
Kavaliers & Ossenkopp [88] (Snail)	0.1 mT - 0.8 mT; 0.5 Hz 15 - 30 min	Inhibited analgesia from opioid agonists
Sisken et al. [44] (Rat)	0.3 mT, 20 msec pulse, 2 Hz repetition 1 hour daily	Regeneration of sciatic nerve
Ieran et al. [38]	2.8 mT, 75 Hz, 1.3 msec 3-4 hrs daily; 90 days	Increased success rate of treating venous skin ulcers; reduced recurrence rate
Mooney [36] (Rabbits)	0.18 mT, 1.5 Hz	Increased success rate of interbody lumbar fusion established effectiveness of bone graft stimulation
Omote et al. [62] (Rats) (Cell culture)	4 mT, 200 Hz, pulse width 2.0 msec 1 hr (once) 4 mT, 250 Hz, pulse width 1.5 msec 2 hrs (once)	Increased survival of rats; survival greatest when PEMF and drug given in combination Colony formation suppressed; greater suppression with Combination PEMF & drug
Tabrah et al. [25]	2.85 mT (peak), 380 μsec quasirectangular, followed by 72 Hz, 6 msec quasitriangular wave 10 hrs daily; 12 weeks	Short-term increased bone mineral density
Bassett & Schink-Ascani [16]	Electo-Biology Inc. (Parsippany, NJ); amplitude set to deliver 1.5 mV/cm for normal cortical bone with periosteum 10-12 hrs daily; 3 months - 4 years	Healed congenital pseudarthrosis of the tibia
Bellossi & Desplaces [59] (Mice)	12 Hz, 9 mT 10 min, 3 non-consecutive days/wk from 2-3 wks after tumours appeared until death	Increased length of survival in early stage of cancer development
Mouchawar et al. [66] (Dogs)	Rectangular pulses 0.1 msec in duration at 50 Hz	Stimulated the heart
Sanseverino et al. [28]	50 Hz solenoid, 3 mT - 6 mT 15-40 min daily; 15 sessions	Removed pain, recovered joint mobility, maintained improved conditions of joints
Stiller et al. [9]	PELUT; ΔB = 2.2 mT; 3 part pulse (+, -, +) of 3.5 msec total width 3 hrs daily; 8 weeks (or earlier is healed); 12 wks total if improvement present at 8 wks	Decreased wound depth and pain intensity
Kanje et al. [43] (Rats)	60 μT or 300 μT, 2 pulse/sec 15 min-24 hrs/day; 1-7 days	Pretreatment increased regeneration of sciatic nerve (not all MFs were effective)
Kavaliers & Ossenkopp [84] (Snails)	0.1 mT - 0.8 mT; 0.5 Hz	Reduced opioid-induced analgesia following administration of naloxone
Roland et al. [48]	0.5 Hz - 17 Hz; 0.1 μT - ~0.15 μT 15 min daily; 1 week	Improved tinnitus
Betancur et al. [87] (Mice)	3 mT - 4 mT	Reduced analgesic effect
Fleming et al. [100] (Rats)	5 μT pulse burst; 1 sec on, 4 sec off 20 minutes	Increased analgesia
Grant et al. [65] (Rabbits)	2.8 mT, 75 Hz, single pulse (280V) 350 min	Lessened cortical ischemic oedema, reduced ischemic neuronal damage

Table 1: Summary of Magnetic Field Effects as Therapeutic Agents in Treatment

Hannan et al. [8] (Mice)	5.2 mT, 250 pulses/sec, 120 μsec ramped pulse 1 hr	Decreased tumour size when in combination with chemotherapy drugs
Jorgensen et al. [39]	1-250 MHz; 2-30 pulses/sec 15-30 min; repeated as necessary	Relief from pelvic pain
Del Seppia et al. [93] (Pigeons)	continuous + 70 μT to - 20 μT; sinusoidal	Hyperalgesia (heightened sensitivity) to painful electrical stimulation
Papi et al. [94]	continuous + 70 μT to - 20 μT; sinusoidal	Increased sensitivity to painful electrical stimulation
Konrad et al. [26]	5 mT, 50 Hz 20 min/session; 20 treatments total	Reduced pain and improved hip movements
Darendeliler et al. [17]	15 Hz, positive duration 200 μsec; 1.8 mT 8 hrs daily; 9 days	Accelerated rate of bone repair
Glazer et al. [35] (Rabbit)	peak (negative) 3 ± 1 T/sec; (positive) 9 ± 4 T/sec 26-msec pulse burst; 670 ± 10-msec burst interval 4 hrs daily; 6 weeks	Reduced the rate of pseudarthrosis
Godley [21]	Electro-biology Inc. (Parsippany, NJ) 10 hrs daily; 3 months	Enabled solid union of carpal scapoid
Harrison & Bassett [27]	PEMF coils 10 hrs nightly; 7.5 to 18.5 months	Not effective in treating Perthes' Disease
Liang et al. [63] (Mice) (Tissue culture)	5.25 mT, 250 pulses/sec, 120 μsec ramped pulse 1 hr weekly; 3 weeks 5.25 mT, 250 pulses/sec, 120 μsec ramped pulse 1 hr weekly; 3 weeks	Decreased tumour volume in combination with anti-cancer drug Enhanced potency of anti-cancer drug only when PEMF was prior to drug injection
Richards et al. [47]	5 μT - 10 μT, 4-13 Hz, 1 msec pulsed waves 10-24 hrs daily; 2 months	Improvement in performance tests; increased alpha EEG during a language task
Sartucci et al. [101]	0.5 Hz; 70 μT to -20 μT; 0.1 msec duration	Reduced pain thresholds and pain-related somatosensory evoked potentials
Thomas et al. [95] (Snail)	100 μT_{pk}, 0.4 T/s 15 min	Induced analgesia, and increased opioid- induced analgesia
DiCarlo et al. [68] (Chick embryos)	60 Hz; 4 μT, 6 μT, 8 μT, or 10 μT 20 min	Increased rate of survival (reduced anoxia- induced mortality)
Jankauskienė et al. [46]	1 mT, 80 kHz 30 min; 10 sessions	Improved soft tissue, reduced inflammation; did not affect visual signs or eye movements
Mann et al. [53]	900 MHz, pulsed with 217 Hz, 577 μsec width 8 hrs (1 night)	Cortisol slightly elevated; no change in growth/ luteinizing hormones, or melatonin
Thomas et al. [98] (Snail)	100 Tpk, 0.4 T/s 15 - 30 min daily; 6 - 9 days	Development of tolerance, and cross- tolerance to repeated MF exposures; effect reduced with novel environmental cues
Albertini et al. [74] (Rats)	Triangular waveform; 75 Hz; 30 mT	Reduced necrotic region of myocardial infarct
DiCarlo et al. [67] (Chick embryos)	60 Hz; 4 μT, 6 μT, 8 μT, or 10 μT 20 min	Increased rate of survival; induced stress response that protected embryo myocardium from anoxia-related mortality
Karasek et al. [57]	2.9 mT, 40 Hz, square impulse shape 20 min daily, 5 days/week; 3 weeks 0.025 mT - 0.08 mT, 200 Hz, complex saw-like impulse shape, bipolar 8 min twice daily, 5 days/week; 3 weeks	Significantly lowered rise in nocturnal melatonin Did not influence melatonin levels
Carmody et al. [73] (Cells)	60 Hz, 8 μT 20 minutes - several hours	Protection from ischemia-reperfusion injuries
Del Seppia et al. [85] (Mice)	hypogeomagnetic field: 4 μT Oscillating magnetic field: 20 μT - 70 μT 90 min in home cage; 30 min restrained	Suppressed stress-induced analgesia
de Seze et al. [61] (Mice)	100 mT, 0.8 Hz square-wave 8 hours daily	Decreased tumour growth;increased survival
Karasek et al. [58]	25 μT - 80 μT, 200 Hz, saw-like impulse shape 8 min twice daily, 5 days/week, 3 week	No effect on melatonin concentrations
Marks [34]	Spinal-Stim (Orthofix Inc., Richardson, TX) 4+ hrs daily, 4-6 months	Enhanced bone bridging in lumbar spinal fusion
Matsumoto et al. [24] (Rabbits)	0.2 mT/ 0.3 mT/ 0.8 mT; 100 Hz; width 25 μsec 4 or 8 hrs daily; 1, 2, or 4 weeks	Promoted bone formation
Jacobson et al. [30]	0.034 μT - 0.274 μT; 0.976 Hz - 7.7 Hz 6 min, 8 sessions; 2 weeks	Reduced knee pain due to osteoarthritis
Pipitone & Scott [14]	50 μT; 3 Hz, 7.8 Hz, or 20 Hz 10 min, 3 times daily; 6 weeks	Improvement from baseline in pain, stiffness, and physical disability
Prato et al. [79] (Mice)	200 μT_{pk}, 0.4 T/s	Increased movement under low intensity light; decreased movement under high intensity light
Thomas, Drost, & Prato [77]	200 μT_{pk}, 0.4 T/s	Improved standing balance
Thomas, White, et al. [78]	200 μT_{pk}, 0.4 T/s	Improved standing balance in fibromyalgics and controls to greater degree than in arthritics during eyes open; all groups had worse standing balance during eyes closed
Williams et al. [60] (Mice)	0, 10 mT, 15 mT, or 20 mT; 120 pulses/s 10 min daily	Reduced tumour growth and vascularization
Robison et al. [64] (Human cell lines)	0.15 ± 0.02 mT_{pk} sinusoidal, 120 W, 60 Hz 4, 12, or 24 hours	Decreased susceptibility to heat-induced apoptosis, leading to proliferation of cancer
Warman et al. [54]	200 μT - 300 μT; 50 Hz 2 hours; 1 night	Changed melatonin onset variability, but not average melatonin onset time

Note: Subjects were humans unless otherwise indicated.

Table 1: Summary of Magnetic Field Effects as Therapeutic Agents in Treatment (continued)

discomfort or known risk associated with this stimulation, it is non-invasive, and the cost of medical treatment is substantially reduced relative to the costs of surgery [2, 6, 7, 11]. The presence of implanted metals does not appear to affect the therapeutic ability of the PEMF exposure [10]. Furthermore, PEMF therapy is simple to use [2]: no surgical procedure is required, the PEMF stimulation can be performed in an office setting, there are no known complications, no anesthetic is required, and the length of treatment is comparable to bone-grafting procedures. However, these advantages of PEMF stimulation are qualified by the cooperation of the patient [12]. Specifically, the patient must sometimes use the PEMF stimulation device for upwards of 10 hours daily, must immobilize the fracture ends, and must ensure no weight-bearing [13].

Of particular concern when considering the use of PEMF stimulation as a clinically therapeutic agent are the health risks associated with exposure to such stimulation. While evidence for carcinogenic effects of magnetic fields (magnetic fields) is small, and there is no evidence supporting the direct damage of DNA by electromagnetic fields, there is some support that magnetic-field stimulation could act as a co-carcinogen in combination with a known genotoxic and/or non-genotoxic carcinogen. There is greater support for the possibility of teratogenic and reproductive effects of ELF magnetic fields [1]. Despite the ongoing debate over the safety of PEMF exposure, it is generally believed and accepted that brief exposure to the fields is safe. Nevertheless, there are still warnings for those with known cancers, those who are pregnant, and those with permanent pacemakers to avoid exposure sessions [7].

This review will examine the therapeutic benefits of PEMF stimulation as used in clinical and experimental settings. Procedures that involve electrode placement in tissue, i.e., capacitive coupling methods, will not be included in this review. Summaries of the discussed findings are provided in Table 1 (studies listed by publication date) and Table 2 (studies listed by disease/ condition category).

2. Musculoskeletal Disorders

To date, the only FDA approvals for the use of PEMF stimulation for clinical treatment are for therapeutically resistant problems of the musculoskeletal system, such as delayed-union bone fractures, failed joint fusions, and congenital pseudarthroses [6, 11, 14]. Several cellular mechanisms, including increases in growth factors, have been implicated as the possible causes of success from PEMF stimulation. For example, fracture non-unions, failed joint fusions, and congenital pseudarthroses are thought to be healed via increases in mineralization [6], angiogenesis, collagen production, and endochondral ossification that result from PEMF stimulation. Congenital pseudarthroses also show decreased osteoclasis following PEMF therapy [11].

2.1 Bone Repair

Bone repair requires the cooperation of bone-specific cell-types: osteoblasts and osteoclasts. Osteoblasts are involved in the formation of bone, while the main function of osteoclasts is in bone resorption. Generally, these two cell types are in normal balance, and the amount of bone is kept constant. When a fracture occurs, osteoblasts and osteoclasts work together to quicken the healing process. However, sometimes healing is not at an optimum, and non-unions result. These types of fractures require an additional stimulus, such as pulsed electromagnetic-stimulation, to assist in the healing process [15].

Pulsed electromagnetic-field stimulation has been shown to have an effect on bone repair via a number of different mechanisms. Firstly, PEMF has been shown to stimulate calcification of the fibrocartilage in the space between the bony segments. Second, the increased blood supply that arises due to PEMF's effects on ionic calcium channels have been implicated as a source of improved bone healing. Thirdly, PEMF has been suggested as having an inhibitory effect on the resorptive phase on wound repair, leading to the early formation of osteoids and calluses [16, 17]. A fourth mechanism by which PEMF is thought to have an effect on bone repair is through its influence on increasing the rate of bone formation by osteoblasts [15].

The degree to which PEMF stimulation is effective is dependent on several factors, including anatomic location, associated surgery, patient age, disability time, date of treatment initiation, adherence to treatment protocol, and infections. In general, non-unions in young adults are more easily stimulated to heal than those in older adults, and stimulation has been found to be more effective if initiated within two years of onset of the original fracture [13].

Non-union fractures are those fractures in which healing does not occur within six months of injury. These fractures represent 3% of all long-bone fractures, and result in a tremendous amount of discomfort and pain. The use of PEMF stimulation as a treatment for non-unions has been very successful, with success rates reaching 80% [7, 12]. The amount of time required prior to having this treatment prescribed is slowly being reduced from its original requirement of nine months following injury. Furthermore, the successful results obtained from this treatment have prompted discussions of the use of these fields for treatment of ordinary fractures. It is anticipated that PEMF stimulation on ordinary fractures would reduce the amount of time that a cast must be worn [7].

The first study to report successful application of PEMF stimulation was conducted by Bassett et al. Using 43 beagle dogs with surgically produced bilateral fibular osteotomies, these researchers were able to demonstrate a non-invasive acceleration of the repair process in the dogs

Disease	Author	Ref.	Effect of MF
Bone			
Osteotomy	Bassett et al.	[18]	Accelerated fibula bone repair
	Bassett et al.	[19]	Accelerated fibula bone repair
	De Haas et al.	[23]	Quickened initiation of long bone healing; did not sig. reduce time for solid union
	Darendeliler et al.	[17]	Increased new bone growth
Non-union bone fracture	Bassett et al.	[20]	Osteogenesis
	Heckman et al.	[13]	Enhanced bone healing
	Barker et al.	[22]	Established tibial unions; not sig. different from controls
	Frykman et al.	[12]	Healed non-union scaphoid fractures
	Godley	[21]	Enabled solid union of carpal scaphoid
Congenital Pseudarthrosis	Bassett et al.	[16]	Healed tibial congenital pseudarthrosis
Bone formation	Matsumoto et al.	[24]	Increased bone contact and bone area with the implant
Osteoporosis	Tabrah et al.	[25]	Initial increase in bone density followed by steady decline
Hip Arthroplasty	Konrad et al.	[26]	Improvement in pain ratings and hip movements
Perthes Disease	Harrison et al.	[27]	No significant difference from controls
Joint			
Joint Disorders	Sanseverino et al.	[28]	Decreased pain ratings and improved mobility of joint
Rheumatoid Arthritis	Ganguly et al.	[29]	Enhanced improvements in pain, swelling, tenderness, and joint function among seronegative relative to seropositive patients
Osteoarthritis	Pipitone et al.	[14]	Improvements in pain, stiffness, and physical disability relative to baseline
	Jacobson et al.	[30]	Greater reduction in pain
Rotator Cuff Tendinitis	Binder et al.	[32]	Decrease in pain ratings, increase in active range
Lateral Epicondylitis	Devereaux et al.	[33]	No significant benefit
Spinal Fusions			
Spinal fusions	Marks	[34]	Enhanced successful bony bridging
Pseudarthrosis	Glazer et al.	[35]	Enhanced solid union, increased stiffness, increased maximum load before fusion failure
Interbody lumbar fusions	Mooney	[36]	Improved success rate of solid fusion
Ulcers			
Venous leg ulcers	Ieran et al.	[38]	Healed ulcers for prolonged period; prevented recurrence
	Stiller et al.	[9]	Decreased wound surface area, wound depth; increased healthy granulation tissue
	Flemming et al.	[37]	Insufficient evidence from reviewed studies to warrant use of PEMF stimulation
Pelvic Pain			
Pelvic Pain	Jorgensen et al.	[39]	Quickened return to normal activities, prevented need for surgery
Nerves			
Median-ulnar nerve	Wilson et al.	[41]	Stimulated and quickened nerve regeneration
Peroneal nerve	Raji	[42]	Quickened toe-spreading reflex, enabled nerve regeneration
Sciatic nerve	Sisken et al.	[44]	Regeneration of nerve
	Kanje et al.	[43]	Enhanced regeneration of nerve
Endocrine ophthalmopathy	Jankauskienė et al.	[46]	Reduced soft tissue involvement and proptosis, improved corneal and optic nerve function
Neurological Disorders			
Multiple Sclerosis	Richards et al.	[47]	Improvement in performance scales;increased alpha EEG during language tasks
Tinnitus	Roland et al.	[48]	Improvements in symptoms; reductions in sensation levels
Neuroendocrine System			
Hormone production	Mann et al.	[53]	Altered cortisol secretion pattern
Melatonin levels	Karasek et al.	[57]	Reduced melatonin profile depending on pulse parameters
	Karasek et al.	[58]	No influence on melatonin concentrations
	Warman et al.	[54]	Changed melatonin onset variability, but not average melatonin onset time
Cancer			
Mammary carcinoma	Bellossi et al.	[59]	Increased length of survival
	Williams et al.	[60]	Reduced tumour growth/ vascularization
KMT-17 / KDH-8 tumours	Omote et al.	[62]	Increased survival rates, decreased colony formation, especially in combination with drug therapy
A431/ HT-29 cell lines	Hannan et al.	[8]	Reduced mean tumour volume, esp. in combination with anti-cancer drugs
Subline KB-ChR-8-5-11	Liang et al.	[63]	Reduced tumour size and enhanced survival
HL-60, HL-60R, and Raji cell lines	Robison et al.	[64]	Decreased susceptibility to heat-induced apoptosis, enabling proliferation of cancerous cell lines
Benzo(a)pyrene- induced tumours	de Seze et al.	[61]	Decreased rates of tumour growth; increased survival
Cerebral Ischemia (Stroke)			
Focal ischemia	Grant et al.	[65]	Reduced extent of cortical oedema, and areas of neocortex and neostriatum
Coronary Protection			
Cardiac Stimulation	Mouchawar et al.	[66]	12 kJ required to achieve closed-chest ectopic beats
Myocardial Protection	DiCarlo et al.	[68]	Incresed survival rates following cardiac anoxia damage
	Albertini et al.	[74]	Reduced necrotic region of myocardial infarct
	DiCarlo et al.	[67]	Increased survival rates following cardiac anoxia damage
	Carmody et al.	[73]	Protection from ischemic-reperfusion injury
Psychophysiological Regulation			
Human Standing Balance	Prato et al.	[79]	Increased movement under low Intensity light; decreased movement under high intensity light
	Thomas, Drost, ...	[77]	Improved standing balance
	Thomas, White,...	[78]	Improved standing balance in fibromyalgics and controls to greater degree than in arthritics during eyes open;all groups had worse standing balance during eyes closed

Table 2: Efficacy of Magnetic Field Therapy, by Disease Category

Pain	Kavaliers et al.	[89]	Abolished morphine-induced analgesia
	Kavaliers & …	[97]	Reduced tolerance to morphine
	Ossenkopp et al.	[83]	Attenuated morphine-induced analgesia in mice
	Prato et al.	[86]	Static component had no effect, radiofrequency component reduced, and pulsed component of MF abolished morphine-induced analgesia
	Kavaliers & …	[88]	Inhibited analgesia from opioid agonists
	Kavaliers & …	[84]	Reduced opioid-induced analgesia following administration of naloxone
	Betancur et al.	[87]	Reduced analgesic effect
	Fleming et al.	[100]	Increased analgesia
	Del Seppia et al.	[93]	Hyperalgesia (heightened sensitivity) to painful electrical stimulation
	Papi et al.	[94]	Increased sensitivity to painful electrical stimulation
	Sartucci et al.	[101]	Reduced pain thresholds and pain-related somatosensory evoked potentials
	Thomas et al.	[95]	Induced analgesia, and increased opioid-induced analgesia
	Thomas et al.	[98]	Development of tolerance and cross-tolerance to repeated MF exposures; effect reduced with presentation of novel environmental cues
	Del Seppia et al.	[85]	Suppressed stress-induced analgesia

Table 2: Efficacy of Magnetic Field Therapy, by Disease Category (Continued)

following 28 days of exposure to low-frequency, low-intensity PEMFs (2 mV/cm, 1.5 ms, 1 Hz, biphasic; or 20 mV/cm, 0.15 ms, 65 Hz, biphasic). The 65 Hz PEMF was more effective in improving healing (i.e., producing new bone tissue) than was the lower-frequency field [18, 19].

2.1.1 Non-Unions

Bassett, Pilla, and Pawluk [20] reported the first account of a therapeutic benefit of ELF PEMFs in humans. These researchers reported that PEMF stimulation (300 ìs pulse width; 75 Hz) on surgically resistant non-unions led to osteogenesis as a result of the therapy. Twenty-five of the 29 patients in the study displayed radiographic evidence of bone formation following one month of stimulation. Furthermore, these researchers were able to prevent several individuals who were recommended for amputations from these painful and debilitating procedures.

Following the success of Bassett et al. [20], further research was conducted investigating PEMF stimulation on fracture healing. Heckman et al. [13], for example, reported a 64.4% success rate in 149 patients who used PEMF stimulation to treat non-unions. For patients who maintained intensive use of the stimulation for three months, effectiveness was seen in 85% of patients. Frykman et al. also reported success of PEMF stimulation. These researchers reported an 80% success rate among 44 patients with non-unions of the scaphoid (a small bone in the wrist joint) treated with PEMF stimulation, and advocated PEMF stimulation as an alternative method for treating non-union scaphoid fractures when long-arm cast treatment proves ineffective [12]. This finding was replicated in 1997 in a case study of a 12-year-old boy with a non-united carpal scaphoid fracture who was successfully treated with PEMF stimulation, such that union of the fracture was established following treatment [21]. The use of PEMF stimulation appears to be effective, and a reasonable choice of treatment, among individuals suffering from non-unions [13].

A more recent reporting by Traina et al. [10] of the successful application of PEMF exposure for the treatment of non-unions claimed a 74% healing rate, with age of patient, site of fracture, type of non-union, and presence of infection as significant factors influencing the results. The presence of infection of the bone tissue or surrounding soft tissue was previously reported to not have an effect on the treatment outcome [10].

The early success of PEMF treatment of non-unions was not replicated in every study. For example, PEMF stimulation (0.3 T/s burst waveform, 15 Hz) was not shown to be effective in the treatment of un-united tibia fractures at 12 months post-injury. Specifically, Barker et al. [22] found that five of the nine patients in the active treatment group, relative to five of the seven patients in the placebo group, displayed united fractures at the end of the 24-week experiment. These data suggest the need for further research; yet, this study included only 16 patients, and so there was very little statistical power to detect a significant difference. Also, the induced electric fields were much lower in this study than in the original work by Bassett [20].

2.1.2 Congenital Pseudoarthrosis

Pulsed electromagnetic-field stimulation has also been shown to have clinical efficacy for the treatment of congenital Pseudoarthrosis [16]. This treatment modality aims at bone consolidation, as well as prevention of re-fracture and misalignment of the bones involved [10]. Specifically, PEMF (8 T/s, 20 pulses repeated at 15 Hz) stimulation, along with immobilization of the fractured area, was found to have an 80% or greater success rate for Type I and Type II lesions (gaps less than 5 mm wide) for which no operations had yet been performed. Type III lesions (lesions which are atrophic, spindled, and had gaps in excess of 5 mm wide) were not as responsive to PEMF stimulation, displaying a 7% success rate in response to treatment that included only PEMF, and an overall 19% success rate for treatments that also included operations. The lesion types were defined according to the lesion's appearance on X-ray photographs [16]. The success of treatment of congenital pseudoarthrosis with PEMF stimulation was outstanding since in the past, amputation was the most frequent outcome for this disorder [6].

2.1.3 Osteotomies

Pulsed electromagnetic field stimulation has been shown to have an additional use in bone repair – one that has yet to be approved by the FDA. Treatment of osteotomies (misaligned bones) in guinea pigs with PEMF therapy (15 Hz, 200 ìs unipolar pulse, 1.8 mT, 3 T/s) has resulted in increased new bone growth in the gap caused by the osteotomy relative to placebo group animals, where loose connective tissue filled the osteotomy sites. This study provides implications to humans about the possibilities of using PEMFs to quicken craniofacial healing [17]. However, disapproval of PEMF stimulation was provided by De Haas et al. [23], who found that recently osteotomized long bones of rabbits given PEMF stimulation experienced a quicker initiation of the healing process, but did not have a significantly reduced time for solid union relative to control rabbits.

The pulse parameters of a magnetic field as well as its duration of use are important characteristics that have been shown to influence the effectiveness of PEMF stimulation. Matsumoto et al. [24] investigated the bone formation surrounding dental implants inserted into the femur of rabbits, and found that bone contact with the implant was greater among PEMF-treated (100 Hz, rise times of 8 T/s, 12 T/s, and 32 T/s for 0.2 mT, 0.3 mT, and 0.8 mT peak, respectively) animals relative to controls. Among treated rabbits, 0.2 mT and 0.3 mT fields had significantly greater bone contact and bone area than the 0.8 mT-treated femurs. No significant difference was observed for bone contact or bone area for those femurs treated four hours/day as opposed to eight hours/day. Furthermore, it was found that two weeks of exposure had a significantly greater effect than one week; yet, the measured outcomes were not significantly lower at two weeks than they were following four weeks of exposure. This study indicated the need to select the proper magnetic-field intensity, duration, and length of treatment to maximize outcome [24].

2.2 Other Orthopedic Disorders

2.2.1 Osteoporosis

Osteoporosis, the most common skeletal disorder, is associated with decreased bone mass. Consequences of this condition include the inability of the skeleton to resist stresses of everyday life, resulting in numerous fractures. The beneficial application of PEMF stimulation in healing non-union bone fractures suggested the possibility that such treatments might be beneficial to patients with osteoporosis. Twenty post-menopausal women participated in an investigation of the effectiveness of PEMF therapy in increasing bone density. During twelve weeks of daily 72 Hz pulsating magnetic field exposure (380 ìs quasirectangular wave, followed by 6 ms quasitriangular wave), bone densities of exposed bone regions increased; however, during the 36 weeks following treatment, bone densities decreased significantly. These rebound results suggest the immediate effectiveness of PEMF therapy, and indicate the need for continued treatment to ensure prolonged increased bone density [25]. A decrease in initial improvement is not exclusive to PEMF treatment; any treatment (including drug therapy) given to improve symptoms associated with osteoporosis is expected to show declines following its removal.

2.2.2 Hip Arthroplasty

Hip arthroplasties are required when individuals are suffering from hip problems. A common side effect of such surgeries, however, is the loosening of the prosthesis that occurs in 15% - 25% of patients within 10 years of the surgery. The successful application of PEMF therapy in orthopedic disorders prompted Konrad et al. [26] to consider its use in a non-blinded, uncontrolled study investigating the treatment of twenty-four patients suffering from aseptic loosening of the hip prostheses. Patients were assessed for levels of pain and hip movements prior to and following exposure to magnetic fields (50 Hz, 5 mT). No patients were randomized to a sham condition. Significant improvements in pain ratings and all hip movements (except for flexion and extension) were noted following exposure sessions in patients suffering from loose hip replacement, but not for those patients suffering from severe pain due to gross loosening of the hip prostheses [26]. This suggests that PEMF therapy may only be beneficial in reducing mild-to-moderate pain associated with hip prostheses, but not severe pain levels.

2.2.3 Perthes Disease

While there have been good results found from the treatment of orthopedic disorders with PEMF, not all diseases or conditions have benefited from such treatment. For example, Perthes' disease, a condition in which young children suffer from a temporary loss of blood supply to the femoral head (the ball part of the hip joint) has not been shown to benefit from PEMF stimulation [27]. Twenty-two boys, randomized to either orthosis plus PEMF treatment or sham treatment, displayed no significant differences in treatment durations (an average of 12.5 months for those receiving PEMF versus an average of 12.0 months for those receiving sham). The treatment time was defined as the amount of time required for the upper femoral epiphysis (the top part of the femoral head) to be resistant to the deforming effects caused by weight-bearing. Based on this controlled study, there does not appear to be a significant effect of PEMF stimulation on the successful treatment of Perthes' disease.

However, there are inconsistencies in the literature with respect to the success of PEMF stimulation in treating diseases associated with the femoral head. For example, research investigating the ways in which PEMF stimulation

enables repair of the dead bone associated with lack of blood supply to the femoral head has found that PEMF exposure enables repair of the dead bone by promoting ingrowth of new blood vessels, while maintaining a balance between the rate of dead bone removal and the formation of new bone [6]. Vallbona and Richards [4], commenting on studies using EMF stimulation to treat femoral-head necrosis, reported that this form of treatment resulted in successful progression for lesions located in the hips, according to both clinical (80% successes) and magnetic resonance (MR) imaging (76.6% successes) evaluations. The combined clinical and MR imaging success rate was reported as 63.3% for the lesions.

2.3 Summary of Orthopedic Literature

Pulsed electromagnetic field exposure has been applied to a variety of orthopedic pathologies, mostly with positive, successful indications. For example, Traina et al. [10] reported that PEMF therapy was a successful modality of treatment of congenital pseudoarthrosis, pseudoarthrosis, delayed union, fracture at risk, recent fracture, bone grafts, vertebral arthrosis, and avascular necrosis. Limb lengthening, however, was not successfully achieved through the use of PEMF stimulation. The reader is directed to the review prepared by Traina et al. [10] on bone healing through pulsed electromagnetic-field exposure and other means of biophysics enhancement for a more comprehensive analysis of bone healing.

3. Rheumatological Disorders

3.1 Joint Diseases

Pulsed electromagnetic-field therapy has been shown to be effective in treating joint diseases; yet, the degree of its success depends on the specific joint disease in question. Specifically, joint diseases involving only one joint, as well as single traumata (suffering from acute lesions), show significant improvement following PEMF stimulation. In contrast, disorders involving multiple joints (e.g., polyarthrosis, rheumatoid arthritis) are much more resistant to the effects of PEMF stimulation, and show less improvement following treatment sessions. In a large 11-year experimental study, 3014 patients suffering from a joint disease were treated with extremely low frequency, low-intensity sinusoidal magnetic fields (0.6 T/s - 1.2 T/s). Patients were given one 15 - 40-minute session daily for 10 - 15 days to assess the effects of the pulsed magnetic field exposure on healing of the joints and associated pain levels. These patients – except females who were pregnant or menstruating, and individuals who carried a pacemaker – were exposed to the magnetic fields. Control patients (in addition to the 3014 patients) were included and provided with sham treatment. Of the 3014 subjects who received PEMF exposure, 78.8% showed good results (i.e., pain disappearance, 40% - 50% increase in degrees of freedom of the sick joint, maintenance of benefit for at least three months, decrease in thermal irradiation of the affected joint after magnetic-field exposure). The best results were obtained with patients who participated in therapeutic exercises following magnetic-field therapy, and maintained control of body weight and bone mineralization. Control patients reported a complete absence of any benefit when (unknowingly) exposed to sham treatment; upon subsequent exposure to the active PEMF unit, these controls obtained the same results as the patients who were exposed to the active unit [28].

3.1.1 Rheumatoid Arthritis

Rheumatoid arthritis (RA) is a chronic condition in which an individual suffers from inflammation of the joints, resulting in feelings of pain, stiffness, and swelling. There is no known cause of this disorder, but it has been implicated as being autoimmune in nature. In testing individuals for the presence of rheumatoid arthritis, screening can be conducted for an antibody known as the rheumatoid factor (RF). The rheumatoid factor is present in the blood of 80% of adults suffering from rheumatoid arthritis [29]; however, its presence or absence does not necessarily indicate that one has rheumatoid arthritis. Individuals who possess the rheumatoid factor are classified as serological-positive, while those lacking the antibody are categorized as serological-negative.

Gunguly et al. [29] conducted a study investigating the effectiveness of PEMF stimulation in reducing pain, tenderness, swelling, joint functional disability, and joint spasm with deformity in 35 patients suffering from rheumatoid polyarthritis (multiple joint disorders). Patients in this study were assessed according to serological grouping. Results indicated that those individuals lacking the rheumatoid factor (i.e., patients who were serological-negative) showed earlier responses to the PEMF (rectangular pulse) for pain and swelling, and a much earlier improvement for pain, tenderness, and joint functional disability relative to serological-positive individuals. The same trend appeared for joint spasm with deformity; however, the overall treatment effect for this symptom was low for both groups. These findings provide empirical support for clinicians to treat individuals with and without the rheumatoid factor differently, as PEMF was not shown to be as effective a therapy for those possessing the antibody [29].

3.1.2 Osteoarthritis

Osteoarthritis is the most common rheumatic disorder, affecting older people in industrial countries [5]. It is characterized by degeneration of articular cartilage (cartilage at a joint), and the presence of hypertrophic (enlargement of organ due to increase in size of constituent cells) tissues [30]. Those suffering from the disorder experience pain, swelling, tenderness, and stiffness in the weight-bearing

joints of the lower extremities [5]. Approximately 80% of the population over 75 years of age displays radiological signs of osteoarthritis, with 40% - 80% of these individuals also having clinical symptoms of the disease [14, 30].

Treatment for osteoarthritis has begun to shift away from drug therapies – which have, in large part, been found to be ineffective and toxic – and towards more unconventional modes of healing [5]. This shift has resulted despite the firm position of the American College of Rheumatology that there is currently inadequate scientific documentation to warrant the use of PEMF therapy for treatment of osteoarthritis of the hips and knees [14]. Nevertheless, PEMF stimulation has been gaining increasing support as a treatment for osteoarthritis. It has been suggested that magnetic fields are beneficial in the treatment of osteoarthritis because they suppress inflammatory responses at the level of the cell membrane [31].

An attempt to demonstrate the clinical importance of magnetic-pulse treatment for knee osteoarthritis was conducted by Pipitone and Scott [14]. These authors found no significant improvement of magnetic-field-treated patients (unipolar pulse, 7.8 Hz in morning, 3 Hz in evening; < 50 T/s) relative to placebo-treated patients at the end of the study. However, the authors did find that magnetic-field-treated patients reported significant improvements in a questionnaire assessing pain, stiffness, and physical disability at the end of the study relative to their baseline scores on these measures. In contrast, no significant changes were observed for placebo-treated patients in these measures between baseline and the end of the study. This work suggests that PEMF stimulation should be included as a part of the treatment protocol for individuals suffering from osteoarthritis; however, further experimentation using different magnetic devices, treatment populations, and experimental protocol should be considered.

3.1.3 Rotator-Cuff Tendinitis

Rotator-cuff tendinitis, inflammation of one or more of the muscles that holds the ball of the shoulder joint tightly against the socket, is a common cause of shoulder pain among adults. Conventional treatments, such as corticosteroid injections, are not always effective; therefore, alternative therapies have been evaluated. A randomized double-blind experiment designed to assess the effect of PEMF stimulation [73 $\pm$2 Hz; 2.7 mT_{pk}, 7.9 T/s] on individuals suffering from rotator-cuff tendonitis was conducted. The design of this experiment consisted of three phases. During the first phase, one group of patients received PEMF treatment, while the other group received sham treatment. The second phase involved the administration of PEMF exposure for *both* groups of patients. In the third phase, no PEMF stimulation was given to either group. This design allowed for obvious group differences to be detected upon the introduction of the second phase, and also enabled all subjects to receive the PEMF treatment following four weeks (the beginning of second phase), as opposed to only offering such therapy to the treatment group. Upon presentation of PEMF stimulation to the control group at the beginning of the second phase, a remarkable decrease in pain ratings and an increase in active range were noted. These scores were in the direction of those of the treatment group, with no significant group differences present following the four-week mark of the study. These findings demonstrate the ability of PEMF stimulation to reduce pain and increase activity among individuals suffering from rotator-cuff tendinitis, and implicate such therapy for individuals who suffer from the disorder, and are unresponsive to, or noncompliant with the administration of, corticosteroid injections. Overall, Binder et al. found that more than 70% of all patients in this study improved following PEMF therapy [32].

3.1.4 Lateral Humeral Epicondylitis

The success of PEMF therapy in treating rotator-cuff tendinitis prompted rheumatologists to consider the use of such therapy for other chronic tendon lesions, such as lateral humeral epicondylitis (better known as “tennis elbow”). A randomized, double-blind assessment of the effectiveness of PEMF therapy in treating this condition (a minimum of eight weeks of treatment) in 30 patients failed to find a significant beneficial effect of PEMF stimulation (single pulse, 200 ìs duration, 15 Hz) to warrant its use over placebo conditions. This conclusion may be related to the 53% spontaneous healing found among patients in the placebo group, or to the use of different pulses in treating lateral humeral epicondylitis relative to other rheumatological disorders [33].

4. Spinal Fusions

Spinal fusions occur when an individual is suffering from a painful vertebral segment, and wishes for the motion at the vertebral region to be reduced to help alleviate the pain. This type of surgery is invasive, and is used only after more conservative methods of treatment have been explored (e.g., bed rest, drug therapy, exercise, massage) [34]. Once spinal fusions are deemed medically necessary, the surgical team wants to ensure that recovery will be as quick as possible, and that minimal pain will be endured. One method in which to achieve these goals is through the use of PEMF stimulation. Marks [34] found that spinal fusions for discogenic low back pain were successful (i.e., incorporation of the graft, no radiolucency between graft and vertebral bone, no motion at level of fusion) in 97.6% of the surgeries of patients in the PEMF stimulation group, as opposed to the low 52.6% success rate among patients in the unstimulated group, indicating that PEMF stimulation allows for bony bridging in lumbar spinal fusions. Furthermore, successful spinal fusions correlated with good or excellent clinical outcomes [34].

A complication of spinal fusions arises when an individual also suffers from Pseudoarthrosis, the failure of a union to develop in fusion. The use of PEMF therapy to

reduce Pseudoarthrosis has been shown to be effective in a rabbit fusion model [35]. Twenty adult white rabbits were randomly assigned to either a PEMF or a sham exposure for four hours daily for six weeks. Characteristics of the electromagnetic field included asymmetric rise and fall times (3 ± 1 T/s and 9 ± 4 T/s) using a 26-ms pulse burst, a 670 ± 10-ms burst interval, and a pulse rise and fall time of 400 ns. The animals were euthanised at six weeks, at which time radiologic and histologic samples were taken. Radiographic analysis indicated that six of the 10 animals in the placebo group, and eight of the 10 animals in the PEMF group, had solid fusions. In the rabbits that demonstrated solid fusion, there was a significant increase in stiffness of the fusion mass, a significant increase in area under the load-displacement curve (representing energy absorbed by each motion segment), as well as a significant increase in the maximum load before fusion failure among the PEMF-exposed animals relative to the placebo controls [35]. The implication of these findings to human studies is of importance, as this study provided preliminary support for the idea that exposure to PEMFs can reduce Pseudoarthrosis, thereby reducing pain among human patients with lower back pain.

4.1 Interbody Lumbar Fusions

Interbody lumbar fusions are performed to help release stress from a damaged disk that has caused a pinched nerve root. The rates of lumbar fusion are unpredictable; however, following evidence that PEMFs have the ability to aid in bone formation, it has been shown that the presence of these fields has a significant effect on spinal fusions. Using a double-blind prospective approach, Mooney [36] assessed the success of spinal fusions in 195 patients who were undergoing initial attempts at interbody spinal fusions. Success rates were defined as radiographic evidence of solid fusion. For those patients who complied with the methodology of using the brace for at least eight hours each day, there was a success rate of 92.2% in the active treatment group (PEMF, 0.18 mT, 1.5 Hz). This rate was significantly higher than the 67.9% success rate found among patients in the placebo group. The patients' age, sex, fusion level, number of grafts, graft type, or internal fixation did not affect these success rates. Smoking made very little difference, yet showed a decreased trend in success rates for both active and placebo group patients [36]. It should be noted that there is controversy as to whether interbody lumbar fusions are an orthopedic indication.

5. Soft-Tissue Regeneration

5.1 Venous Leg Ulcers

Leg ulceration is a chronic, recurring condition, affecting more women than men, and increasing in prevalence with increasing age. Venous leg ulcers are caused by a blockage in the veins of the legs. Compression can heal most of these ulcers; however, it is not an effective form of treatment for all such sores [37]. Pulsed electromagnetic-field stimulation has been investigated as a therapy for wound healing following results that PEMFs can promote healing by potentially increasing collagen synthesis, angiogenesis, and bacteriostasis [9, 37].

The use of PEMF stimulation to reduce the size and eliminate pain associated with venous leg ulcers was investigated in a double-blind study of patients suffering from skin lesions present for at least three months [38]. Using a 2.8-mT magnetic field with a 75-Hz frequency and pulse width of 1.3 ms over a treatment protocol of 90 days, these researchers found a significantly higher success rate (66% versus 32%) among patients exposed to the active PEMF device relative to those exposed to identical dummy devices. Furthermore, the effect of the field exposure was prolonged, evident at follow-up of at least one year, and protected the patient from ulcer recurrence. These findings suggest that PEMF stimulation is a useful complement to the treatment protocol for venous leg ulcers [38].

A prospective, multi-center, double-blind, randomized study was conducted to assess the efficacy of a portable PEMF stimulation device, PELUT (pulsed electromagnetic limb ulcer therapy). The PELUT device (bi-directional $2.2\,mT_{pk}$, three-part pulse of 3.5 ms total width, induces a low-level, non-thermal electrical field of 0.06 mV/cm in the skin above the wound dressing) was modeled after devices that have been successful in treating non-union fractures. Subjects suffering from recalcitrant venous stasis ulcers were randomized into either treatment or placebo group conditions, and were assessed at baseline, four weeks, eight weeks, and 12 weeks from the start of the experiment. All subjects were also given standard wound dressings as part of the treatment protocol. At week eight, relative to placebo group subjects, those individuals treated with the PELUT exhibited a 47.7% (significant) decrease in wound surface area, a significant decrease in wound depth, and a 15% increase in healthy granulation tissue. Those patients who had shown improvement at the eight-week mark, and chose to remain in the treatment program for an additional four weeks, exhibited further improvement, showing a 66% decrease in wound surface area at that time. The investigators' global assessments of the ulcers revealed a 50% improvement among those in the treatment group relative to a 0% improvement rate among the ulcers in the placebo group. None of the ulcers on subjects in the treatment group worsened following treatment; however, 54% of the ulcers in the placebo group worsened over the course of the eight weeks [9].

A review conducted by Flemming and Cullum [37] to investigate the effectiveness of PEMF in treating venous leg ulcers reported that, to date, there is insufficient evidence to warrant the clinical use of PEMF stimulation for the treatment of such ulcers. However, given the results reported in Stiller et al. [9], there is a need to perform further investigations into the true ability of PEMF stimulation in treating these ulcers.

6. Pelvic Pain

Another useful clinical application of PEMF radio-frequency (RF) stimulation is for the treatment of pain arising from pelvic disorders such as dysmenorrhoea, endometriosis, ruptured ovarian cyst, and acute lower urinary tract infection. In a study involving a total of 20 episodes of pain arising from pelvic disorders, pain was reduced following PEMF stimulation in 90% of the cases (i.e., 18 episodes). Pulsed electromagnetic stimulation involved brief 15-minute – 30-minute exposures to short pulses (1.0 MHz - 250 MHz; 2 - 30 pulses/s). The pain relief was evident following PEMF stimulation, and permitted a quicker return to normal life activities and prevented surgery. Patients suffering from the remaining two episodes of pain did not report pain relief following treatment. Recurrence of the pain condition occurred in one, or possibly two, of the treated episodes, and there were no adverse side effects reported either during or following treatment [39].

7. Nerves

Investigations into possible therapies and treatments for damaged nerves are essential, as these injuries can have detrimental and devastating effects in humans. However, human research is not always the best first approach for introducing and subsequently assessing nerve damage. Rather, animal models provide an excellent avenue for establishing an injury to a nerve to enable an understanding into what methods are successful in the repair of the injury. Animal studies investigating repair of damaged peripheral nerves have focused on the use of PEMF exposure sessions to aid in the regeneration of the nerve [40].

Wilson and Jagadeesh [41] conducted an experiment designed to assess regeneration of the median-ulnar nerve in the upper forelimb of 132 rats using PEMF (Diapulse Corporation of America; 65-ms pulse bursts) and sham treatments. Nerve-conduction studies indicated a return to nerve conduction of degenerated nerves following Diapulse treatment, but not following sham treatment. Histology slides revealed regenerating nerve fibers 30 days post-surgery in PEMF-treated rats; slides taken 60 days post-surgery in control rats showed evidence of regeneration, but not to the level found in the treated rats at 30 days post-surgery. Taken together, these results indicated that PEMF treatment is effective in stimulating and quickening the regeneration of the median-ulnar nerve in rats [41].

Further research into treating injured nerves with PEMF treatment investigated a nerve that is fairly important to humans: the peroneal nerve. Located in the leg and used for walking, when damaged, the peroneal nerve prevents individuals from lifting their foot and moving their toes. Raji [42] conducted an animal study investigating the degeneration and regeneration of this nerve using PEMFs. These researchers inflicted injury to the left peroneal nerve of male Lewis rats to assess the effects of both PEMF (Diapulse; 400 pulses/s) and sham treatments on the recovery of the rat's injured leg. Regeneration of functionally complete motor nerves was assessed via a test of reflex spreading of the toes upon being lowered suddenly to the ground. Results from the test suggest the beneficial usage of Diapulse, as the toe-spreading reflex was significantly quicker to appear in treated as opposed to untreated animals. Furthermore, microscope slides revealed accelerated progressive improvement in the appearance of transverse sections of the nerve, as well as increases in the number of nerve fibers, among magnetic-field-treated animals relative to rats in the sham group. These results suggest that PEMF therapy is beneficial in aiding the regeneration of the peroneal nerve in male rats [42].

Continued research on nerve repair and PEMF treatment has enabled investigations into other nerves of interest. In particular, the largest nerve in the body, the sciatic nerve, has followed the success of previous research, and has shown enhanced regeneration following exposure to PEMFs relative to animals (e.g., male and female rats) given sham treatment [43]. Sisken et al. [44] have seen regeneration in the sciatic nerve after a crush lesion following one hour of exposure (0.3 mT, 20 ms pulse, 2-Hz repetition) daily; the regeneration did not improve significantly with longer exposure periods. Furthermore, rats pre-treated with 0.38 T/s PEMFs (20 ms pulse, 2 Hz) were shown to have enhanced regeneration of the sciatic nerve after a crush lesion, while those exposed to 60 ìT fields did not experience this effect. This indicates that the regeneration process might be receptive to specific fields [43]. Overall, these results suggest that PEMF treatment can be used to successfully repair the sciatic nerve.

The animal studies conducted on nerve repair and PEMF treatment, considering nerves from both the arms and the legs, all converged on the finding that PEMF therapy was effective in nerve regeneration relative to results obtained from animals given sham treatment. The regeneration rate following PEMF exposure was enhanced to the same degree as obtained by other treatment methods, including conditioning lesions, hormones, and growth factors [45].

7.1 Endocrine Ophthalmopathy

Endocrine ophthalmopathy is considered an organ-specific autoimmune disorder, caused by an abnormality in immune-response mechanisms. Possible treatments for this disorder include corticosteroids and nonsteroidal anti-inflammatory drugs to reduce the inflammation of the eye (an identifying characteristic of this condition). When these drugs are ineffective, alternative forms of treatment are required. The success of PEMF stimulation in improving metabolic processes in tissues and organs led to the study of the effectiveness of PEMF therapy in treating endocrine ophthalmopathy [46]. Following exposure to magnetic fields of 1 mT with pulses emitted at a frequency of

8.0×10^4 Hz, patients diagnosed with endocrine ophthalmopathy enjoyed reductions in soft-tissue involvement and proptosis (displacement of the eyeball). Limitations in ocular movements were reduced, while corneal and optic-nerve function improved following magnetic-field exposure; these measures did not, however, reach statistical significance. The study showed that PEMF therapy is only useful for those suffering from endocrine ophthalmopathy who show signs of soft-tissue involvement; nonetheless, given the gravity of this disorder, the evidence for the usefulness of and necessity for a non-invasive treatment method is great [46]. Further work is required to determine the significance of PEMF exposure on treating diseases such as endocrine opthalmopathy; a Pubmed search yielded this paper as the only one investigating such effects.

8. Neurological Disorders

8.1 Multiple Sclerosis

The findings that PEMF stimulation was successful in improving nerve conduction and regeneration indicates a possibility that its use might be effective in treating disorders of the central nervous system, such as multiple sclerosis (MS), for example. Multiple sclerosis is a neuro-degenerative disorder in which the myelin sheath surrounding neurons is damaged, and nerve conduction is slowed. In a randomized, double-blind study, Richards et al. [47] found significant improvement in performance scales (assessing bladder control, cognitive function, fatigue level, hand function, mobility, sensation, spasticity, and vision) among magnetic-field-exposed patients (PEMF: 5 - 10 μT, 4 Hz - 13 Hz, 10 - 24 hours daily, two months) relative to non-exposed patients. All subscales of the performance test, except for those of hand function and sensation, as well as the combined performance (all eight tests) were significantly improved among the PEMF-exposed individuals. Electroencephalograph recordings indicated significant improvements among the PEMF-treated individuals between pre- and post-exposure for six of the 19 electrodes, as well as increased alpha EEG during a language task among MS patients exposed to the magnetic fields relative to MS patients who were not exposed to the magnetic fields. No significant differences between pre- and post-treatment scores were found for the test of clinical ratings. These findings suggested that PEMF therapy is a beneficial short-term treatment for individuals suffering from MS [47].

8.2 Auditory Disorders: Tinnitus

Tinnitus, more commonly known as "ringing in the ears," is a disorder of the auditory system in which a bodily condition, such as disturbances of the auditory nerve, causes the affected individual to hear sensations of noises (e.g., ringing) that are only audible to that individual. The high prevalence of this condition warrants alternative methods, such as PEMF stimulation, to be considered as possible treatments [48].

A double-blind randomized trial assessing the effectiveness of PEMF stimulation as a treatment for tinnitus found significant improvements in symptoms and significant reductions in sensation levels among the group of patients treated with the active PEMF device (0.5 Hz - 17 Hz; 0.1 - ~0.15 iT). Overall, significantly more PEMF-group patients (45%) than placebo-group patients (9%) reported subjective improvement throughout the trial [48]. The study thus provided another useful and efficacious application of PEMF stimulation, and submitted evidence that PEMF therapy should be considered among other therapies for use among individuals suffering from tinnitus.

8.3 Psychiatric Disorders: Affective Disease

Transcranial magnetic stimulation (TMS), a safe and non-invasive method of exciting neurons through strong, brief, and focused [49] magnetic-field pulses, is currently the only ethically approved technique of modulating neuronal activity in the human brain. This method of stimulation works via the principle of induction: a capacitor is discharged to enable a strong current to pass through a coil placed over the scalp [50]. The strength of the induced current is a function of the rate of change of the magnetic field, which is affected by the current in the coil. The coils used today have a magnetic field peak intensity of 1.5 T to 2 T at the face of the coil, and neurons can be activated as far as 1.5 cm to 2.0 cm from the surface of the coil in the cortex [51]. The magnetic-field intensity used in TMS is many orders of magnitude larger than that present in ELF magnetic-field exposure, i.e., of the order of 10,000 T/s, as compared to maxima around 10 T/s.

Single-pulse TMS has been distinguished from repetitive TMS (rTMS) in that the latter is a modification of the former in which the magnetic field is repeated over a small time interval, allowing the stimulation of nerves during their refractory period. The multiple pulses that exist in rTMS are discharged through one coil using multiple stimulators, and are classified as fast rTMS if stimulation is greater than or equal to 1 Hz, and slow rTMS if stimulation is less than 1 Hz [49]. The use of rTMS has been considered for the treatment of psychiatric disorders such as depression, as it shares many of the behavioral and biochemical actions as other antidepressive treatments, such as electroconvulsive shock (ECT). Specifically, both treatments use transcranial brain stimulation; however, rTMS produces a localized effect, while ECT's effect is more generalized. Despite promising results with rTMS, its use has not yet produced evidence of beneficial results matching the effectiveness of the more-conventional treatment options [52].

9. Neuroendocrine System

The neuroendocrine system, a combination of hormone secretion and central nervous system activity, can be studied to investigate the biological effects of PEMFs [53]. To assess this link, the effects of an RF PEMF (modulated electromagnetic field) on hormone production were assessed in a healthy male population. Using a 900 MHz PEMF (217 Hz) set to provide a near-homogeneous field distribution, Mann et al. [53] determined nocturnal hormone profiles (growth hormone, luteinizing hormone, cortisol, melatonin) during both sham and RF PEMF exposure. The only significant difference in hormone secretions noted between the placebo and exposure trials was the significant interaction between field exposure and time, suggesting a different cortisol secretion pattern between the two sessions. No difference was reported between total cortisol production between the sham and exposure sessions, indicating a temporal difference in secretion of the hormone while under the influence of the RF PEMF [53]. Another study, designed to determine the effects of magnetic-field exposure on the human melatonin profile, used two-hour pulses of high-level circularly polarized 50 Hz magnetic fields (200 - 300μT) delivered at different circadian times [54]. Blood samples taken every 30 minutes to 60 minutes over a 17-hour overnight period provided multiple plasma melatonin measurements. Results from the study revealed that while no significant changes were present in average melatonin onset time following magnetic-field exposure, melatonin onset was significantly more variable following magnetic-field as opposed to sham exposure. The authors of the study discussed the possibility, on the basis of preliminary data, that the circadian time of the magnetic field might have an influence on the magnitude and direction of the observed response. This possibility might be the mediating link to explain why magnetic-field exposure has not been shown to consistently affect human melatonin profiles [54].

Further investigations of the effects of PEMFs on melatonin levels were conducted. Melatonin, a hormone derived from the pineal gland in the brain and controlled by the light-dark environment [55], is associated with pathological conditions – including cancer – when its levels are altered [56]. Levels of this hormone are generally high at night and low during the day [55]. Melatonin is believed to be important in synchronizing circadian rhythms [56], helping to induce sleep, reducing insomnia, eliminating jet lag, and has been speculated to be a contributor to anti-inflammatory and analgesic responses, as well as to soft-tissue repair [57]. As such, determining ways in which to modify melatonin levels is important to helping with these ailments that melatonin is thought to "cure." The presence of melatonin helps to retard the growth of tumors; however, exposure to ELF PEMFs has an inhibitory effect on the production of melatonin from the pineal glands at night, resulting in increased growth of tumor cells [56].

The ability of melatonin to reduce tumor growth may be a function of its role as hydroxyl ($\bullet$ OH) and peroxyl (ROO $\bullet$) radical scavengers. Free radicals can be toxic and can damage DNA, leading to cancer. Melatonin's antioxidant properties, present through its indole functional group, enable the protection of DNA from oxidative damage (through less free radical attack on the DNA), resulting in reduced incidence of cancer [56]. Since it has been shown that magnetic-field exposure can suppress melatonin secretion, and it has also been shown that melatonin, as a hormone, acts to stop cancer growth, it may be possible to design specific magnetic fields to stimulate melatonin secretion as a treatment for cancer patients [31].

Karasek et al. [57] utilized two PEMFs (2.9 mT, 40 Hz, square impulse shape, bipolar; 0.025 - 0.08 mT, 200 Hz, saw-like impulse shape, bipolar) to assess whether exposure to either had an effect on melatonin levels in men suffering from low back pain. Results from that experiment revealed a significantly reduced melatonin profile following exposure to the 2.9 mT pulse, but no significant difference relative to baseline when exposed to the 0.025 - 0.08 mT pulses. These findings suggested that melatonin levels can be altered by specific PEMF parameters [57], and implied that further research should be conducted to further elucidate the exact "best" parameters for altering melatonin levels in humans.

Further research conducted by Karasek et al. [58], investigating whether the effect of melatonin concentrations in patients with low back pain could be influenced by magnetic-field exposures of different characteristics (e.g., different magnitudes), revealed negative results: chronic exposure to magnetic fields varying in magnitude between 25 and 80μT and having a frequency of 200 Hz did not influence human serum melatonin concentrations. However, those magnetic-field amplitudes were lower than those used by Karasek et al. [57].

10. Cancer

Cancer research is essential, since this disease affects a large proportion of the population, and is one of the major causes of death in North America. Given the promising results of PEMF stimulation in treating other disorders and human conditions, Bellossi and Desplaces [59] conducted an experiment investigating the effects of PEMF exposure on survival rates in C3H/Bi female mice with mammary carcinoma. Using a 9-mT PEMF, set at either 12 Hz or 460 Hz, these researchers found increased length of survival during the early stages of the disease when the mice were exposed to the 12-Hz magnetic field, and increased length of survival during the late stages of the disease when exposed to the 460-Hz field [59]. Williams et al. [60] conducted further work assessing the effects of therapeutic EMF on mammary carcinoma vascularization and growth in C3H/HeJ mice. These researchers found that daily 10-min sessions of 10-, 15-, or 20-mT pulsating magnetic field (120 pulses/s) significantly reduced tumor growth and the extent of vascularization relative to mice not exposed to the

magnetic field. The ability of magnetic-field exposure to prolong survival was replicated in later work conducted by de Seze et al., using tumor-induced male and female mice. These researchers found that eight hours of daily exposure to the 100-mT, 0.8-Hz square-wave magnetic field resulted in significantly decreased rates of tumor growth and increased survival [61]. These three studies indicated that survival and tumor growth can be positively influenced by magnetic-field exposure.

In addition to using PEMF stimulation as a treatment for cancer growth, it is also possible to use this therapy as an adjunct to drug therapy. A combination treatment of pulsing magnetic fields (PMF) and an anti-tumor drug, mitomycin C (MMC), was shown to be successful in treating two experimentally-induced tumors [62]. Either the KMT-17 or the KDH-8 tumor cells were implanted subcutaneously into the right thigh of a male rat. Seven days following implantation, an intravenous injection of MMC was given to the rat; one hour later, PEMFs (2 T/s, 200 Hz) were applied over the thigh region. Rats were placed into one of four experimental groups: no treatment, MMC-only, pulsed-magnetic-field-only, or a combination of MMC and pulsed magnetic field. Survival rates of the KMT-17 implanted rats at 90 days were 0% for the untreated group, 34% in the MMC-only group; 47% in the pulsed-magnetic-field-only group, and 77% in the combination group. None of the rats implanted with the KDH-8 tumor survived to day 90; however, percentages of increased life span relative to untreated rats were 3.4% for the MMC-only group, 7.6% for the pulsed-magnetic-field-only group, and 17.6% for the combination group. Analysis of the cultured cells treated in each of the three treatment groups revealed a significant decrease in colony formation in the combination group relative to either the MMC-only or pulsed-magnetic-field-only (2.7 T/s, 250 Hz) groups. These results indicated the ability of pulsed magnetic field treatment to enhance treatment above that provided solely by MMC injections, and offer hope for possible alternative treatments for cancer [62].

The combination treatment of pulsed magnetic stimulation and anti-tumor drugs has been used in further research following the promising, successful results obtained by Omote et al. [62]. Using an average field strength of 0.525 mT_{rms}, three different chemotherapeutic drugs (cisplatin, carboplatin, and doxorubicin), and either A431 or HT-29 human cell lines implanted into nude or NIH-III female mice, the results consistently showed that mean tumor volume was reduced by the combination treatment (i.e., drug + pulsed magnetic field group). Specifically, the volumes of the tumors in combined-treatment mice were 52%, 34%, and 35% of those found in the cisplatin, carboplatin, and doxorubicin drug-only treatment groups, respectively. This consistent finding demonstrates the generality of pulsed magnetic field augmentation of anti-tumor drug effects [8].

Liang et al. [63] conducted more complex investigations of the combined effect of pulsed magnetic field and drug therapy. These researchers investigated the effects of the combined treatment (i.e., Daunorubicin + pulsed magnetic field) approach on a multi-drug-resistant (MDR) human carcinoma subline KB-Ch^R-8-5-11. For the in vivo part of the study, female mice were inoculated with the KB- Ch^R-8-5-11 cells, and subsequently treated with one hour of pulsed magnetic field treatment (44 T/s, 250 pulses/s) and intravenous injection of Daunorubicin. The only significant differences in tumor volume were between the pulsed-magnetic-field-only and pulsed magnetic field + drug groups at both 39 and 42 days [63]. Thus, it appeared that the combination effect was significantly better at reducing tumor size relative to either treatment alone. The in vitro study revealed that the efficacy of Daunorubicin was enhanced when pulsed magnetic field (44 T/s, 250 pulses/s) was given before the drug was injected, but not when the pulsed magnetic field was given post-injection [63]. These findings provide additional support for the common trend among cancer research that a combination treatment of pulsed magnetic field and drug works best at reducing tumor volume and enhancing survival.

Despite these encouraging results regarding the beneficial effect of PEMF exposure on cancer reduction, the effect of such exposure sessions has not always been found to be positive. Robison et al. [64] have shown that electromagnetic field exposure (54 mT/s, 60 Hz) for 4, 12, or 24 hours resulted in decreased susceptibility to heat-induced apoptosis for three human cancer cell lines, indicating that the cancerous cell lines were able to proliferate. Furthermore, these exposure sessions also resulted in time-dependent decreased DNA repair rates among two of the three cell lines, allowing for propagation of the damaged DNA. Thus, there are conflicting results with respect to the effect of electromagnetic field exposure on cancer, possibly related to the significantly different characteristics of the PEMF exposures (for example, the study by Robison et al. [64] had a much lower T/s than the positive studies).

11. Cerebral Ischemia (Stroke)

The clinical implications of determining a model to protect against cerebral ischemia are important, since strokes have devastating and sometimes life-threatening effects on many individuals in society. Given this importance, Grant et al. [65] designed an experiment to assess the effects of low-frequency PEMF exposure on cerebral injury in a rabbit model of focal ischemia. Twelve male white rabbits underwent occlusion of the left internal carotid, proximal left anterior cerebral, and proximal left middle cerebral arteries for two hours, followed by four hours of reperfusion. Six of these rabbits subsequently underwent treatment, which included PEMF exposure (2.8 mT, 75 Hz) beginning 10 minutes following the onset of ischemia until the end of reperfusion. At the end of the six hours, the 12 rabbits were sacrificed, and magnetic-resonance-imaging (MRI) studies, as well as histological examinations, took place. The MRI studies revealed high-intensity lesions in the anterior and

ventral cortical regions in the middle cerebral artery. PEMF exposure appeared to significantly reduce the extent of cortical oedema at the anterior level by 65% relative to controls. Histology slides revealed ischemic neuronal damage (IND) in the lateral neocortex and neostriatum within the middle cerebral artery ipsilateral to the occlusions. Subsequent to PEMF exposure, the areas of neocortex at the anterior level and the ischemic neuronal damage in the neostriatum were significantly reduced by 69% and 43%, respectively, relative to controls. These results suggest that exposure to PEMFs following focal cerebral ischemia can protect against the development of neuronal damage in the neocortex and neostriatum [65].

The success of combination treatment of pulsed magnetic fields and drugs for cancer treatment might be of benefit to researchers investigating possible therapies for cerebral ischemia. Specifically, given the effectiveness of the combined treatment in cancer, treatment for strokes may also be enhanced by the combined effect of drugs that are effective in treating acute focal ischemia [e.g., N-methyl-D-aspartate (NMDA) antagonists] and PEMF exposure [65]. The success of this approach can possibly be due to the enhanced ability of drugs in the presence of magnetic-field exposure to get across the blood-brain barrier.

12. Coronary Protection

12.1 Cardiac Stimulation

Stimulation of the heart is a necessary medical intervention to prevent coronary failure. Magnetic stimulators are capable of stimulating nerves; however, if the stimulation is too great, cardiac arrhythmias, or alterations in the rhythm of the heartbeat, can occur. Magnetic stimulation of the heart is preferred over stimulation with electrodes in direct contact with the skin since it is a less painful method of achieving the same result. To assess a method in which magnetic-field stimulation can be achieved without causing harm, Mouchawar et al. [66] investigated the threshold for cardiac stimulation by magnetic fields in 11 dogs. The PEMF was delivered via two coplanar coils placed on the surface above which the heart was located in the dogs. To induce a reversible and temporary cardiac arrest in the dogs, the researchers used rectangular pulsed magnetic fields (0.1 ms; 50 Hz) to stimulate the dogs' right vagus nerve. Once arrest was established (after approximately 3 s), electroencephalogram (ECG) and blood-pressure recordings were taken. If the PEMF coils produced an ectopic beat after the dog was in induced cardiac arrest, the voltage was reduced in decrements of 10% until the PEMF no longer evoked ventricular contractions. If, however, no ectopic beat was produced, the voltage was increased by 10% increments until such a beat was produced. The threshold was then defined as the voltage at which 10% less did not stimulate the heart. Results from the study indicated that an average energy of 12 kJ was required to achieve closed-chest ectopic beats via a PEMF. These findings acknowledge the importance of determining safe levels of magnetic fields, and suggest that it is possible to determine safety parameters for PEMF, such as those produced by MRI scanners [66].

12.2 Myocardial Protection

In addition to determining safe levels of PEMF stimulation for the heart, the determination of protective benefits of PEMF treatment for the myocardium would be of great importance to clinicians seeking ways in which to inhibit/ attenuate damage to the heart muscle following reduced oxygen to the area. DiCarlo et al. [67] showed that chick embryos (fertilized White Leghorn eggs) exposed to low-frequency (4-, 6-, 8-, and 10-μT; 60 Hz) PEMFs prior to exposure to an anoxia chamber had significantly increased survival rates (68.7%) relative to the survival rates of control embryos (39.6%) following cardiac anoxia damage. Anoxia was achieved by maintaining oxygen levels below 1% during the experiment. The embryos were subsequently re-exposed to ambient oxygen levels (21%) at the time at which 15% - 45% of control embryo hearts were still beating. Survival was objectively determined as the presence of a heart beat [68]. Further investigations revealed that the protection was due to the PEMF itself, and not due to thermal heating. This research provides encouraging results for human clinical studies, suggesting that preconditioning a human with exposure to PEMF stimulation prior to surgery and transplantation might minimize myocardial damage [67].

Human research, investigating methods in which the myocardium can be protected, is essential to enable potential longevity of human lives. Ischemia (interruption of blood flow) - reperfusion (reintroduction of blood flow) injuries to organs, such as the heart, have potential detrimental effects such as lack of oxygen that can kill ischemic cells, and subsequent reperfusion can introduce harmful oxygen radicals into the organ. Preconditioning a tissue with a non-lethal ischemia-reperfusion (I/R) can help protect the tissue from later, more fatal I/R events. Preconditioning a vital organ by inducing mild heat shock to the tissue or by exposing the tissue to electromagnetic fields helps to protect the organ from subsequent heat shocks [67, 69]. Heat-shock proteins produce heat-shock preconditioning; these proteins act to protect the cell from excess heat, free oxygen radicals, and I/R, and are important for a cell's survival [70, 71]. Heat-shock proteins produced in the presence of PEMFs [72] provide an alternative to other induction methods that are harmful and use non-localized stimuli, such as hyperthermia, or are controversial, such as gene transfection. The production of heat-shock proteins in specific cells is likely dependent on the magnetic-field susceptibility of those cells. For example, cardiomyocytes appear to be consistently stimulated during 60 Hz, 8 ìT magnetic-field exposure [67, 73, 74]. The magnetic-field exposure time required to provide protection from I/R injuries is also cell-

dependent, and ranges from 20 minutes to several hours [73].

Heat-shock proteins are produced within the body, and provide the potential of protecting myocardial tissue from permanent damage due to I/R. However, a problem with the suggested use of PEMF exposure to induce production of heat-shock proteins is that clinically, patients are unlikely to present themselves prior to ischemia. Nonetheless, since most of the damage to the myocardial cells occurs during reperfusion, there is likely still to be a benefit of heat-shock protein production. Specifically, reperfusion therapy and transplantation cause injury that could be ameliorated with heat-shock proteins. It is still unknown whether cardioprotection is conferred by heat-shock proteins, or whether magnetic-field stimulation might be activating opiate agonists that then protect the cell from further damage [75]. Therefore, there is still much more work that must be done to determine the ultimate protector of cardiac cells, to ensure that the best possible, and most appropriate, treatment options are implemented to prevent further cardiac tissue damage.

13. Psychophysiological Regulation

13.1 Human Standing Balance

Pulsed electromagnetic-field stimulation has been shown to produce detectable physiological and behavioral effects in both animals and humans. The investigation of potential magnetic-field effects on human standing balance (postural sway) is important, since disturbances in this behavioral trait can indicate the presence of underlying diseases [76]. "Normal standing balance," defined as "the ability of a human to stand in a fixed position for a period of time [77]," is an automatic behavior in humans. When performed with "eyes open," little perturbations are noted in standing balance; however, this robust behavior is greatly affected when an individual is in the "eyes closed" state. Thomas et al. [77, 78] have extensively investigated this topic. Using a three-dimensional force plate to measure center-of-pressure movements, these researchers have included an objective measure of a behavioral response. Each subject in their studies was given four two-minute exposure conditions (eyes open/eyes closed, sham/magnetic field). Results from these studies suggested that specific ELF PEMF (200 μT_{pk} , 0.4 T/s; generated at head level) exposure has beneficial effects on standing balance, such that standing balance was improved significantly in both the "eyes-open" and "eyes-closed" conditions during magnetic-field exposure sessions relative to sham exposure sessions. The effect of PEMF exposure on standing balance appeared to be mediated by light intensity during the eyes-closed trials, as movement was significantly increased under low-intensity (0.12 W/m^2), and was decreased under high-intensity (0.51 W/m^2) light [79]. These results held for both genders and for all ages (range: 18 - 34 years), despite past findings that postural sway is sensitive to factors such as age and gender [76].

The influence of magnetic-field exposure on standing balance was affected by a subject's physical condition. Specifically, fibromyalgia patients (FM) and normal controls had similar standing balance during eyes open and sham exposure that was better than the standing balance recorded for rheumatoid-arthritis patients (RA). When eyes were closed, the postural sway of all three groups of subjects deteriorated, but to a greater degree in the two patient groups relative to the controls. Magnetic-field exposure was shown to improve the eyes-closed-to-eyes-open ratio in all three groups of subjects [78]. These results suggest that PEMF exposure has the ability to affect behavioral traits in both healthy controls and chronic pain patients.

14. Pain

Most of the therapeutic uses of magnetic-field exposure include a component of pain reduction; however, there are controversial reports in the literature regarding the effects of magnetic-field exposure on specific investigations of pain. Acute pain, or nociception, can be used as an outcome measure to determine sensitivity to stimuli. Nociception is a measure of an animal's or human's sensitivity to an adverse environmental stimulus. An understanding of how an organism responds to such stimuli enables researchers to determine its capacity to perform adaptive behaviors [80].

There is evidence to suggest that endogenous as well as exogenous opioid systems are affected by exposure to magnetic fields [80, 81, 82, 83, 84, 85]. Early investigations of the use of magnetic-field exposure on subsequent pain sensations revealed increases in pain following exposure sessions. Following knowledge that magnetic-resonance exposure can suppress analgesia in mice that have received morphine injections [83], Prato et al. [86] conducted a study to determine which of the various components of the magnetic field (static, time-varying, radio frequency) were responsible for the inhibitory effects of the exposure. The static component from the resistive magnet was 0.15 T; the time-varying component had peak magnetic fields of 0.4 mT and 0.9 mT, which corresponded to rise times of 2 ms and 3 ms, respectively; and the radio-frequency component at 6.25 MHz was a Gaussian-modulated pulse with widths of either 2 ms or 4 ms. Male mice were exposed to 23.2 min of one of the field components both before and after an injection of morphine sulphate (10 mg/kg), and analgesia was defined as the amount of time during which mice were on a hot surface (50 °C) before they displayed an aversive behavior (paw-lick, jump, etc). Results from the study indicated that exposure to the time-varying (pulsed) component of the magnetic field completely abolished, the radio-frequency component significantly reduced, and the static-field component had no effect on morphine-induced

analgesia. These findings may have relevance to humans who have ingested drugs such as morphine, and are subsequently exposed to these components of the magnetic field during magnetic-resonance imaging [86].

Exposures to hypogeomagnetic and oscillating magnetic fields have also been shown to have inhibitory effects in male mice. Specifically, Del Seppia et al. [85] found that mice removed from the normal geomagnetic field and placed in mu-metal boxes showed suppressed stress-induced analgesia; these mice displayed significantly lower latencies than mice exposed to a normal geomagnetic field. Hypogeomagnetic-field exposed mice displayed an effect similar to that observed in mice after exposure to an oscillating magnetic field. These results suggest that in addition to time-varying PEMFs, the presence of hypogeomagnetic fields also has the ability to reduce stress-induced analgesia.

Further work investigating the inhibitory effect of magnetic-field exposure on analgesia in mice focused on the possible modulatory effect of light [87]. Mice, displaying stress-induced analgesia, were found to have significantly lower analgesic levels following stable magnetic-field exposure (3 - 4 mT) under white light, but unaltered analgesic levels following either red light or total darkness. These results replicated previous findings found using lower magnetic-field intensities [86], and suggest that magnetic-field exposure might exert its effect only under certain light-intensity conditions.

Other early work found similar results in the land snail, Cepaea nemoralis. Specifically, Kavaliers and Ossenkopp [88] determined that 15 - 30 min exposures to weak rotating magnetic fields (0.1 - 0.8 mT; 0.5 Hz) inhibited analgesia from opioid agonists. (Absence of analgesia was previously reported in mice following exposure to a similar magnetic field [89]). The latency of nociceptive responses (the elevation of the snail's anterior portion of its extended foot) was shown to increase following the administration of opioid agonists (morphine and U-50, 488H, respectively); however, the concurrent application of an opioid agonist and magnetic-field exposure resulted in significantly reduced nociceptive responses, indicating significant inhibitory effects of magnetic-field exposure on opioid-mediated analgesia. The reduction in opioid-induced analgesia apparent with the magnetic-field exposure sessions was similar to that observed following opioid-antagonist (naloxone) injections [88]. Exposing the snails to 0.1 mT_{rms} , 60 Hz magnetic fields yielded similar results, with reduced opioid-induced analgesia present following magnetic-field exposure sessions. This finding was upheld for a variety of magnetic-field exposure periods (0.5 - 120 hours), and the inhibitory effect was significantly greatest during the dark period of the snail's light-dark cycle [80]. Snails that received daily administrations of naloxone, an opioid antagonist, experienced increased opioid-induced analgesia in a manner similar to that apparent following the magnetic-field exposures [84]. A possible mediating variable that could explain the magnetic field's inhibitory effect on opioid-agonist analgesia is altered calcium channel activity. Calcium-channel antagonists, such as diltiazem, verapamil, and nifedipine, significantly reduced, but did not completely block the inhibitory effects of the magnetic fields on morphine-induced analgesia, while calcium-channel agonists, such as BAY K8644, further inhibited the effects of morphine-induced analgesia that were present with magnetic-field exposure [88]. Administration of either the calcium-channel antagonists or the calcium-channel agonists had no significant effect on the reductions in opioid-induced analgesia achieved by injections of naloxone. It is possible that magnetic-field exposure alters calcium-channel functioning, resulting in differential distribution of calcium ions. This finding was supported by research conducted by Fanelli et al. [90], who showed that exposure to static magnetic fields prevented apoptosis via the flux of calcium into U937 and CEM cells. McCreary et al. [91] provided further support for the change in calcium-ion concentrations following magnetic-field exposure; these authors reported significant changes in cytosolic calcium concentrations following exposure to alternating-current (AC), direct-current (DC), or a combination of AC/DC magnetic fields after cell cycle, pH of suspension medium, and response to monoclonal antibody were controlled. These findings support the possibility that redistribution of calcium ions may have an effect on the functioning of opiates such as morphine [92].

The inhibitory effect of exposure to magnetic fields on analgesic responses was a consistent finding among many researchers. Studies conducted on pigeons [93] found that exposure to weak, oscillating magnetic fields (sinusoidal; continuous induced magnetic flux between +70 and – 20 μT) resulted in hyperalgesia, or heightened sensitivity, to a painful electrical stimulation. The pigeons, when exposed to these specific magnetic-field parameters, displayed significantly decreased thresholds to the electrical stimuli following magnetic-field exposure. In comparison, control pigeons not exposed to the magnetic fields displayed significantly increased thresholds to the stimuli over time, attributable to the formation of stress-induced analgesia. Using the same magnetic-field parameters as in the study on pigeons, Papi et al. [94] reported that humans experienced increased sensitivity, observed via decreased thresholds, following magnetic-field exposure relative to the sham condition. Specifically, assessment of dental sensory threshold (DST), dental-pain threshold (DPT), cutaneous sensory threshold (CST), cutaneous pain threshold (CPT), and cutaneous tolerance value (CTV), revealed no significant change in value for any of these measurements between pre- and post-sham conditions. However, when the same measurements were assessed in the same subjects before and after magnetic-field exposure, significant decreases were observed in the DST, CPT, and CTV measurements post-exposure. These two studies investigating the use of sinusoidal magnetic field exposures on pain sensitivity in pigeons and humans extend past research in showing that in addition to pain sensitivity in animals, such sensitivity in

humans is also negatively affected by magnetic-field exposure.

In contrast to these aforementioned studies, further research has discovered that exposure to magnetic fields does, in fact, have positive pain-relieving, anti-nociceptive, effects. For example, Thomas et al. [95] found that 15-min exposures to an ELFMF (100 μT_{pk}, 0.4 T/s) in land snails induced analgesia, and increased opioid-induced analgesia, rather than producing inhibitory effects. Subsequent injection of an opioid antagonist, naloxone, resulted in a reduced, but not completely abolished, analgesia effect. These results demonstrate the anti-nociceptive action of a specific PEMF via an endogenous opioid mechanism. Specifically, the ability of naloxone to reduce, but not abolish, the analgesia effect suggests the presence of at least partial δ-opioid receptor mediation [96]. Furthermore, the ability of the specific PEMF – but not of the random or burstx PEMFs also tested in the study – to induce analgesia suggests that the opioid analgesia did not arise from a non-specific magnetic-field stress response, but rather seemed to be related to the specific ELF magnetic field pulse form [95].

Further investigation of the effects of the specific PEMF exposure on inducing and augmenting opioid-induced analgesia involved the development of tolerance. Tolerance is defined as reduced drug effectiveness that presents itself following repeated drug exposure. Kavaliers and Ossenkopp showed that tolerance to opioid agonists (e.g., morphine) was reduced if animals are exposed to rotating magnetic fields prior to morphine injections, for animals that had not developed complete tolerance, and that environmental cues provided by magnetic stimuli were important determinants of development of tolerance [97]. Tolerance is apparent in land snails following five to seven days of repeated administration of morphine, and can also extend to other, similar opioids, via an effect known as cross-tolerance [98]. Thomas et al. [98] studied the effects of tolerance to the δ-opioid receptor agonist DPDPE, (D-Pen^2, D-Pen^5) enkephalin in land snails, and found that the magnitude and duration of the magnetic-field-induced analgesia was reduced following repeated (six days - nine days) daily (15 or 30-min) exposures: an effect indicative of the development of tolerance. The same effect of tolerance was present if snails received the nociceptive testing (assessed via the hot-plate test of latency) each day, or only on the first and last days. The effect was nearly completely removed when the land snails were presented with novel environmental cues. Furthermore, snails that received repeated daily exposures of the specific PEMF (100 μT_{pk}) displayed reduced sensitivity to the δ-opioid receptor agonist DPDPE. This reduced sensitivity provides evidence for the development of cross-tolerance of DPDPE to the opioid component of the PEMF [98]. Overall, these results provided insight into the development of tolerance and how magnetic-field exposure sessions can have (negative) effects similar to those of repeated drug administrations. This has great relevance for human clinical trials, as drug tolerance is known to be a problem among humans.

A discrepancy with respect to the effect of ELF magnetic-field exposure on land snails exists, as such exposure sessions have been shown to both increase [95, 96, 98] and decrease [88, 84, 92] analgesia. A possible mediating variable that explains the inconsistency in the literature is the presence of light, as opposed to dark, field conditions [99]. Prato et al. showed that the increases and decreases in opioid analgesia associated with magnetic-field exposure were consistent with the predictions of Lednev's parametric-resonance model (PRM) for the calcium ion [99]. Refer to Section 16.1 for a further explanation of potential mechanisms of action.

The results of increased analgesia following magnetic-field exposure extend beyond the work done on land snails. Briefly, it has also been shown that exposure to a pulsed magnetic field (5 μT burst firing pattern; 1 s on, 4 s off; 20 min) resulted in increased analgesia in female rats, as evaluated via flinch thresholds to electric shock. The analgesia seen was a 50% increase in flinch threshold, and was greater than that seen following the administration of a dose (4 mg/kg) of morphine [100].

In addition to animal research, human research has begun to reveal positive effects of magnetic-field exposure. Sartucci et al. [101] examined the effect of weak, oscillating magnetic-field exposure (constant-current rectangular pulses; 0.5 Hz; 0.1 ms duration; 70 to –20 μT) on human pain perception and pain-related somatosensory evoked potentials (SEPs). Pain thresholds were reduced, and pain-related SEPs were significantly reduced, following magnetic-field exposure; pain thresholds were significantly increased following sham sessions. These results provide the first evidence that human SEPs are influenced by magnetic-field exposure. Ongoing work in our lab is investigating other possible effects of pulsed magnetic-field exposure on human pain perception and analgesia.

15. Discussion and Concluding Remarks

This paper has discussed the effectiveness of magnetic-field stimulation as a treatment for a variety of health-related conditions. To date, of the articles included in this review, magnetic-field stimulation was shown to be effective for treatment of bone disorders (osteotomies, non-union bone fractures, congenital Pseudoarthrosis, bone formation, hip arthroplasty), joint disorders (including rheumatoid arthritis and osteoarthritis), rotator-cuff tendonitis, spinal fusions (including Pseudoarthrosis and interbody lumbar fusions), pelvic pain, neurological disorders (e.g., multiple sclerosis, tinnitus), nerve (median-ulnar, peroneal, sciatic) regeneration, endocrine ophthalmopathy, cancer, focal ischemia, cardiac and myocardial protection, and human standing balance. Stimulation was ineffective for treatment of Perthes Disease and lateral humeral epicondylitis. Magnetic-field stimulation is as yet inconclusive as an effective treatment for conditions such as osteoporosis,

venous leg ulcers, imbalance of the neuroendocrine system (including hormone production and melatonin levels), and pain.

The preceding summary of results discussed in this paper indicates the great success of magnetic-field stimulation in treating a variety of conditions and disorders. Given the study outcomes, the next logical step in understanding what other medical conditions might benefit from this treatment requires a detailed analysis of the mechanisms of action that underlie this form of treatment. The following section will discuss possible mechanisms by which magnetic-field therapy is suggested to work.

15.1 Possible Mechanisms of Action

As can be appreciated from this review, there is a very significant body of literature that supports the idea that therapeutic effects can be achieved from ELF magnetic-field exposure. However, except for application to orthopedics (i.e. non-unions), these therapies have not been accepted as conventional medical practice. One of the reasons is that positive results are often not confirmed when a replication attempt is made, and different magnetic-field exposure conditions are often used. As there are infinite combinations of ELF magnetic-field parameters to choose from, the optimization of treatment regimens is very difficult given the lack of a predictive theoretical framework. This is made more difficult by the nature of the measured endpoints: changing one exposure parameter would require at least two exposure groups along with a sham control where patients must be treated for months (at hours per day), making such experiments strategically and financially almost impossible. Hence, it is not surprising that mechanism discovery has been difficult. However, progress has been made and new tools associated with molecular biology and medical imaging could dramatically accelerate the discovery of mechanism.

What do we mean by mechanism? In this field we are faced with a real challenge, because the mechanism to be discovered includes two discrete steps: a) the initial biophysical mechanism by which the ELF magnetic field is detected and converted to a biological signal; and b) the cascade of events by which the initial biological signal results in the behavioral/physiological event.

For the determination of the initial biophysical detection mechanism, it is generally accepted that the responding system can be treated as a black box. The parameters of the ELF magnetic field can be stepwise changed and the response measured. As there are infinite numbers of possible field combinations, it is helpful to start with some a priori concepts so that exposure conditions can be first set to discriminate between fundamentally different mechanisms, and then further experiments can select between similar mechanisms. For ELF magnetic fields with the parameters used in therapy that have been reviewed here, there are two fundamentally different biophysical transduction mechanisms: induced current and magnetic dipole. The induced current assumes that the time-changing magnetic field induces a current in the conductive tissue (Faraday's Law of Induction). In comparison, the applied magnetic fields could interact directly with the magnetic fields in the tissue associated with an endogenous magnet (e.g., magnetite) or with the magnetic moment produced by a nucleus, atom, or molecule [102]. Engstrom [103] and Engstrom and Fitzsimmons [104] have demonstrated that a limited number of experiments (i.e., five) would be needed. Perhaps the best example of this kind of approach has been undertaken by Prato et al. (see Table 3; [99, 105, 106, 107, 108, 109, 110, 111]). This work suggests that the magnetic fields were detected by a magnetic molecular dipole. However, in the work on snails, using a much different pulse, Thomas et al. [95, 96, 98] presented evidence suggesting that it is an induced-current mechanism. Hence, modification of opioid-like behaviors in land snails using two different magnetic fields (both in the ELF) may involve two very different mechanisms! Note that this work on land snails has been possible in large part because hundreds of animals could be studied in a few hours. A similar approach to study, for example, bone healing in humans would be all but impossible. A different approach is needed if significant mechanism discovery is to be achieved in the majority of therapeutic applications, and especially if validation is to be done in humans.

Fortunately, two significant developments over the last decade have made it possible to dissect the mechanisms, both at the detection/transduction stage and to follow the events to the final behavioral/physiological outcome. These are the combined advances in molecular biology and non-invasive imaging, resulting in the field of molecular imaging.

For example, in 1981 the first commercial bone-density units were being used to evaluate treatments for osteoporosis, but relatively large groups had to be followed for years because of the uncertainty in the measurements. Twenty years later, the success of treatment in a single woman can be determined in six to 12 months. Not only do such advances allow the evaluation of mechanism, but they also allow the fine-tuning of therapy for the individual patient. In the future, ELF magnetic-field therapy will be image-guided. For example, in the treatment of patients with unipolar depression with transcranial magnetic stimulation, positron-emission tomography studies of brain blood flow and metabolism can predict the effectiveness of different magnetic-field parameters, the targeting of different brain structures, and the effectiveness of therapy in the individual patient [112]. These imaging methods are powerful. For example, Huber et al. [113] demonstrated that subtle differences in brain microwave irradiation resulted in significant differences in EEG and regional cerebral blood flow as measured with PET. In the future, advances in molecular imaging, such as the identification of number and activity of opioid receptors [114, 115], will allow

Experiment	Mechanism				Reference
	Induced Current	Free Radical	Magnetite	Parametric Resonance	
Variation of B_{AC} at 60 Hz	x	-	-	✓	Prato et al. 1995 [108]
Variation of frequency for B_{AC} and B_{DC}	x	x	-	✓	Prato et al. 1995 [108]
Variation of angle between B_{AC} and B_{DC}	x	-	x	✓	Prato et al. 1996a [109]
Variation B_{AC} and B_{DC} at 30 Hz	x	x	x	✓	Prato et al. 2000 [102]
Variation of angle between B_{AC} and B_{DC} in light and dark	-	-	x	-	Prato et al. 1996b [110]
Investigation of light/dark effects at 30, 60, 120 Hz	x	x	-	✓	Prato et al. 1997 [111]
Investigation of light/dark effects during day/night	-	-	-	✓	Prato et al. 1998 [112]
Role of nitric oxide synthase and related light/dark effects	-	-	-	✓	Kavaliers et al. 1998 [113], and Kavaliers & Prato 1999 [114]

✓ mechanism supported
x mechanism not supported
- mechanisms neither supported nor unsupported

Table 3: Summary of Evidence Supporting the Parametric Resonance Model as the Detection Mechanism Associated with ELF Magnetic Field Modulation of Opioid-Induced Analgesia

individual tailoring of pulsed ELF PEMFs in the treatment of pain.

Non-invasive anatomical, functional and molecular imaging will provide the platform by which elucidation of mechanisms will be possible and optimization of the treatment for the individual will be routine; these advances will result in a very significant acceptance of magnetic-field therapy for a wide variety of conditions.

15.2 Conclusion

There are many questions that require answers prior to the general acceptance of magnetic-field therapy as a primary treatment, rather than its use mainly as an adjunct therapy. For example, controlled, randomized, and double-blind studies must be used to assess optimal magnetic-field conditions and average duration of effect [4] in producing the best possible treatment. Furthermore, the cost-effectiveness of this form of therapy with respect to more traditional treatment protocols (e.g., non-steroidal anti-inflammatory drugs, analgesics, and massage) must be evaluated prior to use.

Difficulties in bioelectromagnetic research include the inability to reproduce and replicate work conducted in other laboratories. Furthermore, the lack of concrete mechanisms of action has impeded research regarding therapeutics [2]. Through continued research, and more solidified mechanisms of action, it is hoped that the therapeutic value currently associated with some ELF magnetic fields will become a mainstream intervention.

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Dendritic Voltage-Gated Ion Channels Regulate the Action Potential Firing Mode of Hippocampal CA1 Pyramidal Neurons

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Dendritic Voltage-Gated Ion Channels Regulate the Action Potential Firing Mode of Hippocampal CA1 Pyramidal Neurons

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Magee, Jeffrey C. and Michael Carruth. Dendritic voltage-gated ion channels regulate the action potential firing mode of hippocampal CA1 pyramidal neurons. *J. Neurophysiol.* 82: 1895–1901, 1999. The role of dendritic voltage-gated ion channels in the generation of action potential bursting was investigated using whole cell patch-clamp recordings from the soma and dendrites of CA1 pyramidal neurons located in hippocampal slices of adult rats. Under control conditions somatic current injections evoked single action potentials that were associated with an afterhyperpolarization (AHP). After localized application of 4-aminopyridine (4-AP) to the distal apical dendritic arborization, the same current injections resulted in the generation of an afterdepolarization (ADP) and multiple action potentials. This burst firing was not observed after localized application of 4-AP to the soma/proximal dendrites. The dendritic 4-AP application allowed large-amplitude Na^+-dependent action potentials, which were prolonged in duration, to backpropagate into the distal apical dendrites. No change in action potential backpropagation was seen with proximal 4-AP application. Both the ADP and action potential bursting could be inhibited by the bath application of nonspecific concentrations of divalent Ca^{2+} channel blockers (NiCl and CdCl). Ca^{2+} channel blockade also reduced the dendritic action potential duration without significantly affecting spike amplitude. Low concentrations of TTX (10–50 nM) also reduced the ability of the CA1 neurons to fire in the busting mode. This effect was found to be the result of an inhibition of backpropagating dendritic action potentials and could be overcome through the coordinated injection of transient, large-amplitude depolarizing current into the dendrite. Dendritic current injections were able to restore the burst firing mode (represented as a large ADP) even in the presence of high concentrations of TTX (300–500 μM). These data suggest the role of dendritic Na^+ channels in bursting is to allow somatic/axonal action potentials to backpropagate into the dendrites where they then activate dendritic Ca^{2+} channels. Although it appears that most Ca^{2+} channel subtypes are important in burst generation, blockade of T- and R-type Ca^{2+} channels by NiCl (75 μM) inhibited action potential bursting to a greater extent than L-channel (10 μM nimodipine) or N-, P/Q-type (1 μM ω-conotoxin MVIIC) Ca^{2+} channel blockade. This suggest that the Ni-sensitive voltage-gated Ca^{2+} channels have the most important role in action potential burst generation. In summary, these data suggest that the activation of dendritic voltage-gated Ca^{2+} channels, by large-amplitude backpropagating spikes, provides a prolonged inward current that is capable of generating an ADP and burst of multiple action potentials in the soma of CA1 pyramidal neurons. Dendritic voltage-gated ion channels profoundly regulate the processing and storage of incoming information in CA1 pyramidal neurons by modulating the action potential firing mode from single spiking to burst firing.

INTRODUCTION

Under normal, in vivo, conditions hippocampal pyramidal neurons fire either single action potentials or high-frequency bursts of multiple action potentials (Kandel and Spencer 1961; Ranck and Feder 1973; Pavlides and Winson 1989; Ylinen et al. 1995). These are two fundamentally different modes of firing, and a change from one mode to the other can drastically alter the processing of incoming signals. There is, in fact, evidence that action potential bursts are more important units of information than single action potentials (reviewed in Lisman 1997). In support of this concept, it has been observed that the types of computations performed by many neurons are most accurately represented when only the bursts of action potentials are considered (Otto et al. 1991). This informationally rich aspect of burst firing also has been hypothesized to be important in the consolidation of new memories, a process in which the hippocampus is known to be required (Buzsaki 1989; Cattaneo et al. 1981). Burst firing in the hippocampus is, therefore, important in both the processing and storage of neuronal information.

The mechanisms by which hippocampal CA1 pyramidal neurons generate bursts of action potentials are still incompletely characterized. Most studies agree that the generation of an afterdepolarization (ADP) provides the prolonged somatic depolarization required for the initiation of multiple spikes (Jensen et al. 1994, 1996; White et al. 1989; Wong and Prince 1981). There is, however, some discrepancy about the ionic mechanisms underlying this ADP with some studies suggesting that a persistent Na^+ current may be involved (Azouz et al. 1996), whereas others have suggested that Ca^{2+} currents are most important (Andreasen and Lambert 1995; Hoffman et al. 1997; Traub et al. 1993; White et al. 1989; Wong and Prince 1981). There is also considerable evidence that the active membrane properties of the dendritic arborization are involved in the generation of ADPs and action potential bursting (Andreasen and Lambert 1995; Hoffman et al. 1997; Traub et al. 1993; Wong and Stewart 1992; Wong et al. 1979). Therefore the voltage-gated ion channels located in the dendritic arborizations of CA1 pyramidal neurons may be responsible for modulating the mode of action potential firing in these cells.

At the present time the dendrites of CA1 pyramidal neurons have been shown to contain Na^+, Ca^{2+}, K^+, and H-currents (Hoffman et al. 1997; Kavalali et al. 1997; Magee 1998; Magee and Johnston 1995; Mickus et al. 1999; Tsubakawa et al. 1999). One current in particular, a transient outward, A-type, K^+ current, tends to dominate the regenerative active properties of the dendrites and in turn the entire cell itself (Hoffman et al. 1997). The density of this current increases nearly fivefold from the soma to 350 μm out into the apical dendrites and as a result the more distal dendrites are only weakly excitable under control, in vitro, conditions (Hoffman et al. 1997; Magee

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et al. 1998). The activity of the dendritic A-type K^+ channels, and their likely molecular correlate $K_{V4.2}$, are highly modulatable through numerous mechanisms (e.g., neuromodulatory substances, specific subunit composition, and membrane potential) (see Hoffman and Johnston 1998; Magee et al. 1997). Because of these characteristics, the A-type K^+ channel population located in the dendrites appears to be well suited to the task of regulating the firing mode of CA1 pyramidal neurons.

To investigate the role of dendritic voltage-gated ion channels in the generation of action potential bursting, we have used whole cell patch-clamp recordings from the soma and dendrites of CA1 pyramidal neurons located in hippocampal slices of adult rats. We report here that a reduction in the amplitude of dendritic K^+ currents results in the backpropagation of large-amplitude Na-dependent action potentials and the subsequent dendritic-initiation of Ca^{2+}-dependent potentials (see also Hoffman et al. 1997). The dendritic Ca^{2+}-potentials then provide the prolonged inward current required for the generation of the ADP and action potential bursting. The role of backpropagating action potentials and the specific Ca^{2+}-channel subtypes involved also were explored.

METHODS

Preparation

Hippocampal slices (400 μm) were prepared from 6- to 12-wk-old Sprague-Dawley rats using standard procedures that have been described previously (Magee 1998). Individual neurons were visualized with a Zeiss Axioskop fit with differential interference contrast (DIC) optics using infrared illumination. All neurons exhibited resting membrane potentials between −60 and −75 mV. Area CA3 was removed surgically from each slice just before use.

Recordings and solutions

Whole cell patch-clamp recordings were made using two Dagan BVC-700 amplifiers (Minneapolis, MN) in active "bridge" mode. Data was acquired (10–20 kHz, filtered at 1kHz) using an Instrutech ITC-16 interface (Great Neck, NY) and Pulse Control software (Richard Bookman, University of Miami) written for Igor Pro (Wavemetrics, Lake Oswego, OR). External solutions contained (in mM): 125 NaCl, 2.5 KCl, 1.25 NaH_2PO_4, 25 $NaHCO_3$, 2.0 $CaCl_2$, 1.0 $MgCl_2$, 25 dextrose, and 0.01 6,7-dinitroquinoxaline 2,3(1H,4H)-dione (DNQX). The solution was bubbled with 95% O_2-5% CO_2 at ~36°C (pH 7.4) for all recordings. Whole cell recording pipettes (somatic: 2–4 MΩ, dendritic: 5–7 MΩ), were pulled from borosilicate glass. The internal pipette solutions contained (in mM): 120 $KMeSO_4$, 20 KCl, 10 HEPES, 0.1 EGTA, 4.0 Mg_2ATP, 0.3 $Tris_2GTP$, 14 phosphocreatine, and 4 NaCl (pH 7.25 with KOH). Series resistance for somatic recordings was 6–20 MΩ while that for dendritic recordings was 15–40 MΩ. Voltages have not been corrected for the theoretical liquid junction potentials (6–7 mV).

Drug preparation and application

Drug containing perfusion solutions were the same as the external solution with the 25 mM $NaHCO_3$ replaced by 10 mM HEPES and 10 mM 4-AP. Final concentrations of TTX (10–500 nM), ω-conotoxin MVIIC (1 μM), NiCl (750 or 75 μM), CdCl (500–750 μM), and nimodipine (10 μM) were achieved by mixing stock solutions with either the standard external or perfusion external solution. Nimodipine was dissolved in DMSO (final DMSO concentration was 0.1%), whereas all others were dissolved in water to make stock solutions. Because of the light sensitivity of nimodipine, it was used under dark conditions.

We used a pressure ejection system composed of a computer controlled pneumatic pump (Medical Systems, Greenvale, NY) connected to a somatic patch pipette to locally apply 10 mM 4-AP. The pressures (10–20 psi) and durations (5–8 s) required to perfuse an ~200-μm-diam region of the cell were tested using fluorescent dyes (Lucifer yellow or cascade blue). With such settings, a distal region of the cell (~150–350 μm from the soma) could be perfused when the pipette was placed within 20 μm of the dendritic trunk ~250 μm from the soma. When the pipette was placed at a proximal point on the apical trunk (<50 μm from the soma), a proximal region (proximal 150 μm of the apical dendrite, the soma and ~50 μm of the proximal basal dendrite) of the cell could be perfused.

Data analysis

The amount of bursting was quantified by determining the ADP duration and the number of spikes generated during the ADP (for 30 s after 4-AP application). ADP duration was measured after the termination of the current injection as the time the potential stayed above one-half of spike threshold (see Fig. 1*B*). Traces occurring 30 s after 4-AP application (15 sweeps given at 2-s intervals) were averaged, and the ADP was measured from this average trace. The number of spikes fired was the total number of action potentials occurring during the ADP for 30 s after the application of 4-AP (15 total current injections given at 2-s intervals). ANOVA and Fishers post hoc test were performed to test for statistical significance.

RESULTS

Role of dendritic K^+ channels in bursting

It has been shown previously that bath application of millimolar concentrations of 4-AP causes burst and repetitive action potential firing in CA1 pyramidal neurons (Andreasen and Lambert 1995; Hoffman et al. 1997). To test the role of distal, as opposed to proximal, K^+ channels in the regulation of burst firing, 4-AP was applied via a perfusion pipette to either the distal apical dendritic trunk (~250 μm) or to a proximal location (<50 μm; Fig. 1*A*). Under control conditions, somatic current injections (20 ms, 400–800 pA) were given to CA1 pyramidal neurons to evoke a single action potential during each injection period. With distal 4-AP application, the 20-ms-long current injections evoked bursts of multiple action potentials (usually 3 or 4 but ≤8 could be initiated) that generally decreased in amplitude during the burst. No such effect was elicited by proximal application of 4-AP (Fig. 1). The distal application caused a pronounced ADP which appeared as a "hump" in the somatic membrane potential after the current injection. The average duration of the ADP (at half threshold) and the total number of spikes fired (for 30 s after application) was much longer for distal application (41 ± 3 ms, 5 ± 0.5 action potentials, $n = 31$) than for proximal application (18 ± 6 ms, 0 action potentials, $n = 5$) or with no application (16 ± 2 ms, 0 action potentials, $n = 31$; Fig. 1, *B* and *C*). The 4-AP-sensitive K^+ channels located within the distal dendritic arbor of CA1 pyramidal neurons are therefore capable of controlling the firing mode (single spiking or burst firing) of these cells.

By what mechanisms do these distal channels regulate the firing mode of the entire cell? Simultaneous dendritic and somatic voltage recordings from a separate population of neurons revealed that the amplitude and duration of the backpropa-

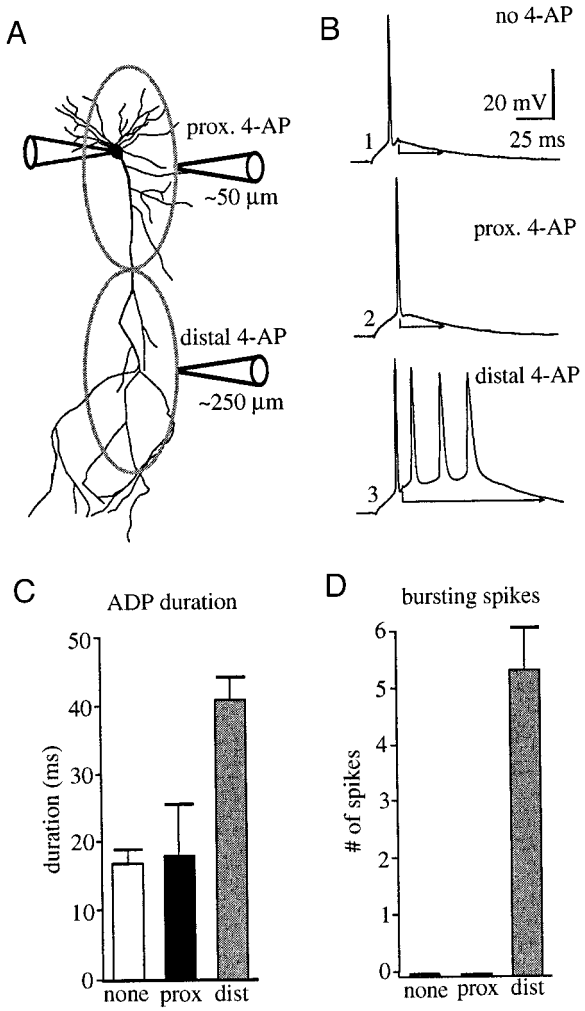

FIG. 1. Blockade of distal dendritic K^+ channels induces burst firing. *A*: schematic diagram of experimental setup showing proximal and distal 4-aminopyridine (4-AP) application areas. *B*: somatic membrane potential under control conditions (*B1*, labeled no 4-AP), with 10 mM 4-AP applied to a proximal area including the soma, proximal apical and basal dendrites (*B2*, labeled prox 4-AP), and with 4-AP applied only to the distal dendritic arborization (*B3*, labeled distal 4-AP). Current injection was 500 pA for 20 ms. →, duration of the afterdepolarization (ADP) measured from the end of the current injection (indicated by —), at a potential that was one-half of action potential threshold. *C*: cumulative averages of ADP duration under control conditions (none, $n = 31$), with proximal 4-AP application (*prox.*, $n = 5$), and distal 4AP application (dist, $n = 31$). *D*: total number of action potentials occurring during the ADP (after the current injection) for 30 s after the 4-AP application (15 current injections) under each condition (numbers of cells are the same as in *C*).

gating dendritic action potential increased significantly during distal 4-AP application (control amplitude: 40 ± 4 mV, duration: 1.3 ± 0.1 ms; 4-AP amplitude: 74 ± 5 mV, duration: 6.8 ± 2.5 ms; $n = 4$; Fig. 2). There was, on the other hand, no change in either amplitude or duration of the dendritic spike with proximal 4-AP application ($n = 2$). The increase in dendritic action potential duration, but not amplitude, during distal 4-AP application was sensitive to nonspecific concentrations of NiCl or CdCl (0.5–0.75 mM) (amplitude: 66 ± 5 mV; duration: 1.9 ± 0.2 ms; $n = 4$). The broadness of the dendritic action potential during bursting, therefore appears to be the result of a Ca^{2+} influx mediated by dendritic voltage-gated Ca^{2+} channels (see also Hoffman et al. 1997).

The ADP likewise was reduced by high concentrations of Cd^{2+} or Ni^{2+}, indicating that it is generated by the inward current carried by dendritic voltage-gated Ca^{2+} channels (4-AP: 48 ± 7 ms; 4-AP and divalents: 22 ± 7 ms; $n = 4$). Together these data indicate that dendritic action potentials are capable of evoking a substantial Ca^{2+} influx through voltage-gated Ca^{2+} channels once they are released from the shunting effect of distal dendritic K^+ channels. This Ca^{2+} current propagates to the soma to generate the somatic ADP that is responsible for the induction of burst firing. Such events were not observed when the proximal K^+ channel population was reduced. The distal dendritic K^+ channels, therefore appear to regulate burst firing in CA1 pyramidal neurons by modulating the backpropagation of dendritic action potentials.

Role of backpropagating action potentials in bursting

It has been reported previously that low concentrations of TTX are capable of blocking burst firing in CA1 pyramidal neurons without affecting somatic spike amplitude (Azouz et al. 1996). Because of the low safety factor of dendritic action potential propagation, low concentrations of TTX also have been shown to greatly inhibit the backpropagation of action potentials into the dendrites (Mackenzie and Murphy 1998; Magee and Johnston 1997). We therefore sought to test the hypothesis that low concentrations of TTX can inhibit burst firing by reducing the amplitude of dendritic action potentials and associated Ca^{2+} current.

Low TTX concentrations were applied to the slice either by bath perfusion of 10 nM TTX or by including 50 nM TTX along with 4-AP in the perfusion pipette. Both methods were equally effective in inhibiting action potential bursting that was induced by distal 4-AP application (43 ± 3 vs. 26 ± 6 ms ADP, 5 ± 0.5 vs. 1 ± 1 action potentials, $n = 6$), without significantly effecting somatic action potential amplitude (94 ± 4 vs. 90 ± 5 mV).

Simultaneous somatic and dendritic recordings in another set of neurons show that bath application of 10 nM TTX reduced the amplitude of the dendritic spike from 76 ± 7 to 21 ± 7 mV (with 4-AP; $n = 4$; Fig. 3). Injection of a large I_{Na}-shaped depolarizing current (10–90% rise time = 0.4 ms, $\tau_{off} = 0.6$ ms, 4-nA peak) into the dendrites during the somatic action potential reestablished the large-amplitude dendritic action potential (69 ± 7 mV, $n = 4$) and the burst firing mode (ADP control: 14 ± 1 ms, +4-AP: 50 ± 12 ms, +4-AP and TTX: 24 ± 5 ms, +4-AP and TTX + I: 44 ± 10 ms, $n = 4$; Fig. 3*D*). Subsequent application of CdCl or NiCl (500–750 μM) blocked the ability of dendritic current injections to restore action potential bursts (ADP: 25 ± 6 ms, $n = 4$; Fig. 3). This indicates that the artificially produced large-amplitude dendritic spikes evoke bursting through the activation of voltage-gated Ca^{2+} channels. These data support the idea that low concentrations of TTX block bursting in CA1 pyramidal neurons by inhibiting the back-propagation of action potentials into the dendritic arbor thereby reducing the prolonged Ca^{2+} influx associated with dendritic spikes.

Large ADPs also could be generated even in the presence of high concentrations of TTX (300–500 μM) as long as I_{Na}-shaped depolarizing currents (10–90% rise time = 0.4 ms, $\tau_{off} = 0.6$ ms, 10- to 15-nA peak) were injected into the apical dendrites (Fig. 4). The amplitude and duration of these ADPs

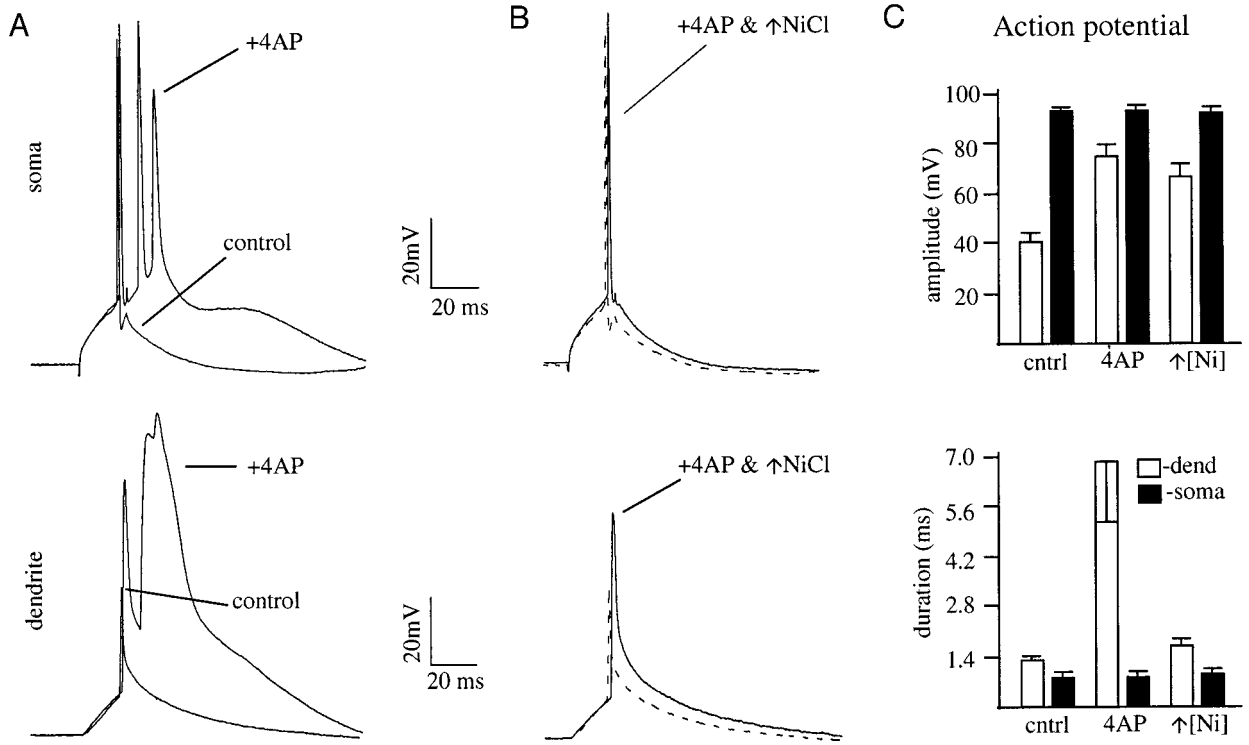

FIG. 2. Large-amplitude dendritic action potentials activate dendritic Ca^{2+} channels. *A*: simultaneous somatic (*top*) and dendritic (*bottom*) recordings of membrane potential in a CA1 pyramidal cell under control conditions (no 4-AP) and with distal application of 4-AP (+4-AP). These traces demonstrate the ability of distal 4-AP application to significantly increase the amplitude and duration of the backpropagating dendritic action potential while not affecting somatic action potential parameters. *B*: simultaneous somatic and dendritic recordings of membrane potential in the same cell as in *A* now with nonspecific concentrations of NiCl (0.75 mM) in bath. - - -, control recordings shown in *A* for comparison. *C*: grouped data of action potential amplitude (*left*) and duration (*right*) for dendritic (□) and somatic recordings (■) under control (cntrl), in the presence of distal 4-AP (4-AP) and with both 4-AP and 0.75 mM NiCl (↑ [Ni]).

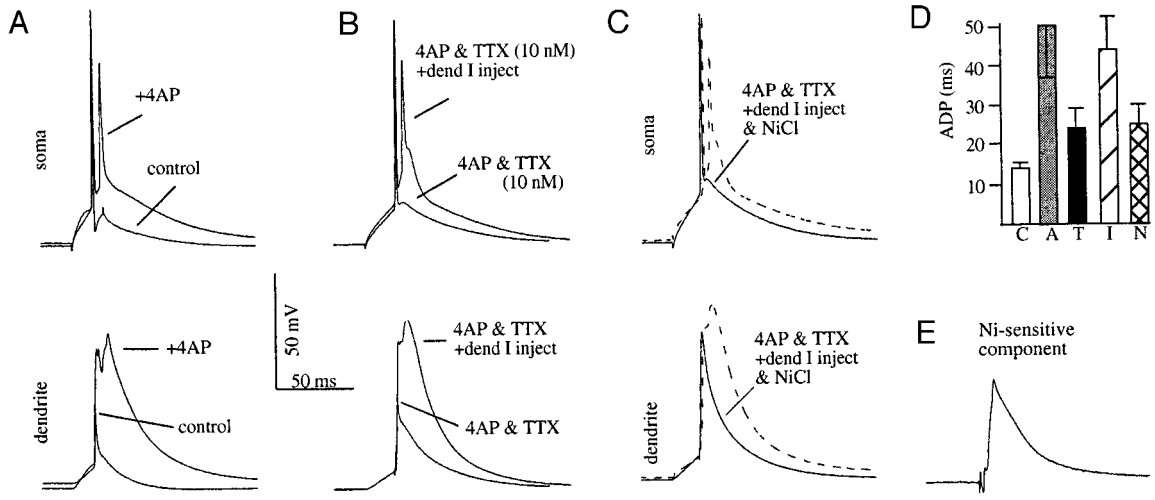

FIG. 3. Dendritic action potential amplitude is reduced by low concentrations of TTX. *A*: simultaneous recordings of somatic (*top*) and dendritic (*bottom*) membrane potential during control conditions (control) and during application of 4-AP. *B*: somatic and dendritic membrane potential showing the reduction in dendritic action potential amplitude induced by bath application of TTX (10 nM; traces labeled 4-AP and TTX). Large, transient depolarizing dendritic current injections (see text) are able to increase dendritic action potential amplitude and overcome the TTX inhibition of burst firing (traces labeled 4-AP and TTX + dend I inject). *C*: traces showing the ability of high NiCl concentrations (0.75 mM) to inhibit the burst firing recovered by dendritic current injection (traces labeled 4-AP and TTX + dend I inject and NiCl). - - -, recordings with dendritic current injection that were shown in *B*. *D*: grouped data of ADP duration under control conditions (C; □), during 4-AP application (A; ▨), 4-AP and TTX application (T; ■), 4-AP and TTX application with dendritic current injection (I; ▨), and 4-AP and TTX application with dendritic current injection plus 0.75 mM NiCl (N; ▩; $n = 4$ for all groups). *E*: difference trace (- - - in *C* minus — in *C*) showing of the effect of Ca^{2+} channel blockade on dendritic voltage. All recordings shown are from the same neuron.

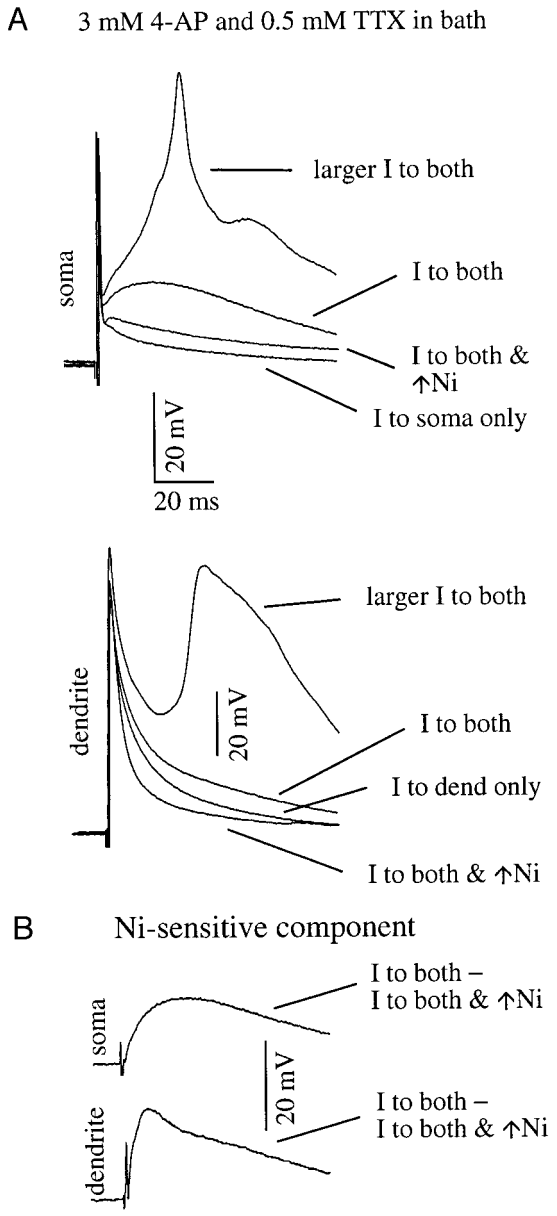

FIG. 4. ADP generation does not require Na^+ channels. *A*: simultaneous recordings of somatic and dendritic membrane potential with 3 mM 4-AP and 0.5 mM TTX in bath. Large transient current injections (see text) into either only the soma or only the dendrite produce spikes that repolarize rapidly. Combined injection of current into both the dendrite and soma compartments produces longer duration dendritic spikes and somatic spikes that are associated with an ADP. This ADP can be made large enough to evoke further spiking by the injection of even larger-amplitude currents. Prolonged dendritic spike duration and ADP are both sensitive to bath application of 0.75 mM NiCl. *B*: difference between somatic (*top*) and dendritic (*bottom*) traces with and without 0.75 mM NiCl in the bath, showing the depolarizations induced by Ca^{2+} channel activation.

could be increased by increasing the amount of current injected. In fact, a presumably Ca^{2+}-mediated spike could be initiated during the ADP if sufficiently large-amplitude currents were used (Fig. 4*A*; trace labeled larger I to both). The ADP again was seen to be sensitive to high concentrations of NiCl (Fig. 4, *A* and *B*). These data demonstrate that the depolarizing component involved in generating action potential bursts (the ADP) is produced primarily by current flowing through dendritic Ca^{2+} channels. Dendritic Na^+ channels, on the other hand, provide the inward current that is needed to depolarize the membrane potential enough to activate the more prolonged dendritic Ca^{2+} current. All of this channel activation is governed by the dendritic K^+ channels, which are able to maintain CA1 pyramidal neurons in a single-spiking mode by counterbalancing the dendritic Na^+ and Ca^{2+} currents. Any reduction in the amplitude of this countering current is capable of moving CA1 neurons into the burst firing mode.

Ca^{2+} channel subtypes involved in bursting

Which specific subtypes of Ca^{2+} channels are involved in the generation of the ADP? The more distal apical dendritic arborizations of CA1 pyramidal neurons have been shown to contain a relatively high density of Ni^{2+}-sensitive (IC_{50} ~50 μM) voltage-gated Ca^{2+} channels along with a lower densities of dihydropyridine-sensitive and ω-conotoxin MVIIC-sensitive Ca^{2+} channels (Avery and Johnston 1996; Christie et al. 1995; Kavalali et al. 1998; Magee and Johnston 1995; Magee et al. 1996). To determine the relative contributions of the various Ca^{2+} channel subtypes to action potential bursting, we observed the ability of various channel blockers (included in either the bathing external solution or along with 4-AP in the perfusion solution) to inhibit the ADP and action potential burst firing (Fig. 5).

L-type Ca^{2+} channels are blocked by dihydropyridines and application of 10 μM nimodipine (a supramaximal concentration) reduced the ADP duration (45 ± 6 vs. 29 ± 3 ms, $n = 10$, $P = 0.04$) and substantially reduced the number of action potential induced (5.8 ± 0.9 vs. 2.0 ± 1.0 action potentials, $n = 10$, $P < 0.01$). T- and R-type Ca^{2+} channels are inhibited by low concentrations of NiCl, and application of 75 μM Ni^{2+} (~70% block) reduced the ADP duration (47 ± 6 vs. 25 ± 6 ms, $n = 7$, $P < 0.01$) and action potential firing (6.2 ± 1.7 vs. 1.5 ± 1.0 action potentials, $n = 7$, $P < 0.01$) to an even greater extent than nimodipine ($P < 0.01$). The combined application of NiCl and nimodipine appeared to have an additive inhibitory effect on bursting (39 ± 2 vs. 16 ± 4 ms ADP, 6.0 ± 1.0 vs. 1.0 ± 1.0 action potentials, $n = 4$). The N- and P/Q-type Ca^{2+} channel blocker, ω-conotoxin MVIIC- (1 μM, a supramaximal concentration) had only a slight and statistically insignificant effect on action potential bursting (ADP: 39 ± 4 vs. 34 ± 5 ms; action potentials: 3.5 ± 1.7 vs. 3.2 ± 1.4, $n = 5$). Together these results suggest that the Ni^{2+}-sensitive Ca^{2+} channels (T and R type) play the most substantial role in the generation of somatic ADPs and action potential burst firing with L channels being next in importance and N and P/Q types playing a lessor role. This fits well with the proposed distribution of voltage-gated Ca^{2+} channels in the dendritic arbor of CA1 pyramidal neurons and further suggests that Ca^{2+} channels located within the dendrites provide the largest contribution of inward current during the ADP.

DISCUSSION

In summary, it appears that dendritic K^+ channels (primarily transient A type) regulate the action potential firing mode of CA1 pyramidal neurons by modulating the backpropagation of dendritic action potentials. Reduction of distal dendritic K^+ current allows large-amplitude dendritic action potentials to

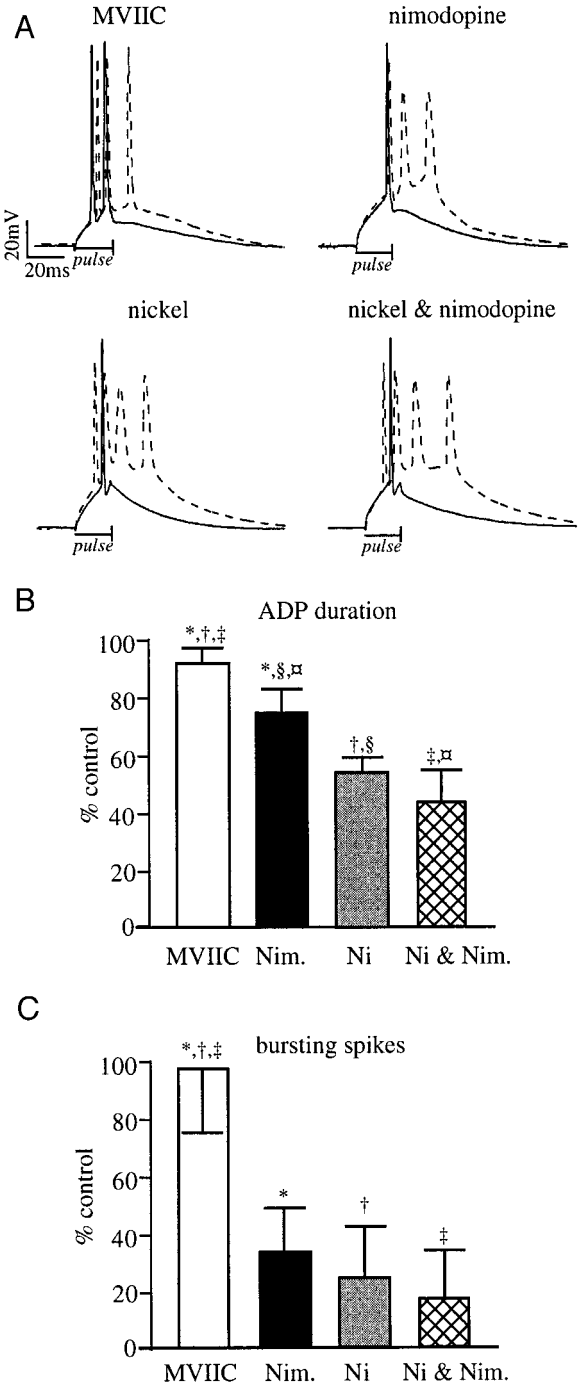

FIG. 5. Ca^{2+} channel subtypes involved in action potential bursting. *A*: somatic membrane potential during normal distal dendritic 4-AP application (· · ·) and in the presence of specific blockers of Ca^{+} channel subtypes (—). Grouped data of ADP duration (*B*) and number of evoked action potentials (*C*) expressed as percent control for 1 μM ω-conotoxin MVIIC (□, $n = 5$), 10 μM nimodopine (■, $n = 10$), 75 μM NiCl (▤, $n = 7$) and nimodopine and NiCl (▩, $n = 4$). In *B*, $P = 0.06$ (*), $P < 0.01$ (†, ‡, §, ¤). In *C*, $P < 0.01$ (*, †, ‡).

proficiently activate dendritic Ca^{2+} channels (primarily T and R type) substantially increasing the duration of dendritic action potentials. The Ca^{2+} current generated by these dendritic spikes propagates to the soma to produce a slow, prolonged membrane depolarization (ADP) that is capable of initiating multiple somatic/axonal action potentials. The action potential firing or output mode of the entire CA1 pyramidal neuron therefore can be regulated through an intricate interplay among the major voltage-gated ion channels (K^{+}, Na^{+}, and Ca^{2+}) located within the distal dendrites. With the transient A-type K^{+} channel population fully intact, the activation of dendritic Na^{+} and Ca^{2+} channels is minimal and the neuron fires single spikes. When, on the other hand, the activatable K^{+} channel population is reduced, the dendrites are allowed to provide both rapidly activating and relatively prolonged inward currents (through the activation of both Na^{+} and Ca^{2+} channels) that induce the cell to fire bursts of action potentials.

Although the importance of the transient Na^{+}-current to burst firing was obvious, we found little evidence of a contribution by a persistent Na^{+} current. Indeed the enhanced sensitivity of burst firing to low TTX concentrations appeared to be associated with the low safety factor of action potential backpropagation and not to the reduction of a persistent Na^{+} current. The observation that full-amplitude ADPs could be generated in the absence of a substantial Na^{+} channel population further supports the idea that the primary role of these channels in burst firing is to allow the backpropagation of action potentials into the dendritic arbor.

The Ca^{2+} dependence of burst firing observed here was not seen in at least one previous study (Azouz et al. 1996) but has been observed in others (Andreasen and Lambert 1995; Traube et al. 1993; White et al. 1989). Although there are many methodological differences between these studies (sharp vs. patch electrodes, recording temperature, $[K^{+}]_o$, species), the most straightforward explanation of the different results is that there are multiple mechanisms of burst generation in CA1 pyramidal neurons. The burst firing induced by dendritic K^{+} channel modulation may involve different mechanisms than those involved in the burst firing studied in other reports.

What physiologically relevant mechanisms exist to lower the density of dendritic K^{+} currents? Numerous neuromodulatory substances (norepinephrine, dopamine, arachidonic acid, and metabotropic glutamate) and events related with ongoing synaptic activity (membrane depolarization and elevated $[K^{+}]_o$, $[Ca^{2+}]_i$) have all been shown to reduce the amplitude of the A-type K^{+} channels that are located in CA1 pyramidal neurons (Anderson et al. 1997; Baldwin et al. 1991; Blair et al. 1991; Chen and Wong 1991; Hoffman and Johnston 1998, 1999; Hoffman et al. 1997; Keros and McBain 1997; Villaroel and Schwarz 1996). Thus the presence of neuromodulators along with specific forms of synaptic input (highly synchronized, high-frequency, distal synaptic input) are likely to result in the generation of action potential bursts. On the other hand, lower frequency, less synchronized input will induce single action potentials. In support of this, CA1 pyramidal neurons have been observed to fire burst of action potentials during sharp wave activity (Buzsaki 1989; Kamondi et al. 1998; Suzuki and Smith 1987). During such activity, populations of CA3 pyramidal neurons provide highly synchronized bursts of high-frequency synaptic input to CA1 pyramidal neurons, which then in turn respond with burst firing and dendritic Ca^{2+} spiking (Buzsaki et al. 1996; Kamondi et al. 1998).

Burst firing has been shown to increase the probability of long-term potentiation (LTP) induction in CA1 pyramidal neurons, suggesting that information storage may be enhanced during this mode of action potential firing (Thomas et al. 1998). Along these lines, memory consolidation is hypothesized to occur primarily during the sharp wave episodes oc-

curring during slow wave sleep (Buzsaki 1989). Thus by modulating the action potential firing mode of CA1 pyramidal neurons dendritic voltage-gated channels may be able to fundamentally regulate hippocampal function.

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The action potential in mammalian central neurons

Bruce P. Bean

Abstract | The action potential of the squid giant axon is formed by just two voltage-dependent conductances in the cell membrane, yet mammalian central neurons typically express more than a dozen different types of voltage-dependent ion channels. This rich repertoire of channels allows neurons to encode information by generating action potentials with a wide range of shapes, frequencies and patterns. Recent work offers an increasingly detailed understanding of how the expression of particular channel types underlies the remarkably diverse firing behaviour of various types of neurons.

Heterologous expression
Expression of protein molecules by the injection of complementary RNA into the cytoplasm (or complementary DNA into the nucleus) of host cells that do not normally express the proteins, such as *Xenopus* oocytes or mammalian cell lines.

Spike
Another term for an action potential (especially the portion with the most rapidly changing voltage).

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In the years since the Hodgkin–Huxley analysis of the squid axon action potential[1], it has become clear that most neurons contain far more than the two voltage-dependent conductances found in the squid axon[2,3]. Action potentials serve a very different function in neuronal cell bodies, where they encode information in their frequency and pattern, than in axons, where they serve primarily to rapidly propagate signals over distance. The membrane of the squid axon is a poor encoder, as it fires only over a narrow range of frequencies when stimulated by the injection of widely-varying current levels[4]. By contrast, most neuronal cell bodies (in both vertebrate and invertebrate animals) can fire over a far wider range of frequencies and can respond to small changes in input currents with significant changes in firing frequency[5–10]. Clearly, this richer firing behaviour depends on the expression of more types of voltage-dependent ion channels. Interestingly, although the squid axon is strikingly deficient as an encoder, some other invertebrate axons can fire over a wide frequency range[11] and have a richer repertoire of ion channel types[12], as do at least some mammalian axons[13].

The presence of multiple channel types in most neurons has been appreciated since at least the 1970s. However, few were prepared for the staggering number of distinct kinds of ion channels revealed over the last two decades by the convergent techniques of patch-clamp recording, heterologous expression of cloned channels and genomic analysis — including, for example, more than 100 principal subunits of potassium channels[14]. Even more surprising, perhaps, was the gradual realization of just how many distinct voltage-dependent conductances are expressed by individual neurons in the mammalian brain — commonly including 2 or 3 components of sodium current, 4 or 5 different components of voltage-dependent calcium currents, at least 4 or 5 different components of voltage-activated potassium current, at least 2 to 3 types of calcium-activated potassium currents, the hyperpolarization-activated current I_h, and others. Because of this complexity, our understanding of how different conductances interact to form the action potentials of even the best-studied central neurons is still incomplete, even though Hodgkin and Huxley devised the basic experimental approach still being used — voltage-clamp analysis of individual time- and voltage-dependent conductances and reconstruction of the whole by numerical modelling — more than half a century ago[1]. In this review I discuss differences in the shape, rate and pattern of firing of action potentials between various types of neurons, focusing on mammalian central neurons, and review recent advances in understanding the role of specific types of ion channels in generating these differences.

All spikes are not alike

The shape of action potentials (BOX 1) differs considerably among various types of neurons in the mammalian brain (FIG. 1). For example, in the cortex and hippocampus, GABA (γ-aminobutyric acid)-releasing interneurons generally have narrower spikes than glutamatergic pyramidal neurons. This is seen most clearly in intracellular recordings, in which spike shape can be determined precisely[8,15,16] (FIG. 1), but the difference in spike width is also evident from extracellular recordings *in vivo*[17]. Cells with narrow spikes also commonly (but not always[8]) display 'fast-spiking' behaviour: being capable of firing at high frequencies with little decrease in frequency during prolonged stimulation[5,6,8,9,15,18–20]. Recently, the fast-spiking phenotype has been related to expression of the Kv3 family of voltage-gated potassium

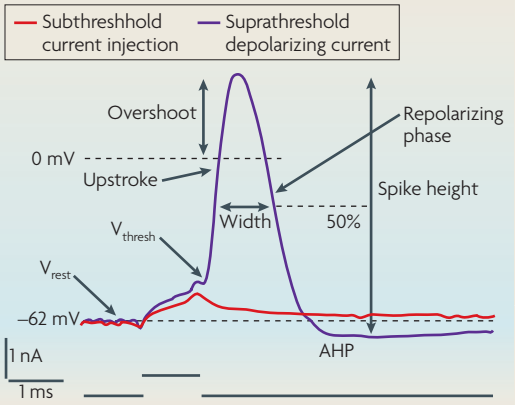
Projection neurons
Neurons with relatively long axons that project out of a local circuit (distinct from interneurons).

Bursting
The firing of a rapid series of several action potentials with very short (less than ~5 ms) interspike intervals.

Adaptation
Slowing or cessation of firing during a maintained stimulus.

Initial segment
The slender initial region of an axon where it originates from an axon hillock of the cell body (or sometimes from a major dendrite), characterized by the fasciculation of microtubules.

Box 1 | **Anatomy of an action potential**



The figure shows an action potential recorded from a pyramidal neuron in the CA1 region of a rat hippocampus[117], illustrating commonly measured parameters. The action potential was elicited by the injection of just-suprathreshold depolarizing current (purple). Use of a brief (1 ms) injection has the advantage that the spike and the afterpotentials are not directly influenced by the current injection. The response to a subthreshold current injection is also shown (red). Resting potential (V_{rest}) is typically in the range of –85 mV to –60 mV in pyramidal neurons. Voltage threshold (V_{thresh}) is the most negative voltage that must be achieved by the current injection for all-or-none firing to occur (in this neuron it is about –53 mV, a typical value). Threshold is less well defined for spontaneously firing neurons, especially in isolated cell bodies where transition from gradual interspike depolarization to spike is typically less abrupt than in slice recordings, and for such cases threshold is more easily estimated from phase-plane plots (FIG. 2). The upstroke (also called the depolarizing phase or rising phase) of the action potential typically reaches a maximum velocity at a voltage near 0 mV. Overshoot is defined as peak relative to 0 mV. Spike height is defined as the peak relative to either resting potential or (more commonly) the most negative voltage reached during the afterhyperpolarization (AHP) immediately after the spike. Spike width is most commonly measured as the width at half-maximal spike amplitude, as illustrated. This measurement is sometimes referred to, confusingly, as 'half-width' or 'half-duration'; 'half-height width' would be clearer. As is typical for pyramidal neurons, the repolarizing phase (also called 'falling phase' or 'downstroke') has a much slower velocity than the rising phase. Figure modified, with permission, from REF. 117 © (1987) Cambridge Univ. Press.

channels, the rapid and steeply voltage-dependent activation and deactivation kinetics of which are well-suited for generating narrow action potentials and short refractory periods[6,21–27]. The fast-spiking phenotype is not confined to interneurons, since Purkinje neurons (which are GABAergic projection neurons) also fire steadily at high frequencies and have narrow action potentials that are repolarized mainly by Kv3-mediated currents[28–31]. Nor are all fast-spiking neurons GABAergic, as neurons of the subthalamic nucleus, which are glutamatergic, have this phenotype[7,32] and express large potassium currents mediated by Kv3-family channels[33]. The calyx of Held, a presynaptic glutamatergic terminal, also has narrow spikes with repolarization by Kv3 channels and can fire at high frequencies[34–36].

The distinctive phenotype of fast-spiking neurons with narrow action potentials is unusual in that it presents a general correlation across many neuronal types between firing behaviour and action potential shape. In general, however, firing behaviour can take many different forms of patterns and frequencies, with little obvious correlation with spike shape[8,15,37,38]. The firing pattern of a neuron (which includes frequency of firing as a function of stimulus strength, bursting versus non-bursting activity and adapting versus non-adapting behaviour) is probably a more important physiological property than the shape of the spike. However, the two cannot be cleanly separated, as differences in ionic conductances producing differences in firing patterns will in general also produce differences in spike shape, although these may be more subtle. Spike shape *per se* is probably most significant at presynaptic terminals, where small differences in shape can produce changes in the timing of presynaptic calcium entry, leading to dramatic changes in postsynaptic currents. Interestingly, spike shape in presynaptic terminals can be quite different to that in the cell body of the same neuron[39] (FIG. 1f,g).

Somatic versus membrane action potentials

The Hodgkin–Huxley analysis of the squid axon action potential[1] was greatly facilitated by creating an artificial situation in which all of the axonal membrane experiences the same voltage at the same time — the 'membrane action potential' — which is achieved by inserting an axial wire to make axial resistance negligible. In mammalian neurons, action potentials are usually recorded from cell bodies in brain slices, in which axons and dendritic trees are largely intact. In this situation, the cell body is roughly isopotential during the spike (that is, the membrane voltage is the same at different places and undergoes simultaneous change), but there may be current flow between the cell body and the dendrites and axon of the cell that alters the shape of the action potential to some extent. In most central neurons, the spike appears to be initiated in the initial segment of the axon[40–47], at a location that in pyramidal neurons is typically 30–50 µm from the cell body. This is far enough away that the shape of the action potential recorded in the cell body can show clear effects arising from non-uniformity of voltage[40,41,47]. Dendrites probably also influence the form of somatically recorded action potentials, partly by serving as a capacitative load that slows and truncates fast spikes (by absorbing currents that would otherwise go to changing somatic voltage) but also by playing an active part in generating afterdepolarizations. Thus, although the action potential recorded in the cell body of a neuron in a brain slice preparation is much closer to a membrane action potential than to a uniformly propagating axonal action potential, non-uniformity of voltage can be significant, especially when it is changing most rapidly.

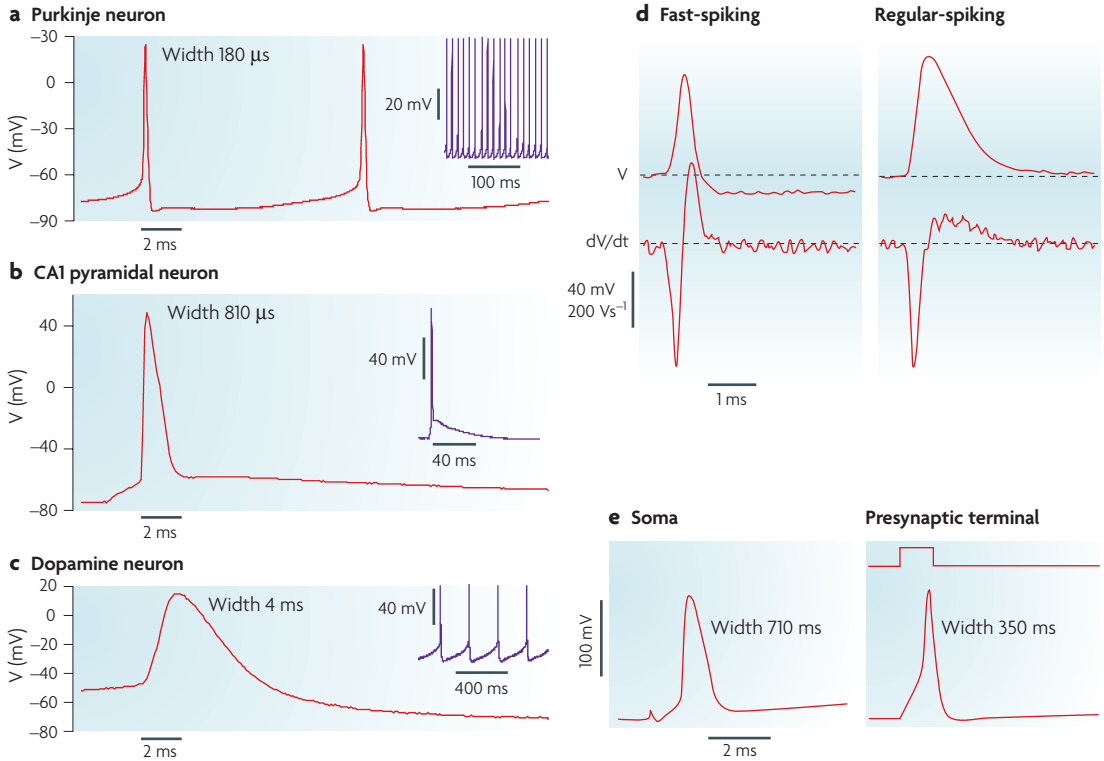


Figure 1 | **Diversity of action potentials in central neurons. a** | Spontaneous action potentials in an acutely dissociated mouse cerebellar Purkinje neuron (A. Swensen, unpublished observations). **b** | Action potential in a hippocampal CA1 pyramidal neuron in brain slice (M. Martina, unpublished observations). **c** | Spontaneous action potentials in a midbrain dopamine neuron (M. Puopolo, unpublished observations). Note the dramatic differences in width between these three action potentials. **d** | Illustration of action potentials from fast-spiking and regular-spiking cortical neurons. Note the faster repolarization rate in the narrow spike of the fast-spiking neuron. V is membrane voltage, and dV/dt is the time derivative of membrane voltage. The signal in extracellular recordings is essentially proportional to dV/dt, and spike widths can be measured from the distance between the peaks in the dV/dt signal. **e** | Different action potential widths in the soma of dentate gyrus granule neurons and in the mossy fibre bouton, a presynaptic terminal made by a granule neuron. Note the narrower action potential in the presynaptic terminal compared to the soma. Panel **d** reproduced, with permission, from REF. 15 © (1985) American Physiological Society. Panel **e** reproduced, with permission, from REF. 39 © (2000) Elsevier Science.

To analyse the ionic currents flowing during action potentials, a particularly useful preparation is provided by acutely dissociated cell bodies, in which action potentials are close to true membrane action potentials and where high-resolution voltage-clamp experiments — including action potential clamp experiments (BOX 2) — can be performed on the same cell in which the action potential is recorded (FIGS 2c,d; 3a–c). Typically, acutely dissociated neurons include a stump of axon as well as varying amounts of proximal dendrites, but the remaining processes are usually short enough that the whole membrane surface is likely to be nearly isopotential, even during spikes. Many neurons, including Purkinje neurons[48], hippocampal pyramidal neurons[49,50], neurons from deep cerebellar nuclei[51], subthalamic nucleus neurons[32], striatal medium spiny neurons[52], globus pallidus neurons[53] and midbrain dopamine neurons[54] (FIG. 2a,b) have action potential shapes and firing properties when dissociated that appear to be very similar to those of the same neuronal type in brain slices. For example, dissociated cerebellar Purkinje neurons fire spontaneously, with typical frequencies near 40 Hz, and also form all-or-none bursts of action potentials when stimulated from hyperpolarized voltages[48,55], similar to the behaviour of intact neurons in brain slices[56,57].

A simple but remarkably informative way of examining the properties of action potentials is to plot the time derivative of the voltage (dV/dt) versus the voltage: a so-called phase-plane plot[58] (FIG. 2a–b). Information about the time course of the spike is lost, but some aspects of the spike are clearer than in a simple display of voltage versus time. For example, the spike threshold is immediately evident as being the voltage at which dV/dt increases suddenly. A particular utility of phase plane plots comes from the relationship between dV/dt and membrane current. For a membrane action potential under conditions of no current injection, ionic current through the membrane (the net current through all channels) is equal and opposite to the capacitative current (since ionic current can go nowhere except to charge or discharge the membrane capacitance). This is expressed in the equation $I_{ionic} = -CdV/dt$, where I_{ionic} is the net ionic current, C is the cell capacitance and dV/dt is the time derivative of voltage[59]. As cell capacitance

Node of Ranvier
Interruption of the myelin sheath in a myelinated nerve fibre.

Outside-out patch
A variant of the patch-clamp technique in which a patch of plasma membrane covers the tip of the electrode, with the outside of the membrane exposed to the bathing solution.

Activation
Conformational change of a channel molecule from a closed (non-conducting) to an open (conducting) state (for voltage-dependent channels, this is usually by depolarization of the membrane).

is an easily measured parameter, the phase plane plot for a membrane action potential gives a direct read-out of net ionic current as a function of voltage during the various phases of the action potential. For example, the maximum sodium current flowing during the spike can be estimated from the maximum CdV/dt reached during the upstroke, when contributions from other channels are likely to be small.

Interpretation of the phase-plane plot for an action potential recorded in the cell body of an intact neuron is more complicated than for a membrane action potential, as the presence of a dendritic tree and axon means that not all ionic current goes to charge or discharge the somatic membrane. In their classic examination of action potentials in spinal motor neurons, Coombs and colleagues[40] observed that the main spike was preceded by a smaller, earlier component (more recently called 'the kink'[47]). This component was interpreted as reflecting initiation of the spike in the initial segment of the axon. Such a component is seen in somatic spikes recorded from a wide variety of central neurons[41,47,60] (FIG. 2a), and direct simultaneous intracellular recordings in pyramidal neurons have confirmed that the spike occurs in the initial segment of the axon before the somatic spike[43,45,47]. The possibility that the spike may occur first even farther from the soma, in the first node of Ranvier, has also been considered[60,61], but at least in cerebellar Purkinje neurons and layer 5 cortical pyramidal neurons the weight of evidence supports initiation in the initial segment[45,46]. In Purkinje neurons, blocking sodium entry at the first node of Ranvier has no effect on the somatic spike waveform (including the kink), whereas inhibiting sodium entry at the initial segment reduces the kink[46].

Box 2 | **Action potential clamp and dynamic clamp**

The contribution of particular currents to the action potential — the 'internal anatomy' of a spike — can be dissected using the action potential clamp technique (FIGS 2,3,5), in which the cell is voltage-clamped using a previously-recorded action potential waveform as the command voltage[110], ideally recorded minutes earlier in the same neuron. The ion channels experience precisely the same trajectory of voltage as during a naturally occurring action potential, and thus each component of current flows with exactly the same time course and size. Individual components of current can be isolated using specific blockers or changes in ionic composition — without changing the voltage trajectory, as occurs when blocking conductances in a current clamp. Accurate measurement of the large, fast currents during the spike requires a fast, spatially uniform voltage clamp, which is possible with acutely isolated cell bodies or outside-out patches. Smaller currents preceding and following the spike can be studied in whole-cell brain slice recordings[54].

Whereas the action potential clamp technique directly measures various currents during normal spiking, the ingenious dynamic clamp technique[198–200] instead examines how spiking is changed by adding, removing or altering the kinetics of particular currents. The effect of adding or removing a conductance on firing in current clamp recordings is simulated by calculating in real time the current that would be produced by the artificial conductance and injecting that current. For voltage- and time-dependent conductances like those underlying the action potential, equations accurately describing the voltage- and time-dependence are needed, and these must be numerically integrated in real time. Update rates up to 50 kHz are possible[26,200], and are adequate for simulating conductances during the spike. Often, relatively non-selective blockers are used to block multiple conductances (for example, tetraethylammonium ion or 4-aminopyridine, which generally block multiple types of potassium channels), and the effect of adding back a particular simulated conductance is tested.

Sodium currents during action potentials

The main contribution of voltage-dependent sodium channels to the action potential is explosive, regenerative activation of inward current during the rising phase[1]. Detailed measurements of sodium current in acutely dissociated cell bodies or outside-out patches of central neurons[48,62–65] show gating properties generally similar to those in squid axons[66,67], with current activating rapidly (hundreds of microseconds) and inactivating with a time constant of less than a millisecond for depolarizations beyond 0 mV. However, the kinetics of sodium currents differ in detail between different types of neurons[62] and, remarkably, even between different regions of the same neuron, with sodium channels in hippocampal mossy fibre boutons (formed by the axons of granule cells of the dentate gyrus) inactivating twice as fast as the sodium channels in the granule cell somata[68]. The molecular basis of these differences is not yet known. The wider action potentials in granule cell bodies (680 µs at half-height) compared to those in mossy fibre boutons (380 µs at half-height)[39] (FIG. 1f,g) could result partly from slower repolarization due to slower-inactivating sodium current continuing to flow during the falling phase of the spike. However, potassium currents may also differ between the two cell regions.

It has recently been reported that sodium currents in mammalian central neurons activate upon step depolarizations with much less delay than is predicted by the Hodgkin–Huxley model, in which activation of sodium current has a sigmoid time course, described by a variable following first-order kinetics raised to the third power ('m^3' kinetics). By contrast, Baranauskas and Martina[65] found that sodium currents in central neurons activate with even less sigmoidicity than predicted by m^2 kinetics[65]. The report's description is consistent with previous studies in other vertebrate sodium channels: when sodium current activation in the frog or rat node of Ranvier was fitted with m^n kinetics, n varied from less than 2 for small depolarizations (where the measurements of Baranauskas and Martina were made) to 4 for large depolarizations[69,70]. These results should prompt a close examination of the kinetics of cloned sodium channels in heterologous expression systems, which avoid complications from the overlap of multiple currents from the multiple sodium channel types probably present in central neurons.

A much more radical proposal concerning sodium channel kinetics in central neurons was recently made: that there is cooperativity between neighbouring sodium

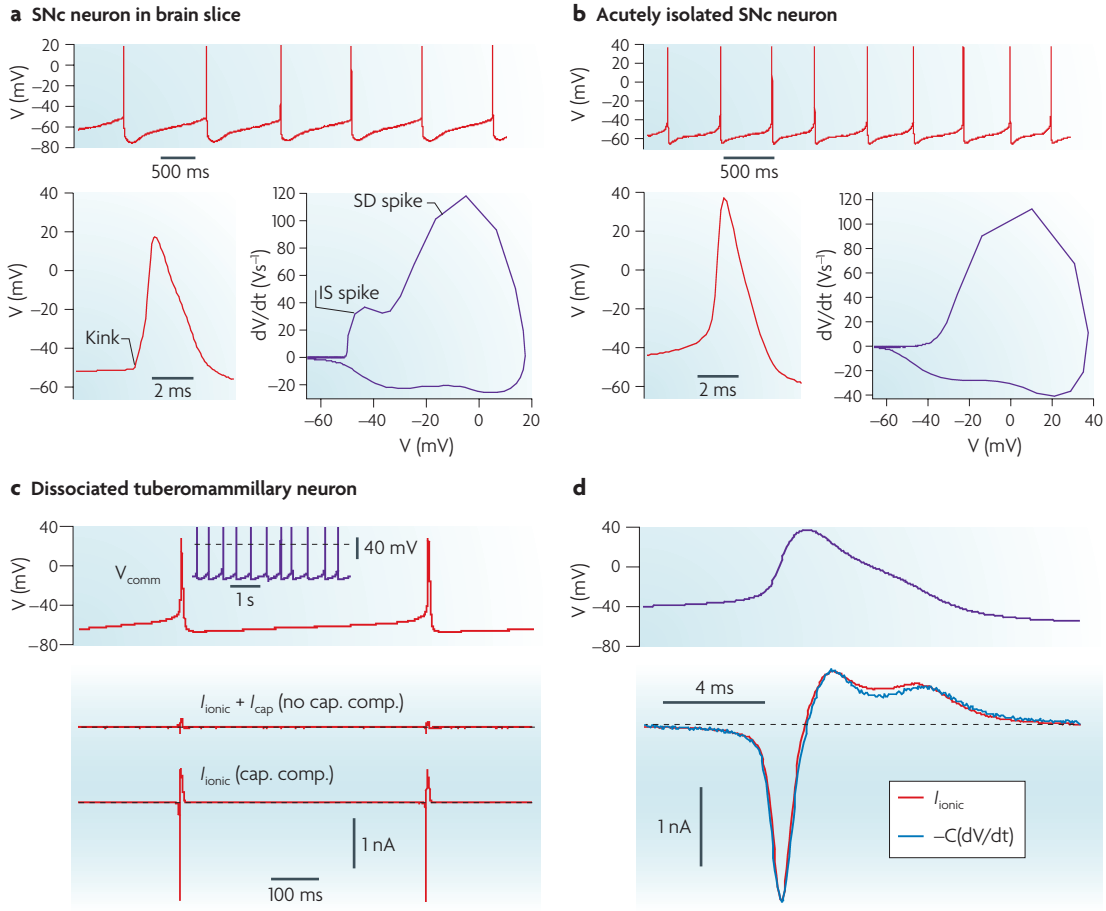


Figure 2 | **Phase-plane plots and action potential clamp. a** | Pacemaking activity recorded from a dopaminergic neuron in the substantia nigra pars compacta (SNc) of a mouse brain slice (M. Puopolo, unpublished observations). Bottom left, action potential at higher resolution, illustrating abrupt rise (kink). Bottom right, phase plane plot, illustrating the distinct components reflecting initiation in the initial segment (IS) and subsequent somatic dendritic (SD) spike[40–42]. **b** | Pacemaking activity in an acutely dissociated dopaminergic neuron (M. Puopolo, unpublished observations). Note lack of kink and IS component. **c** | Action potential clamp (BOX 2) in a dissociated neuron from the tuberomammillary nucleus. Spontaneous action potentials were recorded in current clamp mode (the upper purple trace shows a 5-second segment of spontaneous firing) and then used as the command voltage (V_{comm}) after switching the amplifier to voltage-clamp mode. With no compensation for cell capacitance (no cap. comp., lower panel, upper trace), recorded total current is nearly zero (since ionic (I_{ionic}) and capacitative (I_{cap}) currents are equal and opposite during spontaneous action potentials recorded with no current injection). Ionic current was measured after analogue compensation for the cell capacitance (34 pF) (cap. comp., lower panel, lower trace). **d** | Comparison of the net ionic current (red trace) measured in voltage clamp mode using the action potential waveform (upper purple trace) as the command voltage with predicted ionic current calculated from the voltage waveform as –CdV/dt (blue trace), where C is cell capacitance. Note the shoulder in the action potential associated with an inflection in outward current during repolarization. V, membrane voltage. Panels **c**,**d** modified, with permission, from REF. 98 © (2002) Elsevier Science.

Subthreshold voltages
Voltages negative to the threshold voltage (BOX 1) for action potential firing (which is typically in the range of –55 mV to –40 mV in mammalian central neurons).

Tetrodotoxin
(TTX). Alkaloid toxin derived from *Fugu* puffer fish that is a potent and highly selective blocker of voltage-dependent sodium channels.

Inactivation
Conformational change of a channel molecule to a closed state that differs from the closed 'resting' state in that the channel cannot be opened (for example, by further depolarization).

channels such that the opening of channels shifts the activation curves of neighbouring channels in the hyperpolarized direction[71]. This proposal aimed to account for an abrupt initial rising phase of the action potential, which is seemingly incompatible with predictions of m^3 kinetics, along with trial-to-trial fluctuations in the apparent threshold voltages. The hypothesis of cooperativity among neighbouring channels might best be tested in nodes of Ranvier, where sodium channel density is extremely high and good voltage clamp is possible. Most likely, however, the observed abrupt initial rising phase of the action potential (recorded from cell bodies in slices) simply results from the kink reflecting initiation of the spike in the initial segment[40,47,72]. Apparent variability in threshold can be explained by instantaneous differences between subthreshold potentials in the cell body and the initial segment[72]. Thus, while sodium channel kinetics near threshold may be less sigmoid than Hodgkin–Huxley predictions, the hypothesis of cooperative gating among sodium channels seems unnecessary.

In some central neurons, specialized kinetics of sodium currents give rise to a component of tetrodotoxin (TTX)-sensitive sodium current immediately after the spike. This is the 'resurgent' sodium current, which activates transiently upon repolarization following inactivation by strong depolarizing pulses[48,73]. This current appears to

arise from an unusual mechanism of inactivation: channels are plugged by a positively charged intracellular blocking particle (probably the cytoplasmic tail of the β4 sodium channel subunit[74]) after they open. On repolarization, the block is relieved, and the channels carry an inward sodium current transiently before deactivating[48,73,74]. Recovery from inactivation occurring by this mechanism is faster than recovery from normal inactivation[73], so that as sodium channels pass depolarizing resurgent sodium current after a spike, they also recover from inactivation, producing a pool of channels that are available to give transient sodium current upon depolarization. Both effects promote faster firing of a second spike.

Resurgent current has been found in cerebellar Purkinje neurons[48], cerebellar granule neurons[75,76], neurons in the deep cerebellar nuclei[51,75], cerebellar unipolar brush cells[75], motor neurons of the trigeminal mesencephalic nucleus[77], neurons from the medial nucleus of the trapezoid body[78] and subthalamic nucleus neurons[32], as well as in a subset of neurons in dorsal root ganglia[79]. Consistent with its 'anti-refractory' properties, the presence of resurgent current appears to be generally correlated with the ability of cells to fire rapidly and, at least in the case of Purkinje neurons, to generate bursts[48,80]. It is not yet clear whether a resurgent sodium current is present in all fast-firing cells; fast-spiking interneurons are a case of particular interest not yet examined.

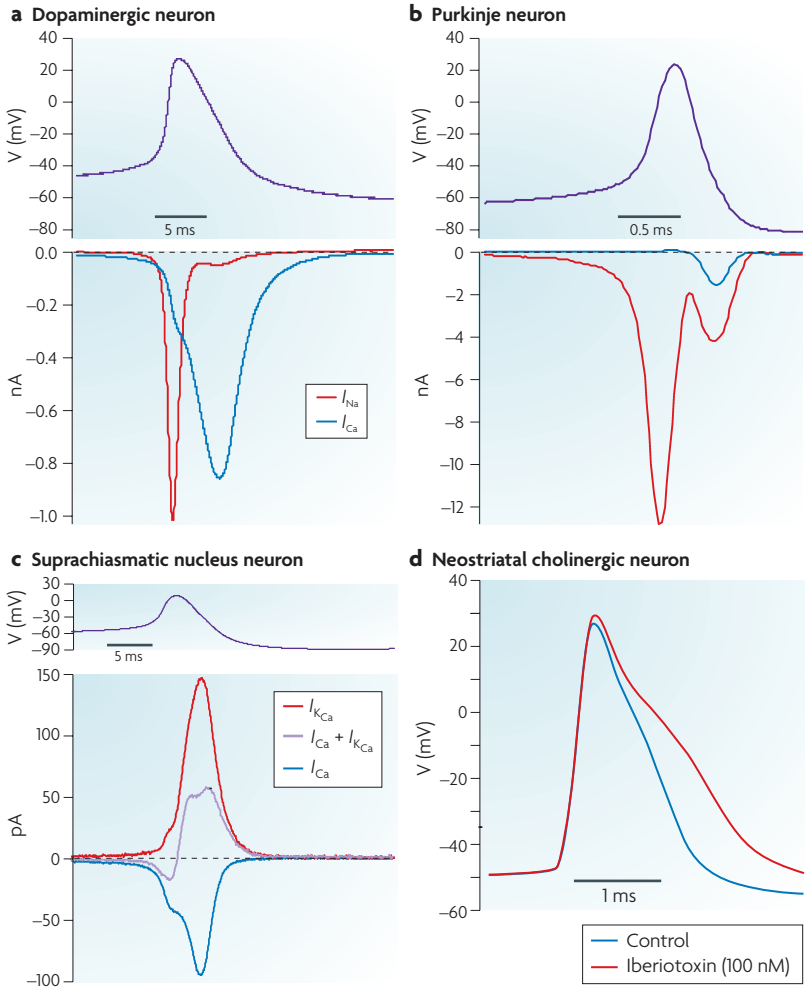


Figure 3 | **Sodium, calcium, and calcium-activated potassium currents during action potentials.** Sodium current (I_{Na}, red) and calcium current (I_{Ca}, blue) in a midbrain dopaminergic neuron (**a**) and a cerebellar Purkinje neuron (**b**), recorded using the action potential clamp technique (B. Carter, unpublished observations). Purple traces show recorded spontaneous action potentials used as command voltages. Block by tetrodotoxin (1 μM) defined the sodium current. Calcium current was determined by replacing calcium by cobalt (in the dopaminergic neuron) or by magnesium (in the Purkinje neuron), in the presence of 5 mM tetraethylammonium chloride to block large conductance calcium-activated potassium (BK) current ($I_{K_{Ca}}$). Note the different timescales. The calcium currents are of a similar magnitude, whereas the sodium current in the Purkinje neuron is much larger than the other currents (so large that voltage clamp is probably imperfect due to series resistance errors). The time course and magnitude of calcium current (blue) and calcium-activated potassium current (red) in a spontaneous action potential of a suprachiasmatic nucleus (SCN) neuron can be seen in panel **c**. Net current resulting from calcium entry is outward except at the beginning of the spike. Calcium current in SCN neurons is unusual in activating significantly during the rising phase of the spike (although still greatly outweighed by sodium current, not shown). The role of BK-mediated calcium-activated potassium current in repolarization of the action potential in a cholinergic neuron from the neostriatum is revealed by the effect of iberiotoxin, a specific blocker (**d**). V, membrane voltage. Panel **a** modified, with permission, from REF. 54 © (2007) Society for Neuroscience. Panel **c** modified, with permission, from REF. 109 © (2004) Society for Neuroscience. Panel **d** modified, with permission, from REF. 97 © (2000) Society for Neuroscience.

Calcium currents during action potentials

Mammalian neurons typically express multiple types of voltage-dependent calcium channels, and these have important roles in determining action potential shapes and firing patterns (in addition to producing the action potential-evoked increases in intracellular calcium used for intracellular signalling). Individual neurons commonly express at least 4 or 5 distinct types of calcium channels, including low-voltage-activated T-type channels (Cav3 family channels) and high-voltage-activated channels that include L-type (Cav1.2 and Cav1.3), P/Q-type (Cav2.1), N-type (Cav2.2) and R-type (Cav2.3) channels. Calcium currents generally make little contribution to the rising phase of action potentials because their activation kinetics are slower than sodium channels. Calcium channels typically begin to be activated near the peak of the action potential and calcium currents are largest during the falling phase, when channels have been opened and the driving force on calcium increases (FIG. 3a,b). Interestingly, although the action potentials of virtually all neurons probably have large inward calcium currents flowing during the falling phase, blocking calcium channels often results in a broadening of the action potential[81–88] (FIG. 3d), which is opposite to the effect expected from blocking entry of positively charged ions. This reflects powerful and rapid coupling of calcium entry to the activation of large conductance calcium-activated potassium channels (BK channels), so that the net effect of blocking calcium entry is to inhibit a net outward current rather than a net inward current — that is, the potassium flux triggered by the calcium entry outweighs the calcium entry itself (FIG. 3c). The contribution of BK channels is typically greatest in the later stages of the repolarization phase[83,89,90]. Biochemical data, experiments with intracellular calcium buffers, and single channel recordings all suggest that this tight coupling reflects colocalization of

4-aminopyridine
Potassium channel blocker that inhibits some potassium channels (including Kv3 family channels and a subset of Kv1 family subunits) with a high relative potency and others (such as Kv4 channels) more weakly, or not all.

Tetraethylammonium ion
(TEA). When applied externally, this blocks some types of voltage-activated potassium channels (notably BK and Kv3 family channels) and not others.

calcium channels and BK calcium-activated potassium channels[91–96]. BK channels can apparently be colocalized in macromolecular complexes with multiple types of calcium channels, including Cav1.2, Cav2.2 and Cav2.1 channels[95].

Neurons that secrete modulatory transmitters such as noradrenaline, serotonin, dopamine, acetylcholine and histamine frequently generate broad action potentials, often with a 'shoulder' in the falling phase (FIGS 1c,2d) that appears to be due to the activation of calcium channels[97,98]. Cells with a shoulder do not necessarily have larger calcium currents during the action potential: cerebellar Purkinje neurons, with exceptionally narrow action potentials and no shoulder, actually have larger calcium currents during repolarization than midbrain dopaminergic neurons, which have broad action potentials with a prominent shoulder (FIG. 3a,b). The difference is presumably in the size and speed of opposing potassium currents, including BK calcium-activated potassium currents, which are very large in Purkinje neurons.

Activation of small conductance calcium-activated potassium channels (SK channels) is also coupled to calcium entry through multiple different types of calcium channels, including Cav2.1, Cav2.2, Cav2.3 and Cav3 family channels, with different specificity of coupling in different types of neurons[88,93,99–104]. However, so far there is no clear biochemical evidence for colocalization of SK channels with calcium channels in macromolecular complexes, and activation of SK channels (unlike activation of BK channels) can often be disrupted by the slow calcium buffer EGTA[99,105], suggesting that they are activated by calcium diffusing over longer distances. Typically, SK current is activated more slowly than BK current and contributes little to the fast repolarization phase of the action potential, but rather helps shape the afterhyperpolarization that follows. The duration of SK conductance following a spike probably reflects the decay of intracellular free calcium, with a typical time course of hundreds of microseconds. By contrast, BK channels deactivate far more quickly, since depolarization as well as high local intracellular calcium is required for activation to be maintained. In several types of pacemaking neurons, block of SK channels has the striking effect of producing substantially less regular firing[10,97,103,106,107]. Although the activation of SK channels is not as rapidly coupled to calcium entry as BK channel activation, coupling is still highly efficacious in that block of calcium entry can produce a net depolarization of the membrane potential between spikes in spontaneously active neurons[108,109], probably reflecting effects on SK rather than BK channels.

Because activation and deactivation kinetics of calcium channels are strongly voltage-dependent, action potential-evoked calcium entry can have a steep dependence on action potential width and shape. This sensitivity is especially significant in presynaptic terminals, where modest changes in action potential shape can translate into significant changes in calcium entry and even more dramatic changes in transmitter release[36,39,110–114].

Potassium currents during action potentials

Most central neurons express such a large variety of voltage-dependent potassium currents that separating and characterizing all the components of total potassium current elicited in voltage-clamp experiments by step depolarizations is daunting (and even mastering the terminology used to name them can be challenging). However, typically only a fraction of the various voltage-dependent potassium currents present in a neuron is significantly activated during normal action potentials. In addition to the BK calcium-activated potassium channels discussed above, two other classes of potassium channels that commonly contribute to spike repolarization in cell bodies are Kv4 family channels, which mediate A-type current (I_A), and Kv3 family channels.

In some fast-spiking neurons, such as cerebellar Purkinje neurons, Kv3-mediated current seems to constitute virtually all of the voltage-dependent potassium current flowing during spike repolarization[23,31]. In most of these cases, other types of potassium channels are present in the membrane, but the activation of the Kv3-mediated currents is so much faster that they activate and repolarize the spike before other potassium channels can activate significantly. The potassium current flowing during the spike may be only a small fraction of the current that can be evoked by a longer depolarizing step (FIG. 4a), providing a powerful feedback element to keep spikes narrow (since any small increase in width would produce more activation of available current).

The functional significance of Kv3 channels in the fast-spiking phenotype is highlighted by the effects of blocking the channels (with low concentrations of 4-aminopyridine (FIG. 4a,d) or tetraethylammonium ion (TEA) (FIG. 4c)), which often results in the slowing of high-frequency firing[6,26,27,35,115] (FIG. 4c,d). It is counter-intuitive that removing a potassium conductance would decrease the excitability of a neuron. The reasons for this are not clear but they almost certainly involve changes in the activation of other channels secondary to the changes in spike waveform produced by block of Kv3 current. For example, a smaller afterhyperpolarization (FIG. 4c,d) might result in slower recovery from inactivation of sodium channels, or broadening of spikes might activate types of potassium channels not activated by normal narrow spikes[115]. If these channels deactivate more slowly, they could retard firing of a subsequent action potential. In fast-spiking hippocampal oriens-alveus interneurons, Lien and Jonas[26] found that when dynamic clamp was used to add Kv3-like currents back to neurons in which native currents were pharmacologically blocked, the restored currents produced faster spiking only if the deactivation rate was near that of native channels in the neurons, neither faster nor slower[26] (FIG. 4). The reasons for this remarkable tuning are not clear, but must involve complex interactions with other channels mediated by changes in spike shape. In Purkinje neurons, it has been proposed that the shape of the repolarization waveform produced by Kv3 currents leads to enhanced activation of post-spike resurgent sodium current[116].

In glutamatergic neurons in the hippocampus and cortex, at least three types of potassium currents have major roles in action potential repolarization: two types of purely voltage-dependent potassium currents, known as I_A and I_D, and BK calcium-activated currents[50,89,117–126]. The term 'I_A' has been used broadly to refer to potassium currents showing relatively rapid inactivation. In cell bodies and dendrites, the current called I_A is formed primarily by Kv4 family channels[125–127]. Kv1 family channels that include Kv1.4 subunits or the β-subunit Kvβ1 can also mediate an inactivating current that has been called I_A[14], which appears to be prominently expressed in at least some presynaptic terminals[39,128]. The term 'I_D' also has some ambiguity. The term was originally introduced to denote a 'delay' current in hippocampal pyramidal neurons that prolongs the approach to threshold[129]. This current is activated by subthreshold depolarizations, inactivates slowly, and is blocked by low concentrations (10–100 μM) of 4-aminopyridine. Dendrotoxin, which is selective for a subset of Kv1 family subunits, blocks a current with these properties[122,130], and 'I_D' is now often used to denote dendrotoxin-sensitive current. However, dendrotoxin generally has little[38] or no[131,132] effect on spike width, while low concentrations of 4-aminopyridine have a greater effect[19,50,117,119,122,131,133]. The effects of low concentrations of 4-aminopyridine on spike width in pyramidal neurons have often been ascribed to block of I_D, but in retrospect these effects may also represent block of other currents, possibly mediated by Kv3 family channels[22] or Kv1.5 channels, which are blocked by low concentrations of 4-aminopyridine but not dendrotoxin.

In a number of neurons, the action potential becomes broader in response to increased frequency of firing because of the cumulative inactivation of potassium channels. A particularly well-studied example occurs in the neuron R20 of the mollusc *Aplysia*, where frequency-dependent broadening of the action potential produces facilitation of synaptic transmission[134].

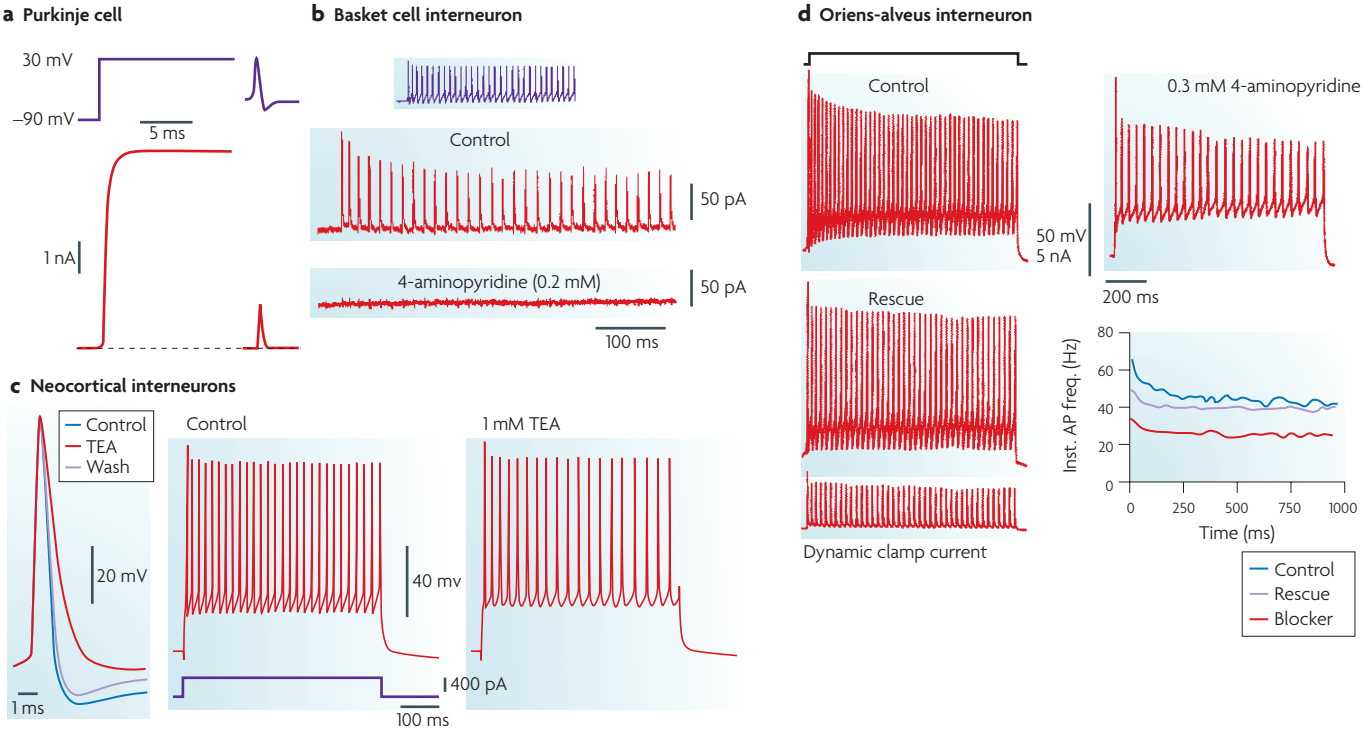


Figure 4 | **Role of Kv3 potassium currents in fast-spiking neurons. a** | Potassium currents in a nucleated patch from a Purkinje neuron elicited by a step to +30 mV and by an action potential waveform. Spike-evoked current is only a small fraction of total available current (which is almost all Kv3-mediated[28]). Any small increase in spike width can activate more current; this keeps the spikes narrow. **b** | In a nucleated patch from a dentate gyrus basket cell interneuron, potassium current activated by action potential waveforms (inset) is completely inhibited by 0.2 mM 4-aminopyridine (which, in these cells, is probably selective for Kv3-mediated current). **c** | Effects on the firing of fast-spiking neocortical interneurons of 1 mM tetraethylammonium (TEA), which, in these neurons, appears to be selective for Kv3 channels. TEA produces broadening, a decreased rate of repolarization and a reduction in the afterhyperpolarization of spikes, along with slowing the firing frequency evoked by a constant current pulse, suggesting a major role for Kv3-mediated current in the firing of fast-spiking neocortical interneurons. **d** | Rescue of the fast-firing phenotype in oriens-alveus hippocampal interneurons by the addition of Kv3-like conductance with a dynamic clamp (BOX 2). 0.3 mM 4-aminopyridine slowed firing, but high-frequency firing was restored by the addition of artificial Kv3 conductance (rescue) by a dynamic clamp in the presence of 4-aminopyridine (giving ~95% restoration of action potential (AP) half-duration). Inst. AP freq., instantaneous AP frequency. Panel **a** modified, with permission, from REF. 31 © (2007) American Physiological Society. Panel **b** reproduced, with permission, from REF. 23 © (1998) Society for Neuroscience. Panel **c** reproduced, with permission, from REF. 6 © (1999) American Physiological Society. Panel **d** reproduced, with permission, from REF. 26 (2003) Society for Neuroscience.

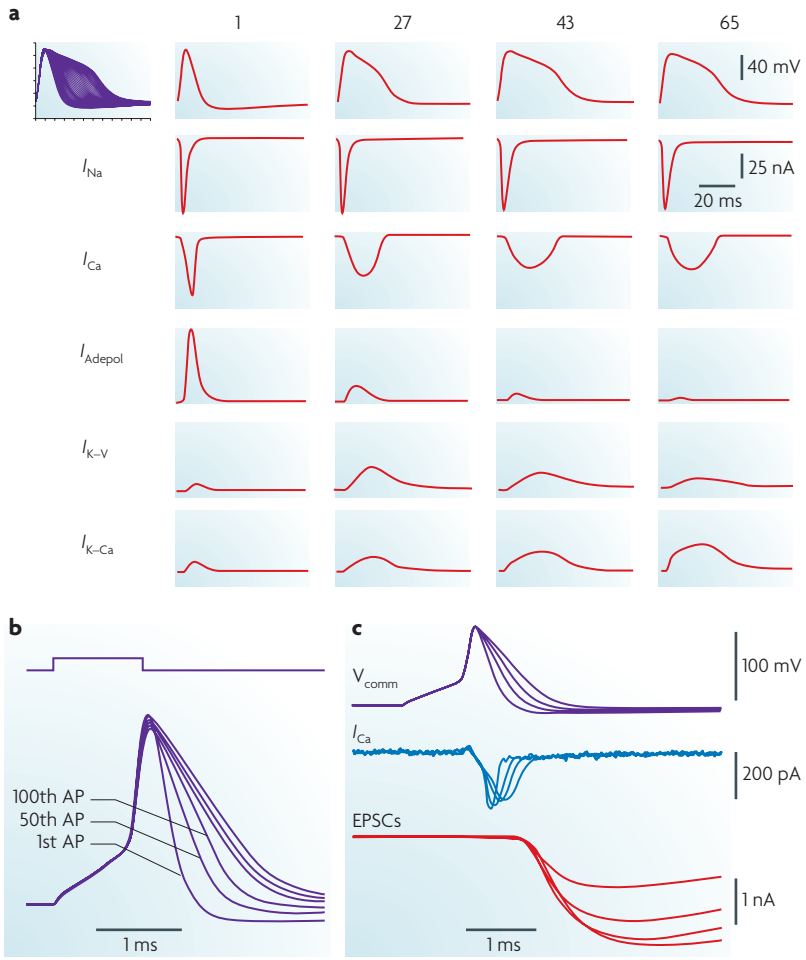


Figure 5 | **Frequency-dependent spike broadening from inactivation of potassium current. a** | Changes in currents accompanying frequency-dependent spike broadening in neuron R20 of *Aplysia*. A train of action potentials (first row) was evoked by stimulation at 7 Hz. Five major ionic currents were isolated by action potential clamp. Subtraction of currents before and after successive application of 60 μM tetrodotoxin, 3 mM tetraethylammonium (TEA), 40 mM TEA, 1 mM 4-aminopyridine, and 2 mM $CdCl_2$ was used to define I_{Na}, $I_{K–Ca}$ (calcium-activated K^+ current), $I_{K–V}$ (delayed-rectifier K^+ current), I_{Adepol} (I_A K^+ current), and I_{Ca}, respectively. Currents during the first spike, 27th spike (at which point $I_{K–V}$ reached its peak), 43rd spike (at which point the maximum broadening was reached) and last spike (65th) are shown. **b** | Frequency-dependent broadening of action potentials in mossy fibre boutons in the hippocampus. Every fiftieth action potential is shown, superimposed with the first. **c** | Potentiation of transmitter release at mossy fibre boutons by spike broadening. The waveforms recorded in panel **b** were applied as command waveforms (V_{comm}, top) in a voltage clamp to a different mossy fibre bouton, while both presynaptic calcium currents (middle) and excitatory postsynaptic currents (EPSCs, bottom) were recorded. Waveforms of action potentials 1, 25, 50 and 100 in the 50 Hz train were applied. The largest EPSC corresponds to the broadest presynaptic spike. Panel **a** reproduced, with permission, from REF. 135 © (1996) Society for Neuroscience. Panels **b**,**c** reproduced, with permission, from REF. 39 © (2000) Elsevier Science.

An elegant analysis of the mechanism of frequency-dependent broadening in this neuron[135] (FIG. 5a) provides an instructive example of how the techniques of action potential clamp and dynamic clamp (BOX 2) can be combined with conventional voltage clamp to experimentally attack a problem that otherwise might be approached purely through computer modelling. Action potential clamp showed that the current that changed most during frequency-dependent broadening was an I_A current that normally provides most of the repolarizing drive[134]. Puzzlingly, however, complete block of I_A (either pharmacologically or by dynamic clamp) produced much less broadening of the spike than occurs during frequency-dependent broadening. This is because modest increases in spike width result in increased activation of two other potassium currents, the activation of which during the spike is normally minimal: a delayed-rectifier current and a calcium-activated potassium current. Dynamic clamp analysis showed that a crucial factor in the greater degree of broadening seen during frequency-dependent changes is gradual use-dependent inactivation of the delayed rectifier current. This analysis illustrates several key aspects of the interaction of multiple currents in forming the action potential: first, the effect of pharmacologically blocking a single current may cause the role of that current to be underestimated, since the role of other currents can be drastically enhanced as a result of relatively modest changes in spike shape. Second, the role of any ionic current depends crucially on the context of all the other voltage-activated (and calcium-activated) channels in the membrane, and multiple currents may undergo frequency-dependent changes.

Frequency-dependent broadening of the action potential is also prominent in some mammalian neurons[19,37,83,86]. In hippocampal CA1 pyramidal neurons[83] and pyramidal-like projection neurons in the lateral amygdala[86], progressive inactivation of BK channels helps to produce frequency-dependent spike broadening. Inactivation of Kv4-mediated I_A also contributes to spike broadening in cell bodies of pyramidal neurons[125]. A particularly dramatic example of frequency-dependent spike broadening occurs at mossy fibre boutons in the hippocampus; this is probably mediated by inactivation of Kv1 family channels[39] (FIG. 5b) and is associated with pronounced synaptic facilitation (FIG. 5c).

Prelude to the spike: subthreshold currents

The ability of central neuron cell bodies to encode firing over a wide frequency range depends on multiple small currents flowing at subthreshold voltages, which speed or retard the approach to threshold and thereby influence the spike rate and pattern of firing. Subthreshold currents common in central neurons include I_A and I_D potassium currents, steady-state 'persistent' sodium currents, the current known as 'I_h' carried by hyperpolarization-activated cyclic nucleotide-gated (HCN) channels, and the current mediated by T-type (low-voltage-activated) calcium channels. Classic papers by Connor and Stevens[136,137] described a potassium current in molluscan neurons that they named 'A-type' current (I_A), which both activates and inactivates at subthreshold voltages, and explained how it enables repetitive firing at low frequencies: during the relative hyperpolarization after a spike, some inactivation is removed, then as the membrane depolarizes (still at subthreshold voltages), I_A increasingly activates (retarding the approach to threshold) but eventually inactivates, allowing threshold to be reached. I_D produces a subthreshold current with a similar role, but inactivates more slowly[50,129]. In many neurons, Kv1-mediated currents (in some cases called I_D

Delayed-rectifier current
Depolarization-activated potassium current similar to that of the squid axon, with relatively slow activation and minimal (or very slow) inactivation.

by virtue of dendrotoxin-sensitivity, but not necessarily showing the inactivation of I_D as originally described) have a major influence in determining firing patterns by flowing at subthreshold voltages before and between spikes, although they are swamped by other currents during the spike itself[34,36,132,138,139].

Virtually all central neurons appear to have a steady-state inward sodium current, sensitive to TTX, that flows at voltages between about –65 mV and –40 mV [7,48,63,76,77,97,98,140–145]. This 'persistent' sodium current activates with a voltage-dependence (e-fold for 4–6 mV) that is as steep as the voltage dependence of activation of the transient sodium current, but with a midpoint (typically ~–55 mV) about 30 mV below that of transient sodium current. Although maximum steady-state sodium current is only a tiny fraction (typically 0.5–5%) of the maximum transient sodium current, the resulting current of 5–200 pA is very significant functionally at subthreshold voltages. It has been argued that steady-state current amounting to ~0.5% of transient current is a necessary consequence of the normal gating behaviour of conventional sodium channels[98] (although an apparent counter-example of transient sodium current with no detectable persistent current has recently been described[144]). In addition to a persistent sodium current originating from the same population of channels underlying the transient sodium current[64,98,146], some neurons also clearly have specialized sodium channels that give subthreshold sodium currents far larger than 0.5% of the transient sodium current[147,148]. The molecular basis of these channels is not known. By producing a regenerative depolarizing current in the voltage range between the resting potential and spike threshold, persistent sodium current has a major role in determining the frequency and pattern of firing of many neurons[3,7,77,97,140,142,143,145,149–153].

T-type or low-voltage-activated calcium currents, originating from Cav3 family gene products, are also active at subthreshold voltages. One major function of the T-type calcium current is to produce rebound bursting following hyperpolarization (such as that from prolonged inhibitory input), which removes resting inactivation of the channels[154,155]. However, smaller steady currents can flow through T-type channels at resting potentials even without dynamic hyperpolarization and can influence firing patterns[156].

Because some currents flowing during the spike (including the transient sodium current and I_A potassium current) are sensitive to inactivation by changes in subthreshold voltages, spike shape can be significantly affected by the preceding voltage trajectory[39,157–159]. Remarkably, the electrotonic length constant of some central axons is sufficiently long that changes in somatic resting potential can affect the voltage in axons and nerve terminals hundreds of micrometres away[160,161] and, in some cases, alter the shape of presynaptic action potentials[161].

A remarkably poorly understood aspect of neuronal cellular neurophysiology is the collection of non-voltage-dependent conductances that determine resting potential, the foundation on which voltage-dependent subthreshold and suprathreshold currents exert their effects. The resting potassium conductance of neurons (and all cells) appears to depend on two-pore domain family (KCNK) potassium channels[162–165]. However, most neurons have resting potentials that are considerably more depolarized than the potassium equilibrium potential. In some neurons, there is evidence for a basal, non-voltage-dependent permeability to sodium ions[51,109,166], but the molecular basis for this is unknown.

The CNS includes many neurons that fire spontaneously in the absence of synaptic input[3]. The subthreshold current most closely identified with this electrical pacemaking is I_h [167,168]. Although I_h clearly has a major role in driving pacemaking in some central neurons[10,53,97,169,170], in others it appears that a TTX-sensitive persistent sodium current flowing at voltages positive to –65 mV is the major element driving the membrane to threshold[7,32,48,51,98,109]. Midbrain dopamine neurons are an interesting exception, where pacemaking seems to be driven mainly by a subthreshold calcium current[54,171].

After the spike is over

In the squid axon, the action potential is followed by an afterhyperpolarization, produced by the voltage-dependent potassium conductance activated during the spike, which transiently hyperpolarizes the membrane and then deactivates slowly over about 10 ms. For action potentials in mammalian neurons, afterhyperpolarizations are common but by no means universal. Distinct fast, medium and slow afterhyperpolarizations can often be recognized (FIG. 6a). Potassium channels contributing to afterhyperpolarizations include BK channels, SK channels[84,172,173] and Kv7 channels that mediate the M-current[89,174,175]. Usually, any afterhyperpolarization mediated by BK channels is brief[85,117,176,177], whereas those mediated by SK channels can last for hundreds of microseconds to seconds[172], reflecting the slow decay of intracellular calcium. Many pyramidal neurons have a slow component of the afterhyperpolarization that lasts for seconds, is not mediated by SK channels and has a molecular basis which remains a mystery[172,178–181].

In many neurons, including many pyramidal neurons in the cortex and hippocampus, the opposite of an afterhyperpolarization, an afterdepolarization, occurs: the membrane potential is depolarized relative to the resting potential[37,39,76,182–184]. Sometimes the afterdepolarization appears simply as a slow phase of repolarization at the end of the spike (FIG. 1b), but in other cases the voltage trajectory following the action potential has a clear rising phase (FIG. 6a). If the afterdepolarization is large enough to reach threshold, the result is all-or-none burst firing (FIG. 6b,c). Ionic currents contributing to afterdepolarizations include persistent sodium currents[140,149], resurgent sodium currents[48,76,185], T-type calcium currents[185], R-type calcium currents[186] and currents generated by calcium-activated non-selective cation channels[187].

The dendritic tree may also make important contributions to the afterdepolarization[188–191]. A purely electrotonic (non-active) mechanism is possible, in which an action potential in the cell body depolarizes the dendritic membrane (which has a much larger surface area and capacitance) and, after the somatic spike has repolarized, charge

e-fold
Measure of steepness of voltage-dependent activation, associated with a description by the Boltzmann function; e-fold increase is a 2.72-fold increase.

Midpoint
Voltage at which activation is half-maximal.

Rebound bursting
Firing of a burst of action potentials when a hyperpolarizing influence (such as inhibitory postsynaptic potential) is terminated.

Electrotonic length constant
Measure of the distance over which a voltage change imposed at one point in a cable-like structure decays to 1/e (~37%).

Pacemaking neurons
Neurons that fire spontaneous action potentials in a regular, rhythmic manner.

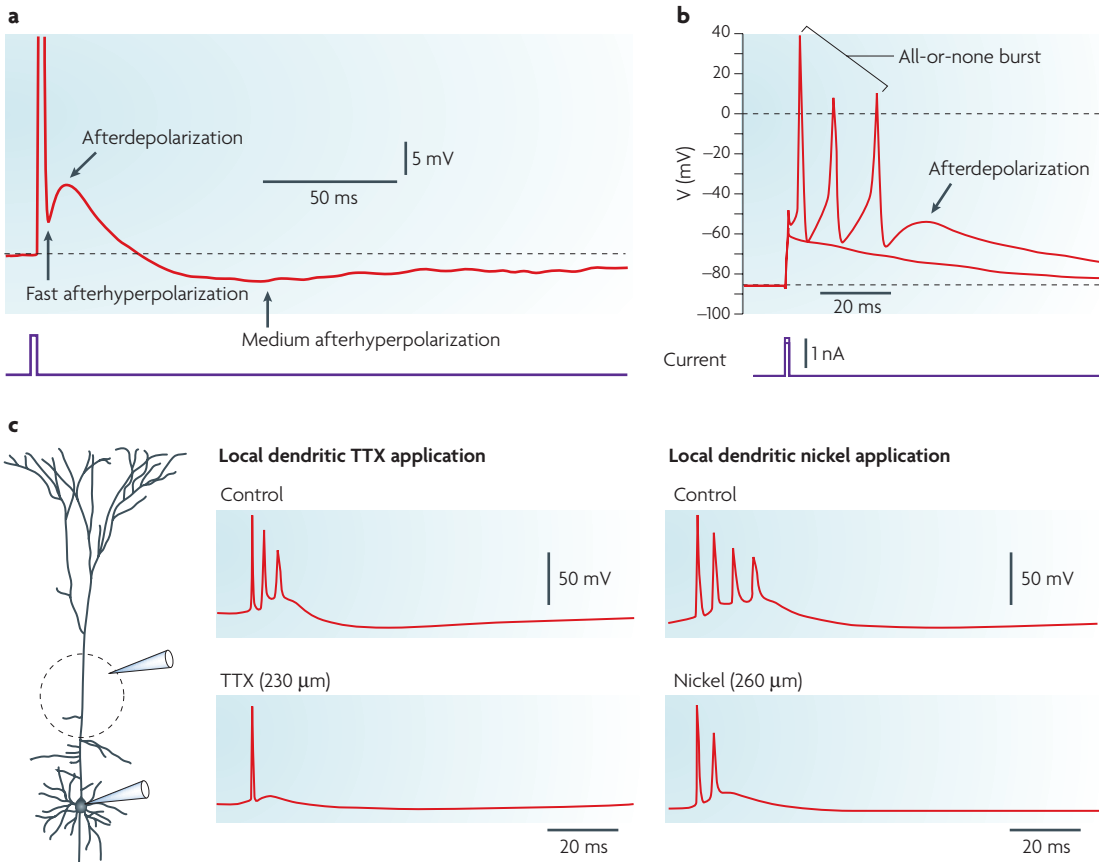


Figure 6 | **Afterhyperpolarizations, afterdepolarizations, and all-or-none burst firing. a** | Afterpotentials in a CA1 pyramidal neuron studied in brain slice. A slow afterhyperpolarization continues after the time shown, lasting more than a second (not shown). **b** | All-or-none burst firing resulting from afterdepolarizations in a dissociated cerebellar Purkinje neuron. The cell was held at –85 mV by a steady holding current of –40 pA (to stop spontaneous firing) and stimulated by brief (1 ms) current injections of 1.2 nA (which produced a subthreshold response) or 1.4 nA (which produced all-or-none burst firing). All-or-none burst firing results from afterdepolarizations that reach spike threshold. Arrow, afterdepolarization following the final spike in the burst. **c** | The contribution of dendritic sodium and calcium currents to burst firing in cortical layer 5 pyramidal neurons. Traces recorded at the soma show the effect in different cells of tetrodotoxin (TTX) (100 nM) or nickel (1 mM) applied locally to the apical dendrite 230–260 µm from the soma. Note that TTX did not change the amplitude, threshold or waveform of the first somatic action potential, verifying the confinement of TTX to the dendrite. The holding potential was –65 mV for both experiments. V, membrane voltage. Panel **a** reproduced, with permission, from REF. 117 © (1987) Cambridge Univ. Press. Panel **b** reproduced, with permission, from REF. 48 © (1997) Society for Neuroscience. Panel **c** reproduced, with permission, from REF. 191 © (1999) Cambridge Univ. Press.

from the depolarized dendritic membrane passes back to the soma and produces an afterdepolarization. This effect can be amplified by active dendritic conductances mediated by sodium and calcium channels[189–191] (FIG. 6c), which are commonly present in dendrites and produce large active depolarizing responses that are slower and occur later relative to the somatic action potential. However, even in the absence of contributions from dendritic depolarization, afterdepolarizations can be produced solely by ionic conductances in the cell body[140] and are prominent in acutely dissociated cell bodies from both pyramidal neurons[50] and Purkinje neurons[48,185].

Perspectives

Merely cataloguing all the components of ionic currents for any neuron is extremely challenging, as is characterizing the gating kinetics of any single component (as illustrated by the evolution of increasingly complex kinetic models for sodium channels, BOX 3). For currents such as I_A, I_D and TTX-sensitive sodium currents, the same channels can underlie both small subthreshold currents between spikes and large currents during the spike, producing highly complex gating behaviour as channels activate and inactivate on slow and fast timescales and influence both the approach to the spike and the spike itself. Accurately describing channel gating on both fast and slow timescales is difficult. However, the far greater challenge is to understand how all the voltage-dependent currents interact — with each conductance element both controlled by voltage and causing voltage to change — to control the generation of action potentials. Because of the two-way interactions between any pair of conductances, mediated by changes in membrane voltage on a sub-millisecond timescale, the role of any particular

Box 3 | **Limitations of the Hodgkin–Huxley model for channel kinetics**

The Hodgkin–Huxley explanation of the action potential — regenerative depolarization due to a steeply voltage-dependent sodium-selective conductance followed by repolarization due to inactivation of this conductance along with activation of a slower potassium conductance[1] — has been confirmed beyond doubt. In devising a mathematical model to predict the shape and propagation of the axon potential from their voltage-clamp data, Hodgkin and Huxley invented ingeniously simple mathematical expressions to account for the complex time- and voltage-dependent changes in sodium and potassium conductances. The simplicity of these expressions facilitated the tedious process of numerical integration. However, the Hodgkin–Huxley model of sodium current gating kinetics has subsequently been found to be at odds with the actual behaviour of sodium channels. In this model, the inactivation of channels is governed by a separate voltage-dependent process than that governing the activation of channels. In fact, various experiments have shown that inactivation derives its voltage-dependence from the same voltage-dependent steps as activation[2,66,67,201–203]. In modern models for sodium channel gating, inactivation is viewed as an inherently non-voltage-dependent process that is promoted by activation because activated channels can more readily undergo inactivation than non-activated channels[51,98,203,204]. The revised view of channel gating is most significant for predictions of the relative speed and completeness of activation and inactivation at subthreshold voltages — key for accurate predictions of repetitive firing — because the time-dependence and voltage-dependence of activation and inactivation are inherently intertwined. The same principles probably apply to other inactivating channels, such as T-type calcium channels and A-type potassium channels, and similar Markov models based on an allosteric relationship between activation and inactivation have been formulated to capture the kinetics of these channels[205,206]. However, kinetic models based on these advances in understanding channel gating are only beginning to be used in models of action potentials and firing patterns[46,76,146].

ionic conductance in influencing the action potential shape and firing pattern of a neuron depends crucially on what other ionic conductances are also present, and the effects of adding or deleting a given conductance can be counter-intuitive[6,135,145]. Although each neuronal type is different, there are undoubtedly general principles involving interactions among particular conductances waiting to be discovered.

Despite its complexity, the system of ionic currents in a neuron has remarkable advantages as a problem in 'systems biology', including highly quantifiable elements, highly quantifiable outputs (not just action potential shape but also firing rates and patterns) and a dynamic feedback mechanism based on a primary central quantity (membrane voltage) that can be measured extremely accurately. Because of these features, the system is ideally suited for mathematical modelling, probably more so than any other biological system, and relatively detailed models (including at least approximate kinetic descriptions of up to 11 different voltage-dependent conductances) have been formulated for the best-studied mammalian neurons[76,80,83,140,143,145,146,192,193]. Already, such modelling has given insight into an important general question in systems biology — how tolerant is the overall behaviour of the system to alterations in the magnitude or properties of system elements? In the case of burst firing of a particular neuron in the crab stomatogastric ganglion, electrophysiological experimentation and modelling have been combined to yield a remarkable finding: individual neurons with very similar firing properties can have densities of individual currents that vary several-fold[194–196], even though modest changes of a single conductance are capable of altering the firing of any given cell. Similar findings have been reported for the firing of cerebellar Purkinje neurons[193,197]. Related questions in a broad array of neurons with widely different functions in information processing can be attacked with an increasing array of powerful experimental tools. Knockout mouse models, RNA interference and new pharmacological agents allow the increasingly accurate definition of individual conductances, while the action potential clamp technique provides a direct, purely experimental integration between current clamp behaviour and voltage-clamp measurements. Such experimental advances will facilitate increasing detail and realism of computer models, with the dynamic clamp technique providing an especially powerful link between model and experiment.

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Competing interests statement

The author declares no competing financial interests.

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A Pilot Investigation of the Effect of Extremely Low Frequency Pulsed Electromagnetic Fields on Humans' Heart Rate Variability

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The question whether pulsed electromagnetic field (PEMF) can affect the heart rhythm is still controversial. This study investigates the effects on the cardiocirculatory system of ELF-PEMFs. It is a follow-up to an investigation made of the possible therapeutic effect ELF-PEMFs, using a commercially available magneto therapeutic unit, had on soft tissue injury repair in humans. Modulation of heart rate (HR) or heart rate variability (HRV) can be detected from changes in periodicity of the R-R interval and/or from changes in the numbers of heart-beat/min (bpm), however, R-R interval analysis gives only a quantitative insight into HRV. A qualitative understanding of HRV can be obtained considering the power spectral density (PSD) of the R-R intervals Fourier transform. In this study PSD is the investigative tool used, more specifically the low frequency (LF) PSD and high frequency (HF) PSD ratio (LF/HF) which is an indicator of sympatho-vagal balance. To obtain the PSD value, variations of the R-R time intervals were evaluated from a continuously recorded ECG. The results show a HR variation in all the subjects when they are exposed to the same ELF-PEMF. This variation can be detected by observing the change in the sympatho-vagal equilibrium, which is an indicator of modulation of heart activity. Variation of the LF/HF PSD ratio mainly occurs at transition times from exposure to nonexposure, or vice versa. Also of interest are the results obtained during the exposure of one subject to a range of different ELF-PEMFs. This pilot study suggests that a full investigation into the effect of ELF-PEMFs on the cardiovascular system is justified. Bioelectromagnetics 28:64–68, 2007.

Key words: ELF; PEMF; heart rate; cardiocirculation; magnetotherapy

INTRODUCTION

Although several studies have been made into the effects of extremely low frequency electro magnetic field, both in continuous (ELF-EMF) and/or in pulsed form (ELF-PEMF), on human biological system, published results do not offer a clear or consistent indication of such effects. As an indicator of the ELF-EMF/ELF-PEMF induced effects different parameters have been chosen for analysis. Among them the principal ones are heart rate variability (HRV) [Tabor et al., 2004], biochemistry [Kurokawa et al., 2003b], and cognitive functions [Kurokawa et al., 2003c; Sait et al., 2006]. For this study, HRV has been chosen as the main indicator of ELF-PEMF effect. Regulations have been proposed and accepted [Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996] to facilitate the interpretation and repeatability of the HRV results.

In a random double-blind study [Baldi et al., 1999] on the possible therapeutic effect of extremely low frequency pulsed electro magnetic fields (ELF-PEMF) on soft tissue injuries, half of the subjects (25) were exposed to an active PEMF unit and the other half was exposed to a sham unit. Each subject was exposed for 5 consecutive days to the therapeutic cycle (see below) lasting 1 h. The results of that study [Baldi et al., 1999] did not show clearly a therapeutic effect. A second study [Baldi et al., 2001] indicated a possible relationship

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between blood pressure (BP) and heart rate variability (HRV) as an effect of PEMF stimuli. However, the relationship between HRV and BP was not clear.

Considering that no similar or comparable research has been published, the aim of this pilot study is to investigate if research into the effect of ELF-PEMF on heart rate (HR) using a larger number of subjects, is justified. Specifically it aimed: (a) to test the response to exposure to a PLF-PEMF with constant pulsing frequency (PRF) on a small number of healthy subjects (five males age 30–65 years) and (b) to test responses to an ELF-PEMF pulsed at various frequencies in one subject exposed 5 consecutive days, a different PRF each day [Baldi et al., 1999]. The resultant data are presented in tabulated and graphic form, as the limited number of subjects did not allow for a significant statistical data analysis.

This study was approved by the Monash University Standing Committee on Ethics in Research on Humans (SCERH).

METHOD

The importance of the HR variations and the methods for signal analysis have already been described elsewhere [Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996; Stein et al., 1999; Tabor et al., 2004]. In this brief communication we present only the power spectrum density (PSD) data obtained from the electrocardiogram. In this study we have duplicated the previous method [Baldi et al., 1999]. Figure 1 shows the subject position with reference to a PEMF radiating pad, the direction of the magnetic field (B field), and the intensity of the PEMF at specific locations. The PEMF stimulating unit is the Magnafield® Magnetic Induction Unit, Clinical Model MF998 (Magnacare Pty. Ltd, Prospect, South Australia). This specific unit has been used in all the previous investigations. The radiating pad is located on the floor under the chair upon which the subject is seated (Fig. 1)

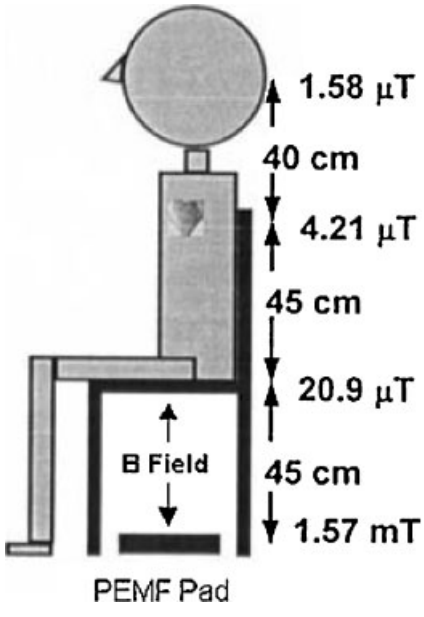


Fig. 1. Average distances from radiating pad, located on the floor under the seat, to heart and brain with the relative averaged PEMF intensity. The bipolar PEMF field (B) is shown.

Instrumentation

For data acquisition and analysis the following hardware and software were used:

Hardware:

(a) Magnetometer: Wandel & Goltermann (Germany) EM Field Analyser EFA-2. Figure 2 shows the "shape" of the PEMF as detected at the radiating pad using a 20 turn coil, 0.75 mm enameled copper wire wound on a 2 mm diameter support. The coil axis is oriented along the normal of the radiating pad.
(b) Electro cardiogram (ECG): BioPac (Goleta, CA, USA) System MP 100WSW with acquisition module ECG100C. The module is connected to two "Red Dot" 2249 Ag/AgCl electrodes in a Lead I configuration. A third Ag/AgCl electrode at the subject's right calf is used as ground signal.
(c) Heart rate and blood pressure (HR/BP): OMRON (Sydney, Australia) mod T5. The cuff is located on the subject's left arm.

Software:

(a) BioPac software package for the evaluation of PSD.
(b) Excel for data elaboration and for graphical presentation of the tables.

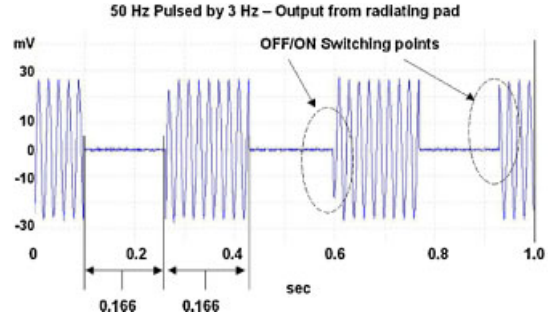


Fig. 2. The "shape" of the PEMF, 50 Hz pulsed by a 3 Hz signal. The highlighted points on the waveform show the pseudo-random pulse-controlled switching points.

Protocol

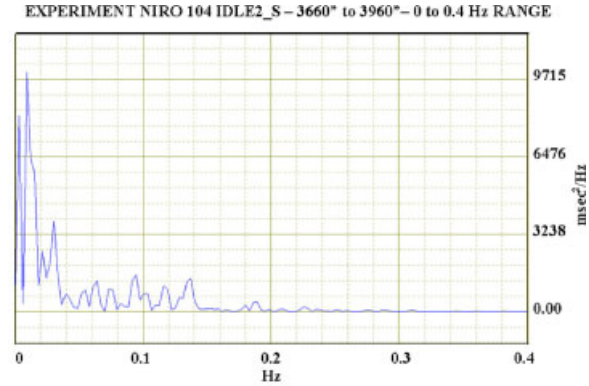
The protocol used for a previous therapeutic investigation [Baldi et al., 1999] was the guideline for this study. This study used one subject only to evaluate the subject's response to different PEMF frequencies and five subjects to evaluate the response to the PEMF of 50 Hz pulsed by a 3 Hz frequency of Figure 2. This specific PRF, 3 Hz, was chosen because in a previous investigation [Baldi, 2001] it was found that the intensity of the PEMF has its maximum value, as measured at the sitting point (Fig. 1) when the 50 Hz field is pulsed by a 3 Hz frequency. It is also of interest to consider that the period of the 3 Hz pulsing sinewave is an irrational number, 0.3333 s, and this might be the reason why the on/off switching (Fig. 2) occurs at pseudo-random points of the pulsed frequency (each pulsed period contains 8.3333 waveforms of the pulsed signal).

In this study the subjects had no visual or auditive stimuli from the PEMF source and/or from the BioPac unit during the test. Also, we introduced uncertainty in the starting/ending times and two treatments so that the complete experiment consisted of:

(a) Pre-Test: no radiation. Exposure time variable between 5 and 10 min;
(b) EMF 1: first exposure to ELF-PEMF exposure time 20 min;
(c) IDLE 1: no fields. Exposure time 20 min;
(d) EMF 2: Second exposure to ELF-PEMF. Exposure time 20 min. Same exposure as EMF1;
(d) IDLE 2: no fields. Exposure time between 5 and 15 min.

Data Elaboration

Heart rate data were recorded continuously for the duration of each experiment. Data were evaluated using a 5 min block method to conform to the suggested PSD evaluation short-term procedure [Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996]. The 5 min blocks (BioPac = 300 s) were chosen at the start (S) and at the end (E) of each radiation (field on)/nonradiation (no field) (20 min) section. The reason for this choice is to investigate HR variations at the transient points between radiation/nonradiation/radiation sections. These blocks are identified by the name of the section and the extension S (for Start) or E (for End) to indicate their position in the section. Eg EMF1_E refers to the last 5 min block of the EMF1 section (Fig. 3).



Frequency Band	Peak (Hz)	Power (ms^2)	Power %	Power (n.u.)
All Range	0.00916	212	100	
VLF	0.00916	137	64.6	
LF	0.09460	62	29.2	83
HF	0.18921	12	5.6	16

LF/HF Ratio: 5.17

Fig. 3. Sample of data elaboration. The graph refers to the whole 0–0.4 Hz frequency range for a 5 min ECG section; the table refers to the range of frequencies [Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996] in the same 0–0.4 Hz range.

BioPac software has been used to evaluate the PSD for all the 0+ to 0.4 Hz range and for each of the frequency bands in the range 0+ to 0.4 Hz [Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996] using the continuously recorded ECG data. The following steps were followed: (a) evaluation of the R-R intervals (ms), (b) evaluation of the Fast Fourier Transform (FFT) of the R-R intervals (ms/Hz) using the following protocol: Windows: Hamming; Packing: use end points; Mean & Trend: removed; Graph: Linear, (c) evaluation of the PSD (ms^2/Hz) for the whole FFT using the BioPac software (Fig. 3).

The PSD of the signal obtained from the R-R intervals shows the contribution of rhythmic fluctuations that may affect the heart period. The autonomic nervous system (ANS) [Stein et al., 1999] controls the HRV with sympathetic and parasympathetic afferents. This control can be detected in the low frequency (LF) range, 0.04–0.15 Hz, for the sympathetic branch of ANS and in the high frequency (HF) band, 0.15–0.4 Hz, for the parasympathetic or vagal branch of the ANS. Consequently, the LF/HF ratio is considered to mirror sympatho-vagal balance. Following the guidelines [Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996], LF and HF PSD values were normalized: LF (HF) n.u. = LF (HF)/(totalpower− VLF) × 100.

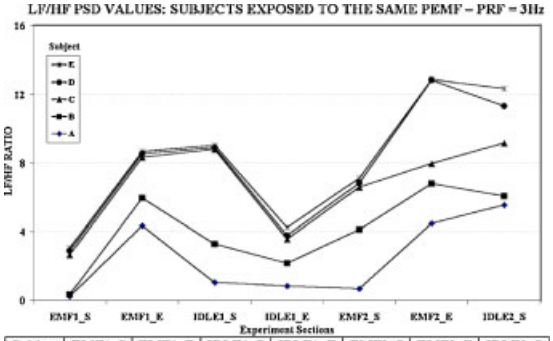
No statistical elaboration has been attempted due to the small number of subjects. Data are presented as

observation of variation of HRV as a function of subjects and as a function of PRF.

RESULTS

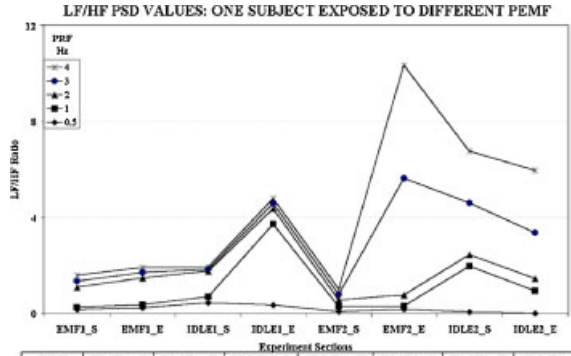
For each of the 5 min sections data were evaluated and tabulated as in Figure 3. As previously specified, Pre-Test and IDLE2 sections are of variable length: for this reason data comparison is limited to the time span between EMF1_S and IDLE2_S sections. Data are presented in tabulated and graphical format. The graphical format uses the Excel ''stacking'' method to show the differences between the results. In the Figure 3 graph, the PSD for the whole 0–0.4 Hz frequency band [Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996] is shown together with the peak frequencies. In the Figure 3 table the PSDs for the component frequency bands [Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996] are shown together with the relative peak frequencies. The LF/HF PSD ratio is also shown.

Figure 4 shows LF/HF PSD ratios referring to five subjects, identified with the letters A–E, exposed to the same ELF-PEMF pulsed at 3 Hz. The LF/HF PSD ratios are tabulated for each of the 5 min interval selected. The LH/HF PSD ratios are also shown in graphic form. Figure 5 shows data referring to the subject exposed to ELF-PEMF with PRF sequentially varying. The exposure was for 5 consecutive days, each day with a different PRF. The LH/HF PSD ratio results are tabulated as a function of the varying PEMF and a graphic interpretation of the result is also presented.



Subject	EMF1_S	EMF1_E	IDLE1_S	IDLE1_E	EMF2_S	EMF2_E	IDLE2_S
A	0.23	4.34	1.03	0.84	0.68	4.48	5.55
B	0.12	1.63	2.25	1.32	3.43	2.31	0.52
C	2.30	2.35	5.52	1.41	2.47	1.17	3.10
D	0.28	0.27	0.11	0.22	0.21	11.27	5.14
E	0.15	0.16	0.15	0.62	3.16	0.02	5.20

Fig. 4. Graphical and numerical synoptic result for five subjects exposed to the same EMF 50 Hz field pulsed with PRF of 3 Hz. Graphics are in Excel "stacked" form to emphasize the LF/HF PSD ratio variations. Graphs are in a sequence starting, at the bottom, with subject A's data. The LF/HF PSD ratio variation is noticeable at the end of each cycle. Note the values for subject C.



PRF Hz	EMF1_S	EMF1_E	IDLE1_S	IDLE1_E	EMF2_S	EMF2_E	IDLE2_S
0.5	0.18	0.22	0.44	0.35	0.08	0.17	0.06
1	0.09	0.14	0.24	3.37	0.31	0.14	1.90
2	0.83	1.12	1.07	0.65	0.26	0.46	0.49
3	0.28	0.27	0.11	0.22	0.21	11.27	5.14
4	0.25	0.22	0.09	0.21	0.24	4.72	2.16

Fig. 5. Graphical and numerical synoptic result for one subject exposed to EMF 50 Hz pulsed with different PRFs. Graphics are in Excel "stacked" form to emphasize the LF/HF PSD ratio variations. Graphs are in a sequence starting, at the bottom, with result for exposure to PEMF with a PRF of 0.5 Hz. The LF/HF PSD ratio variation is noticeable when the EMF is pulsed with 3 Hz and 4 Hz.

No statistical data analysis has been attempted, given the small number of subjects. However, inspecting the tables it can be noted that in the case of five different subjects exposed to the same PRF, the response to the stimulus is similar: the only difference is the magnitude of the response. In the case of the same subject exposed to different PRFs, the responses to the stimuli with a PRF of 3 Hz and 4 Hz show a noticeable variation in PSD ratio during the second period of exposure (EMF2) when compared to other PEMFs.

DISCUSSION AND CONCLUSIONS

Low frequency/high frequency PSD ratio values have been obtained using LF and HF normalized units (n.u.) [Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996]. We recall that dominance of the sympathetic branch of ANS, LF/HF > 1, might indicate vasodilation and cardiac acceleration; dominance of the parasympathetic or vagal branch of (ANS), LF/HF < 1, might indicate vasoconstriction and cardiac inhibition. By inspecting the tabulated results it can be seen that the variation in LF/HF PSD ratio mainly occurs at transition time from exposure to nonexposure to exposure sections. In particular, Figure 4 shows an increase in sympathetic dominance towards the end of the exposure time (EMFx sections). Subject C's response shows a different pattern. At start, all the

other subjects show a vagal dominance (LF/HF < 1), C shows a sympathetic dominance (LF/HF > 1); the LF/HF ratio shows a pattern that is similar to the other subjects' ones, but shifted in time. The qualitative implication of this behavior should be investigated.

Figure 5 shows changes in LF/HF PSD ratios for all the PRF, except for PRF $= 0.5$ Hz. The intensity of the PEMF is as shown in Figure 1. The values represent the averages of the readings taken for each of the PRF. The standard deviation of the averaged data is between 0.5 and 0.1 μT as a function of the measuring location starting from the sitting point. Considering the variation between data obtained from exposure to a single PRF versus the data obtained from exposure to the range of PRFs, we hypothesize that variations in cardiocirculatory system might have a pattern as a function of the PRF. The result shown in Figure 5 seems to support our hypothesis. For exposure to EMF pulsed at 3 Hz and 4 Hz, the variation in LF/HF PSD ratio is similar (increase at the end of EMF2 and decrease at the beginning of IDLE2); however, the amplitude is different, showing for PRF of 4 Hz a variation of nearly 50% of the one detected for exposure to an EMF pulsed with a PRF of 3 Hz.

As previously stated, we were unsuccessful in finding similar or comparable published studies we could refer to, and this is one of the reasons for the limited number of subjects in this pilot study. The results of this preliminary study, however, justify an investigation with a larger number of subjects of the effects of ELF-PEMF on the human biological and physiological systems with particular reference to the cardiovascular system. It will be of interest, in such a study, to investigate if fluctuations in HR, in particular presence of ectopic beats, might be a function of the PRF and if it might be duplicated using a suitable Windkessel model [Ursino, 2003], in particular with reference to variations in the arterial-venous system compliance—vasoconstriction/vasodilation—induced by the ELF-PEMF, as may be indicated by variations in sympatho-vagal balance.

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Extremely Low-Frequency Pulsed Magnetic Fields and Multiple Sclerosis: Effects on Neurotransmission Alone or Also on Immunomodulation? Building a Working Hypothesis

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Key words: ELF pulsed magnetic fields, multiple sclerosis, neurotransmission, immunomodulation, cell membrane alterations, picotesla fields, millitesla fields.

SUMMARY – *This paper outlines the current state of knowledge on the pathology and treatment of multiple sclerosis (MS) and critically analyses the vast clinical experience of Sandyk in the use of pulsed magnetic fields of 5 Hz at 7.5 pT to treat many symptoms of MS. A complete regression of symptoms, or at least a major improvement, is sometimes so rapid as to suggest that ELF fields exert a greater effect on axonal and synaptic neurotransmission than on the processes leading to demyelination. Pulsed magnetic fields of 50-100 Hz and a few mT (whose flux intensity is 10^9 times greater than that of the fields used by Sandyk) have been seen to induce profound morphological changes (the Marinozzi effect) in the plasma membrane of several cell types, including Raji human lymphoblastoid cells. These observations underlie the author's hypothesis on the possible use of such fields in the treatment of MS. Indeed, these fields should induce the functional arrest of the cells (B- and T-lymphocytes, macrophages, microglia, dendritic cells) of the MS plaque, thereby providing an "electromagnetic immunomodulatory boost" to the effects of drug therapy. To test this working hypothesis, it is suggested that preliminary experimental research be carried out to ascertain:* 1) *the Marinozzi effect* in vivo*;* 2) *the Marinozzi effect on microglia and dendritic cells; and* 3) *the duration of the membrane changes and their relaxation rate. ELF magnetic fields in the picotesla and millitesla ranges are aimed at improving neurotransmission and correcting local immune pathology, respectively. Both types of field might find application in the treatment of MS patients who no longer respond to or tolerate currently used drugs.*

Introduction

Multiple sclerosis (MS) is a chronic inflammatory demyelinating disease of autoimmune origin and is highly invalidating in subjects aged between 15 and 50 years. MS develops in genetically susceptible individuals and displays a broad spectrum of biological events, not all of which can be explained in terms of demyelination alone [1,2,3].

The severe impact on patients and their families, the large number of cases, the long course of the disease and the high mean annual cost for every person affected warrant research into new therapeutic approaches.

Such approaches, however, must be founded on rational bases. After some necessary reminders on the pathology of MS (section 2), its clinical course and the drug treatments currently implemented (section 3), the therapeutic possibilities of extremely low-frequency pulsed magnetic fields (ELF PEMF) in the picotesla (pT) range will be examined (section 4) and their possible mechanisms of action discussed. A working hypothesis will then be construed based both on the morphological changes that pulsed magnetic fields in the millitesla (mT) range can induce in the cell membrane (section 5) and on the ensuing functional changes (section 6).

Lastly (section 8), the paper addressed how the use of both drugs and pulsed magnetic fields of a few pT, according to Sandyk's technique, and pulsed magnetic fields of a few mT, according to our hypothesis, may be integrated in the treatment of MS.

The Pathological Features of MS and the Cells of the Immune System

Varying in size from 1 mm to a few cm, pink and turgid if recent, shrunken by gliosis if older, MS lesions are multifocal and scattered throughout the white matter [4]. However, it has recently been demonstrated that demyelination also involves the grey matter of the cortex and the deep grey nuclei even in the earliest stages of the disease, and that this *pathological heterogeneity* is reflected in the variety of clinical manifestations [2,5].

Moreover, new imaging techniques have revealed a *structural heterogeneity* of the lesions, which prompts the hypothesis that different mechanisms are active in the pathogenesis of MS [6]. According to Dammacco [7], the MS plaque displays hypocellularity at the centre and hypercellularity at the periphery, where B-lymphocytes, activated T-lymphocytes, macrophages and microglia are observed. In addition, immunohistochemical research conducted on patients who have died of MS has revealed that *the seemingly unaffected white matter* adjacent to the plaques contains endothelial cells that test positive for class I HLA antigens and scattered cells, 70% of which are macrophages and 30% of which are microglia cells positive for class II HLA antigens; the latter contain fragments of myelin indicating that they can process the antigen and present it to the effector cells. Thus, we have a sort of peripheral "metastasis", which is held to be responsible for the radial growth of the single plaques. These findings were recently confirmed by Lassmann et Al [1] who described diffuse injury to the *seemingly normal white matter*.

Bruck [8] reported on a recent *neurodegenerative model* of MS which complements the inflammatory hypothesis. According to this model, axonal damage is already visible in the early stages of the disease during acute inflammatory attacks. In the late-stage disease, slowly progressing axonal damage persists, even in the absence of inflammatory signs. The antigens that trigger the autoimmune response may differ from one patient to another. Not only do they include myelin proteins, but also oligodendrocyte precursor proteins or axonal constituents themselves. In addition to confirming that lymphocytes, macrophages and plasmacells are in close contact with the myelin sheets in the inflammatory phase, an ultrastructural electron microscopy analysis conducted by Rodriguez and Scheithauer [9] on 11 stereotaxic biopsies demonstrated that, in areas of chronic, established demyelination, the *oligodendrocytes* (figure 1) are greatly reduced in number. By contrast, at the edges of acute lesions with demyelinated axons, the oligodendrocytes appear morphologically preserved.

In addition to the importance of the oligodendrocytes, Zawadzka and Franklin [10] recognized the importance of the *oligodendrocyte precursor cells* (OPC) – stem cells that may differentiate into astrocytes and oligodendrocytes. A set of cytokines and growth factors act upon the OPC, causing them to differentiate into remyelinating oligodendrocytes [11]. It is therefore believed that areas of chronic demyelination develop as a result of the concurrent loss of oligodendrocytes and their progenitor cells.

Having mentioned the most recent insights into the pathology of MS, attention will now focus on the plaque-forming cells [12,13,14,15,16], with a view to their possible role as *targets* of ELF pulsed magnetic fields in the mT range.

The *B-lymphocytes* are the precursors of the antibody-producing cells: the plasmacells. A recent immunohistochemical and morphometric analysis conducted by Magliozzi et Al [17] demonstrated that *meningeal B-cell follicles entering the cerebral sulci* were present in 41.4% of 29 patients who had died of SPMS, but in none of seven patients who had died of PPMS. Moreover, both the clinical course and cortical demyelination were more severe in SPMS patients than in PPMS patients, so much so that the authors concluded that the *intrathecal production of antibodies* plays an important role in the inflammatory response and in the development of demyelinated lesions.

The *T-lymphocytes* are responsible for cell-mediated immune responses. In addition to directly eliminating tumour cells and cells infected by pathogens, they control functions of other cells, such as B-lymphocytes and effector cells (macrophages, granulocytes, cytotoxic T-lymphocytes and NK cells). Three main subgroups are distinguished: 1) cytotoxic T-cells; 2) helper T-cells, which help the B-cells to produce antibodies, stimulate the proliferation of activated T-cells and activate macrophages; 3) suppressor T-cells, which regulate the functions of other T- and B-cells. From the morphological point of view, B- and T-cells cannot be distinguished from one another under the optical microscope. Even on scanning electron microscopy (SEM) *resting* B- and T-cells (figure 2) are indistinguishable in that they are densely carpeted by microvilli. SEM can, however, distinguish *activated* cells, the surface of which is smooth and displays few microvilli. The presence or absence of microvilli therefore seems to indicate a functional stage of B- and T-cells rather than a stable condition [13,14].

Mononucleated phagocytes, or *macrophagic monocytes*, include the monocytes in the blood and the macrophages residing in the various regions of the body, both of which are endowed with a long life. The mononucleated phagocytes respond to chemotactic signals from the lymphokines secreted by the T-cells. They bind antigens by means of membrane receptors, process them by demolishing the ingested structures and release fragments through a continuous turnover of the cellular membrane. Monocytes and macrophages also have an irregular cell surface, which displays various types of folds and abundant microvilli (figures 3, 4).

The *cells* of the *microglia* are resident macrophages originat-

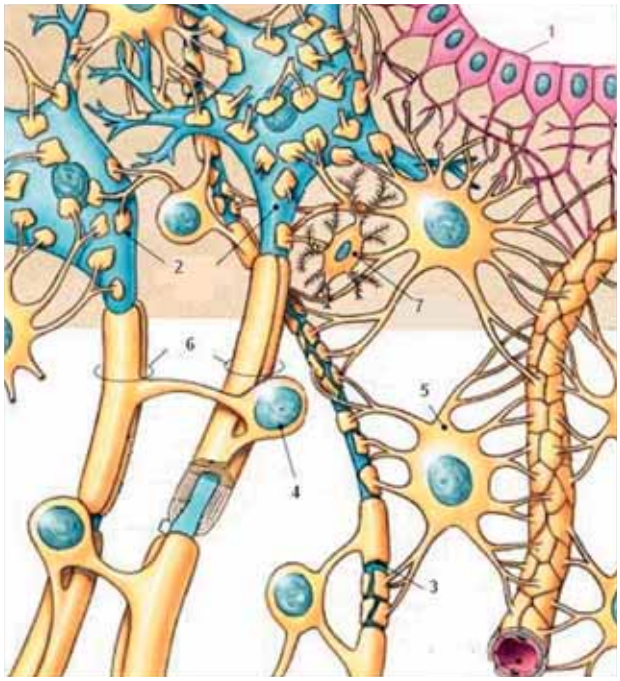


Figure 1 1) Ependymal cell; 2) neuron; 3) axon; 4) oligodendrocyte; 5) astrocyte; 6) myelin sheet; 7) microglia cell. From Consejeria de Educacion y Ciencia, Plaza de España 5, 33007 Oviedo, Spain.

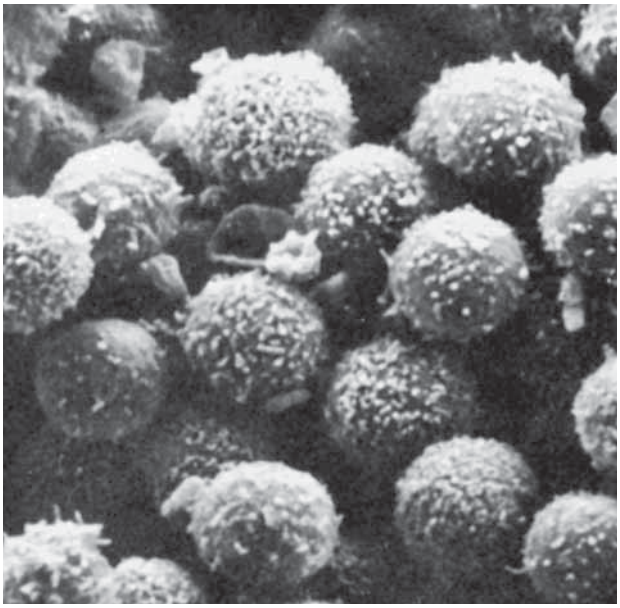
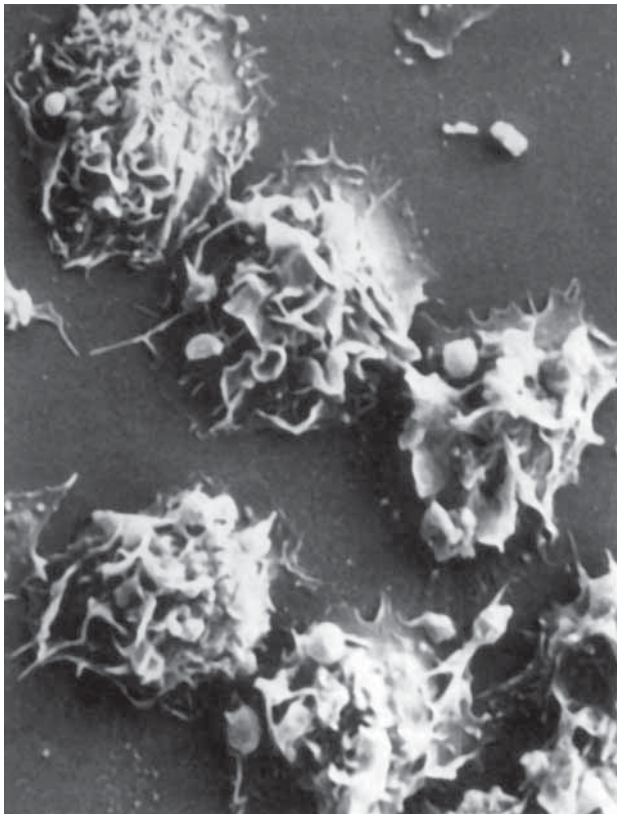
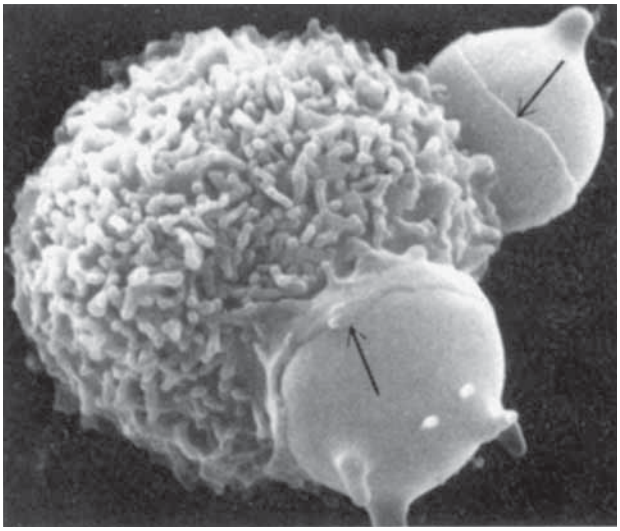


Figure 2 SEM images of lymphocytes in peripheral blood. From R. Laschi et Al. in L. Oliva (ed.) "Radiobiologia del Linfocita", Piccin Editore, Padova 1975: 55. With the editor's permission.





↑ Figure 4 SEM image of a mouse macrophage phagocytizing two altered erythrocytes. From B. Alberts et Al. "Biologia Molecolare della Cellula", Italian translation by M. Guardo, G. Corte and E. Melloni, Zanichelli, Bologna 1991, 2nd edit: 399.

← Figure 3 SEM images of monocytes (×3200) carpeted with microvilli, lamellar villi and blebs. From R. Laschi (ed.) "Patologia Ultrastrutturale". Editrice Compositori, Bologna 1980: 54.

ing from bone-marrow monocytes. Activated microglial cells (figure 5 [A1-A6]) are involved in the inflammatory processes of the CNS and respond to neuronal damage by removing the damaged cells through phagocytosis. However, chronic activation of the microglia (figure 5[B1-B3]) may in turn damage neurons through the release of cytotoxic molecules: pro-inflammatory cytokines, reactive oxygen intermediates and proteinases. Consequently, the suppression of microglia-mediated inflammation is regarded as an important therapeutic strategy in the treatment of neurodegenerative diseases [18]. This is, however, difficult to achieve as the activation mechanisms of the microglia remain elusive [19]. The *dendritic cells* (figure 6), which are responsible for presenting the antigen to the T-cells, are extremely important in MS [20]. Present within the healthy CNS in association with the cerebral spinal fluid space and with the microvasculature, they are able to sample CNS antigens. As they are also present in MS plaques, attempts are being made to identify therapeutic approaches directed at these cells in order to treat multiple sclerosis [20]. Interactions among the various cells of the immune system take place through the release of interleukins and through direct membrane interactions [7,16]. It therefore follows that, at the centre of immune interactions, *the membrane of the cells involved always plays a leading role*. This observation reveals the importance of the profound membrane changes produced by exposure to ELF fields of a few mT. These changes are described in section 5 and discussed in section 7.

The Clinical Course of MS and the Pharmacological Therapies Currently Undertaken

MS displays various types of clinical course [21,22]. The *relapsing-remitting form* (RRMS) (figure 7A), in which acute episodes are followed by spontaneous remission, is the most frequent. In the early stages, remission is complete; over time, however, serious neurological deficits remain. The pattern of RRMS gradually changes, and the disease takes on a slow, but continuous progressive course (figure 7B, C): relapses become rare and remission practically no longer occurs (*secondary progressive form*, SPMS). According to Dammaco [23], this chronic active feature suggests a "permanent autofeeding phlogosis". *Primary progressive relapsing MS* (PPMS) (figure 7D) is less common. The pharmacological strategies currently adopted can be divided into four groups [24]:

1) The *first group* comprises measures aimed at reducing the severity and duration of acute attacks through the use of high-dose glucocorticoid steroids.

2) The *second group* of measures strives to slow down the biological activity of the disease to reduce or prevent further neurological damage by means of immunomodulators and immunosuppressors. Beta 1-a interferon [25] and especially beta 1-b interferon [26] are useful in both RRMS (figure 7A) and SPMS (figure 7 B, C). Recently, the synthetic protein glatiramer acetate has proved efficacious [27]. Previously known as copolymer-1, glatiramer acetate simulates the basic myelin protein, is multifaceted and is able to effectively control both the inflammatory and degenerative components of experimental autoimmune encephalomyelitis [28]. Immunosuppressive treatments, both with antiblastic drugs and by means of total lymphoid irradiation are also efficacious. However, being highly toxic, they are undertaken only in highly selected cases [24].

3) The *third group* comprises symptomatic treatments. While unable to modify the biological course of the disease, these improve the patient's quality of life.

4) The strategies that make up the *fourth group* aim to repair the damage to the CNS by means of experimental approaches, such as myelin transplantation and the use of oligodendrocyte precursor cells, which have characteristics of stem cells.

ELF Pulsed Magnetic Fields in the Treatment of MS. Clinical Results and Possible Mechanisms of Action

Extremely low-frequency magnetic fields (ELF fields) interact readily with the CNS. While the high-intensity ELF fields encountered in industry can expose workers to an increased risk of Alzheimer's disease [29], amyotrophic lateral sclerosis [29,30] and multiple sclerosis [30], ELF magnetic fields of weak and very weak intensity can exert interesting and proven therapeutic effects on the CNS [34-69]. Such differential interactions are also shared by other chemical and physical agents to which humans are exposed. In the last 15 years, several publications have dealt with the treatment of multiple sclerosis by means of extremely low-frequency pulsed magnetic fields (ELF PEMF). A 1994 publication by Jerabek [31] on the use of ELF PEMF in Czechoslovakia for more than ten years defined the results obtained in MS and other spastic conditions as "promising". In 2002, Brola et Al [32] reported on a study conducted in Poland on 76 patients who had been ill for a mean 8.5 years; these patients were divided into two groups: one treated with pulsed magnetic fields and the other a control group. After 21 days of therapy, the quality of life of the patients in the treatment arm was seen to have improved significantly ($p<0.01$), especially with regard to their mental condition, muscle tone, dysaesthesia and painful sensations; moreover, no side-effects were recorded. In 2003 in the USA, Lappin et Al [33] published a placebo-controlled, double-blind, multicentre pilot study conducted on 117 patients who were exposed daily for four weeks to ELF fields produced by a small portable pulsing e.m. field generator. The authors concluded that weak pulsed magnetic fields could alleviate the symptoms of MS, but that the effects were modest and required further confirmation. They also suggested investigating the possibility that patients on treatment with beta interferon

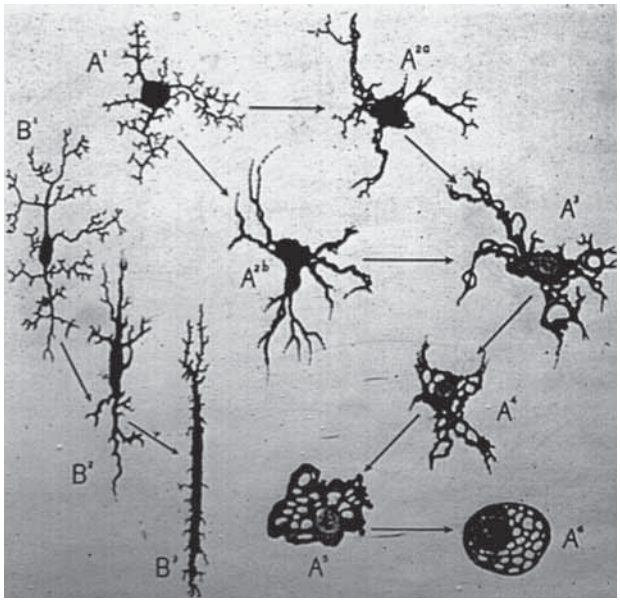


Figure 5 Schematic activation of a resting microglia cell (A^1) about to become a mobile scavenger cell. The pleomorphic response of the cell to necrotic stimuli (from A^2 to A^5) finally gives rise to a rounded macrophagic cell (A^6). $B^1B^2B^3$ chronically activated microglia cells. Copyright © 2000, by Department of Neurology. Neuropathology and Neuroimaging Laboratory. University of Rochester. http:// www.urmc.rochester.edu/neuroslides/index.html. Permission obtained.

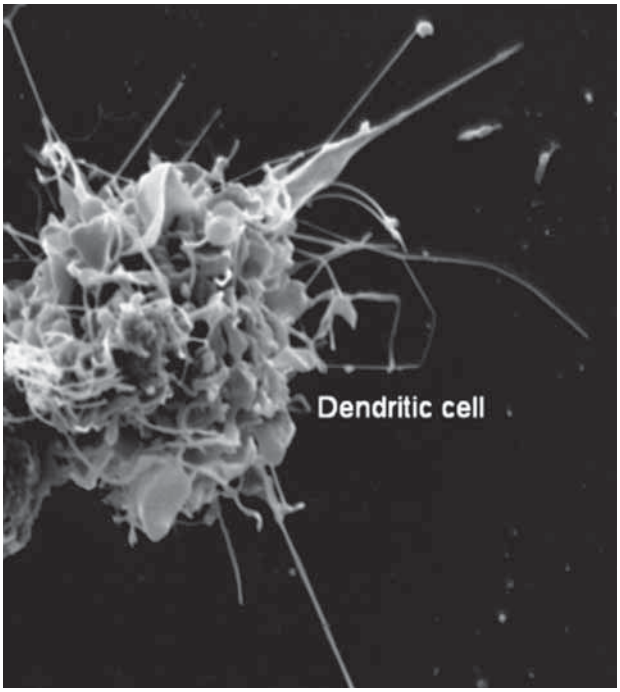


Figure 6 SEM image of a dendritic cell carpeted with a number of microvilli. From S. Hayley, W. Bowers and R. Hunt, partially modified. In R. Hunt "Microbiology and Immunology on line". University of South Carolina, School of Medicine.

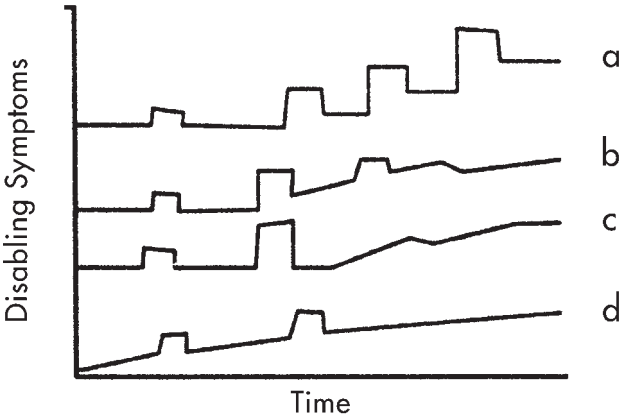


Figure 7 A-D) Clinical course of multiple sclerosis (MS). The rising lines indicate an increase in disabling symptoms; the falling lines a decrease. A) Worsening relapsing-remitting MS; B,C) two types of secondary progressive MS; D) primary progressive-relapsing MS. From ref. 21, partially modified.

might be more responsive to ELF field therapy.

Clinical Results of Treatment with ELF Pulsed Magnetic Fields in the Picotesla (pT) Range

The research conducted by Sandyk deserves to be examined in greater detail. Between 1992 and 1999, this author published numerous papers in the *International Journal of Neuroscience* [34-69], some in collaboration with Derpapas [35,36], Iacono [37,38,42] and Dann [44,45]. Sixty-four cases of MS are described in patients with variable clinical histories spanning five to 37 years, who were mainly affected by the progressive chronic form of the disease.

The therapeutic technique used consists of the extracranial application of a very weak sinusoidal magnetic field of 7.5 picotesla (7.5×10^{-12} T) at a frequency of 4-5 Hz. These exposure conditions must be regarded as "physiological", not only because they match the EEG frequencies, but also because they simulate the spontaneous biomagnetic signals emitted by the human brain (10^{-12} T for the alpha rhythm) [70], thereby enabling interactions according to Jacobson resonance [71] to occur between the applied field and some cerebral functions.

The duration of the applications

Table 1 Therapeutic results on some MS symptoms obtained with sinusoidal magnetic fields of 4-5 Hz and 7.5 pT according to Sandyk [34-69]

A1 *Very Rapid Complete Resolution*	
• pretreatment latencies of the visual and auditory evoked potentials • Lhermitte's sign • acute parkinsonian syndrome	– within the first week [35] after two sessions of 20' [67] after one session of 20' [41] – in 3 pts [65] after 2 short sessions – in 1 pt [56] after 2 short sessions
A2 *Complete Resolution Obtained after Weeks or Months of Treatment*	
• impairment of dual-task performance (talking while walking)	– in 3 pts [55] after brief treatment courses
• acute exacerbation of various MS symptoms	– in 1 pt [36] within 2 wks of treatment
• loss of dream recall on waking	– in 4 pts [46] after 1 course of sessions
• alexia	– in 3 pts [50] after a few months
• severe dysarthria	– in 2 pts [51] after 3-4 wks
• premenstrual exacerbation of MS symptoms	– in 2 pts [52] after two months
• partial cataplexy	– in 1 pt [53] after three wks
• long-standing suicidal tendency	– in 3 pts [59] within a few wks, maintained for 3.5 years
• sleep-related paralysis	– in 1 pt [64] during treatment, maintained for > 3 years
B1 *Very Rapid Improvement*	
• headache and other neuralgia during acute exacerbation of MS	– in 1 pt [36] after the first session
• deficit of cognitive functions	– in 3 pts [38,42,63] almost immediately
• various MS symptoms	– in 1 pt [47] after two sessions of 20'
• various MS symptoms	– in 1 pt [37] after two sessions of 30'
• impairment of dual-task performance (talking while walking)	– in 1 pt [66] after two sessions of 45'
• speech impairment	– in 3 pts [43] after 4-5 sessions
• intention tremor and postural tremor	– in 3 pts [44] after brief sessions
B2 *Improvement after Weeks or Months of Treatment*	
• various cognitive deficits	– in 7 cases [40,62] slow but progressive
• body-image perception	– in 5 [48] and 2 pts [58] after one course
• severe fatigue	– in 3 pts [54,63] after a few months
• carbohydrate craving	– in 1 pt [57] after one treatment course
• impairment of depth perception with postural instability	– in 1 pt [69] after one treatment course

is brief, from a few minutes to 20, 30, 45 minutes, but their time sequence may vary from two to three sessions on consecutive days to two to three sessions per week or even a single session per week in cycles that are sometimes repeated for one or more years. This is indicative of the fundamental importance attached to neurological evaluation of the individual patient and to careful clinical control throughout the treatment with ELF PEMF, the *experimental nature* of which, notwithstanding its proven therapeutic efficacy, was frequently stressed by Sandyk himself. While all of the cases described were multi-symptomatic, Sandyk each time highlighted one or more symptoms and their response to the magnetic field, with a view to clarifying the possible mechanisms of action.

Table 1 summarises the symptoms specifically analysed and their responses (type A_1 A_2 and B_1 B_2) to treatment. Treatment was always applied by means of a 4 or 5 Hz sinusoidal field and intensity of magnetic flux of 7.5 pT, though with considerable differences in length, number and fractionation of the single applications. Another two papers by Sandyk [60,65] merit special attention. These show that weekly treatment prolonged for years with very weak ELF magnetic fields can alter the clinical course of chronic progressive MS, *arresting progression of the disease* for as long as four years. This ob-

Table 2 Marinozzi effect induced by ELF time-varying magnetic fields in the mT range

Marinozzi et Al 1982 [84]	Hep 2 cells	Three waveforms (see figure 8) 50–100 Hz	7 mT ×1–32 h	Disappearance of microvilli, filopodia and blebs; membrane has rough appearance and evident breaks (see figure 9)
Paradisi et Al 1993 [86]	K562 cells	sinusoidal 50 Hz	2.5 mT ×24–72 h	Disappearance of microvilli and diffuse blebbing of the membrane (see figure 10)
Santoro et Al 1997 [81]	Raji cells	sinusoidal 50 Hz	2 mT ×24, 48, 72 h	Reduced membrane fluidity, disappearance of microvilli, redistribution of actin filaments in the cytoskeleton
Lisi et Al 2000 [82]	Raji cells	sinusoidal 50 Hz	1 mT ×13–64 h	Disappearance of microvilli; progressive appearance of deep membrane infolding; redistribution of actin filaments in the cytoskeleton (see figure 11)
Grimaldi et Al 2004 [87]	Raji cells	sinusoidal 50 Hz	2 mT ×9–64 h	Disappearance of microvilli and pseudopodia; appearance of roughness and narrow membrane infolding

Table 3 Marinozzi effect induced by static magnetic fields in the order of mT

Chionna et Al 2003 [88]	lymphocytes	6 mT	24 h	round cells → irregularly elongated cells appearance of lamellar microvilli when simultaneously exposed to apoptogenic agents
	U937 cells promonocytes carpeted with microvilli	6 mT	24 h	numerous lamellar microvilli rearrangement of F-actin filaments
Chionna et Al 2005 [89]	Hep G2 polyhedral cells carpeted with short microvilli	6 mT	24 h	polyhedral cells → elongated cells short m.villi → irregular m. villi distributed at random changes in microfilaments and microtubules
Dini et Al 2005 [90]	various cell types	6 mT	24-48 h	appearance of irregular lamellar microvilli time-dependent changes in microfilaments and microtubules
Teodori et Al 2006 [91]	Human glioblastoma cells with long microvilli	8 up to 300 mT		loss of long villi disappearance of surface ripples and furrows appearance of membrane roughness and blebs

servation prompts the hypothesis that, in addition to effects on axonal and synaptic neurotransmission, effects may also be exerted on the immune mechanisms responsible for demyelination.

On the Mechanism of Action of ELF Pulsed Magnetic Fields in the Picotesla (pT) Range

At the centre of the mechanism of action of ELF fields of a few pT and 4-5 Hz, Sandyk sees the pineal gland, a magneto-sensitive organ secreting melatonin [34,35,41]. Magnetic fields are thought to stimulate the pineal gland to release melatonin, a neurohormone that in turn acts upon the synthesis and release of serotonin (5-HT) [35,41,51]. As the symptoms of MS seem to be conditioned by neurotransmission deficiency, and particularly by serotonin deficiency [37,38,39,47], the action of these "physiological" magnetic fields might be mediated by the increased synthesis of 5-HT through resynchronisation of the circadian secretion of melatonin by the pineal gland [59,64,67].

The sensitivity of the pineal gland to very weak magnetic fields was demonstrated in 1980 by Semm et Al with regard to the geomagnetic field [72], while its regulatory effect on circadian rhythms was demonstrated in 1978 by Brown et Al [73] on experimental animals. It should also be mentioned that in 1983 in the vicinity of the hypophysis and pineal

gland, Baker et Al [74] discovered tiny *magnetosomes*, which might play a role in the interaction between magnetic fields and the hypophysis and pineal gland.

The notion that magnetic fields exert their effect by stimulating the pineal gland to secrete melatonin may be accepted in the case of a *hypofunctioning pineal gland*, though not necessarily in the case of a normally functioning gland. Indeed, a recent study conducted by Graham et Al [75] on 46 healthy subjects of both sexes exposed overnight to magnetic fields of 60 Hz and 28.3 μT showed that the concentrations of melatonin and its 6-OHMS metabolite in morning urine samples were no different from those seen in control subjects. These results confirm what has often been reported in the literature, namely that the effects of low flux density magnetic fields are *exerted on altered functional states*, in the sense of *hyper-* or *hypo-function*, rather than on normal functional states. Sandyk's neurophysiological interpretation is that *neurotransmission is favoured* at various sites: partially demyelinated axons [39], synapses [62], the cerebellum [51,64], and interhemisphere transcallosal connections [50], an idea which is *strongly supported by the rapid regression seen in certain symptoms* in patients treated with only one or very few sessions of 20', 30' or 45' (see cases listed in A_1 and B_1). Such rapidity of effect certainly cannot be attributed to remyelination, but rather to the correction of perturbations of synaptic conductivity due to the deficit of serotonin (5-HT) [47,61]. Finally, Sandyk admits that part of the mechanism of action of magnetic fields may be attributed to an increased hypophyseal secretion of ACTH [68], an immunomodulator hormone which is also used in the treatment of multiple sclerosis. The following sections will address our personal hypothesis that ELF magnetic fields in the mT range can also be proposed for the treatment of MS on the basis of an action mechanism centred around the *immunomodulation of the disease*. As suggested in the Discussion (section 7), this hypothesis should be adequately tested.

Morphological Changes in the Membrane of Cells Exposed to ELF Magnetic Fields in the Millitesla (mT) Range: the Marinozzi Effect

The cell membrane is endowed with nanometric "protein organelles" (receptors, enzymes, ion channels, active pumps). Ligand-receptor bonds, all ion exchanges with the extracellular environment, and the transduction of external signals and their conduction into the cell depend on these. Since the 1980s, it has been hypothesised, particularly by Adey [80], that the cell membrane is the primary site of interaction between the cell and low frequency magnetic fields [76,77,78,79,80]. Indeed, such fields are believed to modulate events which take place on the cell surface by modifying the signals arising from the bond between extracellular ligands and membrane receptors. *Distorted signals* are thought to send *erroneous messages* to the intracellular organelles, thereby giving rise to functional alterations in the cell [77]. If, then, an ELF magnetic field with given characteristics is able to *induce the profound changes in membrane morphology and structure* described here, largely characterised by the "loss of microvilli", it is reasonable to suppose that, at the very least, the cells undergo changes in the initial processes of the transduction cascade of external signals [81,82]. The microvilli are membrane protrusions endowed with a nucleus of F-actin filaments that communicate with the interior of the cell. In addition to the functions of cell migration, the microvilli also have biophysical properties of true sensors of electromagnetic fields [83]. Their "disappearance" from the cell surface as a result of the action of ELF magnetic fields (and also, as will be seen, of static magnetic fields) eliminates their function as "cellular antennae", thereby altering those intercellular interactions that take place at the membrane level. The working hypothesis advanced in the present paper stems from lengthy reflection on the research conducted by Marinozzi et Al [84] and from having found subsequent confirmation in studies on membrane changes induced by both ELF and static magnetic fields (see tables 2 and 3, respectively).

Induction of the Marinozzi Effect by ELF Magnetic Fields and Static Magnetic Fields

In 1982, Marinozzi et Al (84) studied the effects produced on Hep 2 human epidermoid carcinoma cultures by magnetic fields of 50 or 100 Hz and 7 mT with three different waveforms (figure 8):

- Pulsed semi-sinusoidal at 50 Hz (one half-wave) (figure 8a)
- Pulsed semi-sinusoidal at 100 Hz (rectified half-wave) (figure 8b)
- Sinusoidal at 50 Hz (one full-wave) (figure 8c)

The cultures were treated with the three types of wave for 1 h to 32 h at a constant temperature. SEM observation revealed constant and dramatic changes in all cell membrane protrusions (disappearance of microvilli, filopodia and blebs) even in cells undergoing mitosis (figure 9). The most significant changes followed exposure to the *pulsed wave at 100 Hz* (figure 8 b) for 1 h or for 1 h in repeated cycles interrupted by brief pauses. Of the three waveforms, the pulsed wave showed the greatest ability to induce unidirectional magneto-mechanical effects very like those produced by a static magnetic field [85].

The *disappearance of the microvilli*, as observed by Marinozzi et Al (henceforth shortened to "the Marinozzi effect") was confirmed in 1993 by Paradisi et Al [86] on human K562 erythroleukaemia cells, by Santoro et Al [81] in 1997 on human lymphoid cells (Raji) and again, on the same cells, by Lisi et Al [82] in 2000 and by Grimaldi et Al [87] in 2004. The experimental conditions used by these authors [81,82,86,87] are summarised in table 2 and differed slightly from those used by Marinozzi et Al in that the mag-

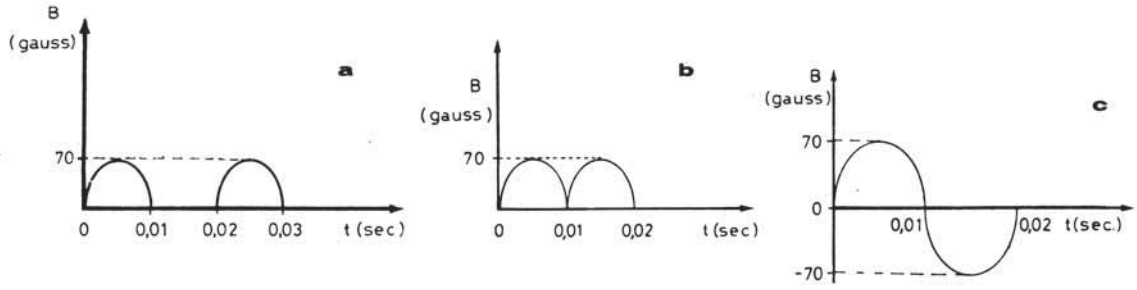


Figure 8 Three waveforms of the 7 mT magnetic fields used by Marinozzi et Al [84] to obtain the results depicted in Fig. 9. Maximal magneto-mechanical effects occur with waveform b. From F. Bistolfi (ed.) "Campi Magnetici in Medicina. Biologia Diagnostica Terapia". Edizioni Minerva Medica, Torino 1986 [85]: p. 256.

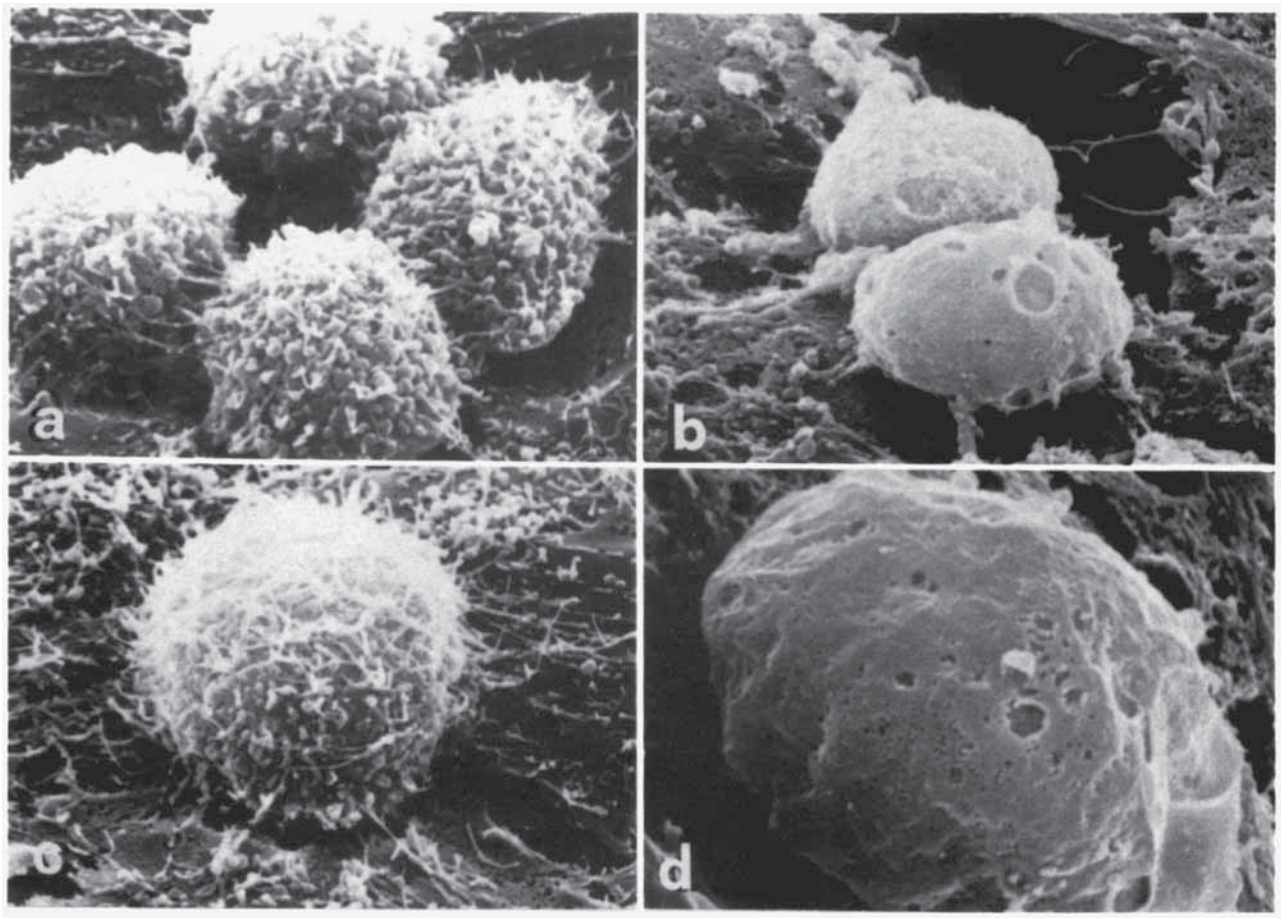


Figure 9 A,C) SEM images (x 7500) of Hep 2 human carcinoma cells not exposed to magnetic fields. B,D) SEM images (x 10000) of the same culture after 1 h exposure to a 100 Hz, 7 mT, pulsed magnetic field. See text and Table 2. From Marinozzi G. et Al [84] in F. Bistolfi (ed.) [85] p. 290-291.

netic fields were only of sinusoidal form and always had a frequency of 50 Hz, with a lower magnetic flux density and a longer exposure time. With regard to the membrane changes induced by ELF fields, Paradisi et Al [86] observed the complete disappearance of microvilli and the subsequent appearance of diffuse blebbing [1] (figure 10) directly proportional to the exposure time.

[1] According to some authors quoted by Paradisi et Al, blebs are a fairly general response to stress; induced early by toxic substances, they are sometimes followed by cell death (various authors quoted by Paradisi et Al). However, it should be noted that in Marinozzi's research the blebs were present *before* exposure to the magnetic field and *disappeared* after exposure.

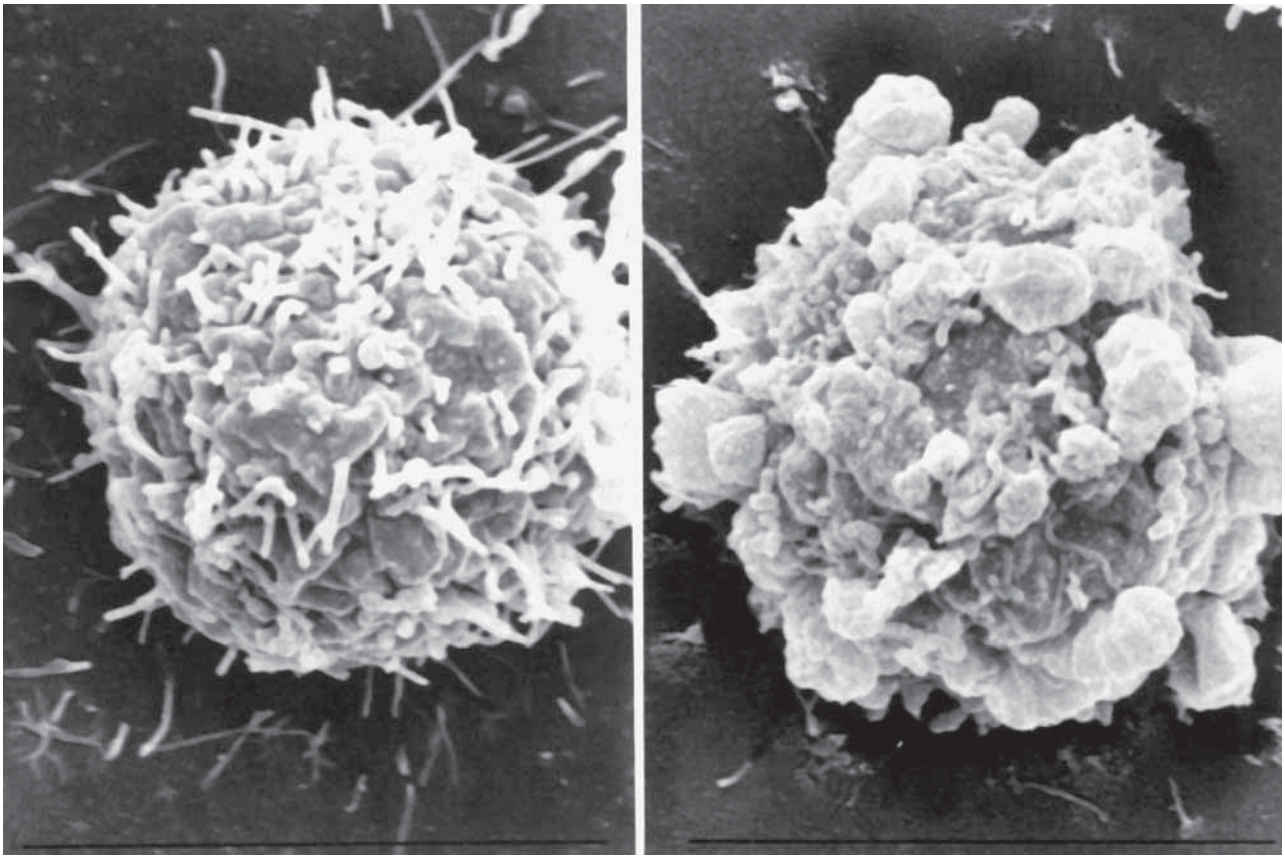


Figure 10 Left: SEM image of a K562 human erythroleukaemia cell not exposed to magnetic fields. Right: SEM image after 72 h exposure to a 50 Hz, 2.5 mT, sinusoidal magnetic field. Diffuse blebbing has appeared. See text and table 2. From Paradisi et Al [86], partially modified.

Santoro et Al [81] observed reduced membrane fluidity and the disappearance of the microvilli, accompanied by a different distribution of actin filaments in the cytoskeleton (examined by means of phalloidin-fluorescence). Using an Atomic Force Microscope, Lisi et Al [82] also confirmed the disappearance of the microvilli from the Raji cells, followed by the gradual appearance of deep corrugations in the plasma membrane (figure 11). All of these alterations were accompanied by a redistribution of actin filaments, clearly demonstrated by means of phalloidin-fluorescence. Similar results were obtained by Grimaldi et Al [87] in 2004.

In the last few years, further experimental studies have documented marked membrane changes largely similar to those we have called "the Marinozzi effect". These, however, have been produced by exposing cells in culture to *static magnetic fields* of a few mT for 24 or 48 hours (see table 3). The changes described include: the transformation of round or polyhedral cells into elongated cells [88,89], the loss of long villi from the membrane [91], the disappearance of the regular surface undulations and their substitution by membrane roughness and blebs [91], and the appearance of irregular microvilli arranged at random [89] or even of lamellar microvilli [88,90]. All of these alterations are evident expressions of a *magneto-mechanical effect* exerted by the static magnetic field and of the consequent spatial rearrangement of the actin microfilaments [88,89,90] known to be endowed with anisotropy of diamagnetic susceptibility [85]. These last results confirm the interpretation that we gave in 1986 for the original Marinozzi effect (observed in 1982), which was obtained by means of ELF fields with different waveforms. As mentioned above, the most intensive effect was obtained under the pulsed 100 Hz semi-sinusoidal wave (see figure 8B), which approximates the interaction conditions of a static magnetic field better than the other two waveforms used (see figures 8A and C [85].

The series of studies involving static magnetic fields, however, brought to light some bioeffects that were not observed when ELF fields were used. Specifically, these involve the substitution of thin microvilli by irregular [89] or lamellar [88,90] microvilli. This may depend on the more uniform and constant magneto-mechani-

cal forcing induced by the static magnetic field. In any case, the radiobiological sense of the whole series of experiments conducted with both ELF fields (table 2) and with static magnetic fields (table 3) remains unmodified.

Thus, on the basis of the studies examined [81-91], we can today define the Marinozzi effect as follows:

- high-degree *morphological changes* in the cell membrane;
- *induced* by time-varying ELF fields, but also by static magnetic fields, in the mT range;
- *characterised* by one or more of the following partial effects:
  - disappearance of microvilli;
  - their transformation into irregular or lamellar microvilli;
  - disappearance of pre-existing blebs;
  - induction of diffuse blebbing;
  - appearance of deep wrinkles;
  - smoothing of a previously irregular membrane;
  - appearance of apparent breaks in the membrane.
- due to a magneto-mechanical rearrangement of actin filaments, whose anisotropy of diamagnetic susceptibility is well known.

Functional Changes in Cells Undergoing the Marinozzi Effect Due to ELF Magnetic Fields or Static Magnetic Fields

In the studies consulted [81-91] the functional changes accompanying the profound membrane changes that we have called the *Marinozzi effect* have been considered only in part. In the experiments utilising ELF magnetic fields, according to both Paradisi et Al [86] and Lisi et Al [82], the *growth curves* of the cells exposed (K562 and Raji, respectively) did not display significant differences from sham exposed cells. Moreover, in their experiments involving static magnetic fields, both Chionna et Al [89] and Dini et Al [90] also concluded that *cell proliferation* was only partially modified. According to Chionna et Al [88], static magnetic fields induce an increase in $[Ca^{++}]i$. However, the effect of this increase in endocellular calcium on apoptosis does not seem to be well defined. Indeed, it has been regarded as an anti-apoptotic factor for some cells (lymphocytes and U937) [88], but also as an apoptosis-inducing factor in another study [90]. The fact is that, in cells exposed to static magnetic fields, the effect on apoptosis, whether increasing or reducing, seems to be influenced in a cell-dependent manner [90].

An important observation was made by Dini et Al [90], who found that the *recognition by liver macrophagic cells of apoptotic lymphocytes* exposed to a static magnetic field of 6 mT for 24 h was *influenced by the very membrane changes induced by the magnetic field on the cells undergoing apoptosis*. This observation attributes to the Marinozzi effect at least a sound role from a functional point of view, and is in line with the findings of Grimaldi et Al [87] that in cells displaying membrane changes as a result of treatment with ELF fields, it is reasonable to hypothesise that "some functional alterations occur, for instance in cell motility or target recognition".

In our view, too, significant functional alterations must accompany the Marinozzi effect, inasmuch as they are linked to the inevitable distortion of the quaternary structure of the membrane proteins which form the receptors, ion channels and active pumps. Nevertheless, it must be ascertained whether the Marinozzi effect is reversible and, if so, what the relaxation rate of the changes induced is. Indeed, this could determine the possible fractionation schedules of treatment with ELF fields of a few mT aimed at reducing the activity of the immune cells present in MS plaques.

Functional Alterations Induced by ELF Magnetic Fields in the mT Range on Cells of the Immune System. The Need to Establish Links with the Marinozzi Effect

Although relatively few experimental studies have been conducted on the morphological membrane alterations induced by ELF magnetic fields and by static magnetic fields (see section 5), a number of papers have addressed the specific functional effects induced by magnetic fields on various biosystems, as well as a few excellent critical reviews: Walleczek J, 1992; Hong FT, 1995; Lacy-Hulbert et Al, 1998; Zhadin MN, 2001 [92,93,94,95]. Among these reviews, Walleczek's [92] is particularly relevant to the present study, as it focuses on the cells of the immune system. The review reports that the in vivo exposure of animal organisms to non-thermal ELF magnetic fields induces demonstrated effects on the leukocyte count in blood [96,97], the inflammatory response [98,99] and the activity of NK cells in the peripheral blood [100].

What is more interesting for us, however, is the fact that, up to 1992, at least ten laboratories had independently demonstrated *non-thermal effects on cells of the immune system* exposed *in vitro* to ELF magnetic fields of a few mT (from 0.1 mT to 10 mT). In other words, cells exposed to magnetic fluxes of the same order of magnitude as those used in the experiments which induced the Marinozzi effect (from 1 mT to 7 mT) and 10^9 times higher than the few pT used by Sandyk in the therapy of multiple sclerosis (see sect. 4). The endpoints analysed by Walleczek were principally: the metabolism of Ca^{++} (intracellular free calcium concentration and mitogen-dependent $^{45}Ca^{++}$ uptake); [^{3}H] Uridine uptake and the consequent gene transcript levels; and [^{3}H] Thymidine uptake and the consequent cell-cycle kinetics. Although it is very difficult to establish precise dose-effect relationships in this sector of biophysics, some correlations between dose and effect can be discerned in studies dealing with DNA synthesis [101-107]. *Non-sinusoidal* magnetic fields (square or saw-tooth wave) at both 3 Hz and 50 Hz elicit an increase in [^{3}H] Thymidine uptake at a low peak flux density (2.5 mT×66 h), but a reduction in uptake at a high peak flux density (4.5 mT, 6 mT, 10 mT×72 h). With *sinusoi-*

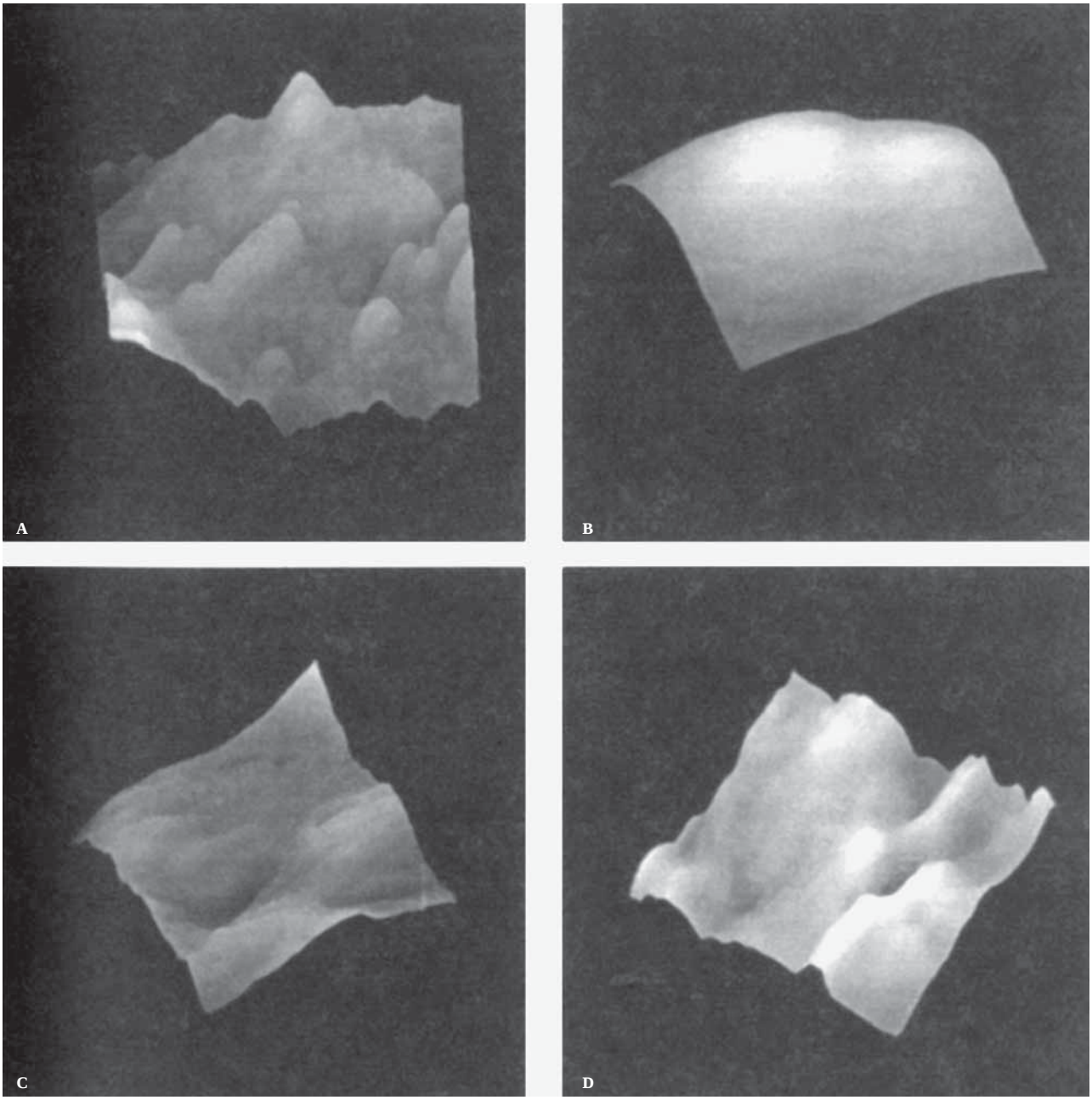


Figure 11 A-D) Atomic Force Microscope 3-D images of square membrane fragments (4 µm x 4 µm) of Raji cells not exposed (A) and exposed (B-D) to a 50 Hz,1 mT sinusoidal magnetic field for 13 h, 36 h and 64 h, respectively. See text and Table 2. From Lisi A. et Al (ref. 82), partially modified.

dal fields of 50/60 Hz, the study of "cell cycle progression" shows no effect at very low flux (0.2 mT×69 h), but reveals a heightened effect at a flux of 5 mT×48 h.

In simple terms, it would seem possible to pick out growing mT exposure levels responsible for a *null effect*, a *stimulatory effect* or an *inhibitory effect*. In reality, however, the situation is far more complex in this sector. ELF magnetic fields can either inhibit or stimulate lymphocyte activity as a function not only of the exposure data [107,108], but also of the biological conditions of the cells exposed, *mitogen-activated cells* being more responsive than *resting cells* [103-107, 109, 110].

To explain this *ambivalence of the effects* of ELF magnetic fields on the immune system, Marino et Al [111] started from the hypothesis that the biological effects of ELF magnetic fields are governed by non-linear laws, and that *deter-*

ministic responses may therefore occur that are both *real* and *inconsistent*, thereby yielding two conflicting types of results. They tested this interesting hypothesis on laboratory animals by applying a novel statistical procedure that avoids averaging out the contradictory changes recorded in various animals. A particular role in the interaction of ELF fields with lymphocytes seems to be played by the mobilisation of intracellular Ca^{++} from the calciosomes and of extracellular Ca^{++} through the membrane channels [103,104,112,113,114]. The action of ELF fields on lymphoid cells, however, can also be exerted on the functions of the plasma membrane: the duration of the ligand-receptor bond [115], the clustering of membrane proteins [116] the activity of enzymatic macromolecules [117,118], and the active ion pumps (Ca^{++} ATPase and $Na^{+}K^{+}$ ATPase). In this regard, it should be borne in mind that the membrane micro-organelles are complex quaternary protein structures made up of α-helices and β-planes, elements which determine their specificity. Endowed with anisotropy of diamagnetic susceptibility, the α-helices and β-planes are the sites of intense electrical fields [119]. It therefore follows that the cells of the immune system *can carry out their specific functions only if the various membrane organelles remain morphologically and functionally intact*, in such a way as to correctly receive extracellular signals, transduce them and transfer them to the cytoplasm and the nucleus by means of the cytoskeleton, which must, in turn, be intact.

It seems highly likely that the profound morphological alterations that make up the Marinozzi effect (figures 9, 10, 11) impact on membrane organelles (ion channels, receptors, active pumps) *by distorting their quaternary protein structure* and eliciting cascade effects of a chiefly inhibitory, even if not cytocidal, nature. In the future, it will therefore be necessary to carry out dual investigations simultaneously by means of biochemical techniques and the most modern imaging methods (SEM, Atomic Force Microscopy, etc) to *ascertain and analyse the correlations between the functional alterations induced in the cells of the immune system and the appearance of a Marinozzi effect*.

Discussion

As we have seen (section 4), ELF pulsed magnetic fields of a few picotesla (pT) induce demonstrable, and sometimes very rapid, positive effects on a variety of MS symptoms [34-69]. They therefore fall within the third category of treatments considered in section 3, which are aimed at relieving the patient's symptoms. By contrast, our proposal that the therapy of MS should include the use of ELF pulsed magnetic fields of a few millitesla (mT) falls within the second category of treatments, i.e. those aimed at correcting the biology of the disease through processes of immunomodulation and/or immunosuppression.

While the action of ELF fields of a few pT is characterised by an *improvement in neurotransmission*, the use of ELF fields of a few mT aims to exert an action of *local immunomodulation* on the cells of the MS plaque through the induction of the Marinozzi effect (figures 9, 10, 11). It therefore follows that the targets of ELF fields in the mT range will be the plaque cells (B- and T-lymphocytes, macrophagic monocytes, microglia cells and dendritic cells), those cells disseminated in the seemingly normal nervous tissue (the peripheral "metastasis") [1,7] mentioned in section 2 (macrophages and microglia cells), and also the B-cell follicles found in the meninges of the cerebral sulci [17].

More specifically, the target will be the plasma membrane of these cells, which is almost always carpeted with microvilli and protrusions of various types (filopodia, lamellipodia, pseudopodia, blebs) (figures 2, 3, 4, 5, 6). Since the plasma membrane is central to the relationships among cells of the immune system (section 2), and since it has been seen to be the elective target of ELF fields of a few mT [81-91], the rationale of our working hypothesis is based on the *induction of the Marinozzi effect in the plaque cells in order to slow down their activity, thus obtaining an effect of local immunomodulation* (on the brain) *or locoregional immunomodulation* (on the entire CNS).

The addition of ELF magnetic fields of a few mT would therefore provide an "electromagnetic immunomodulatory *boost*" (a term borrowed from radiotherapy), which would tend to potentiate the general action of drugs on the individual plaques without worsening the side-effects, which may sometimes be severe. Our hope that this may be possible is also based on some observations by Sandyk [60,65]. As mentioned in section 4, this author found that, in two MS patients treated periodically for some years with magnetic fields of 7.5 pT at 5 Hz, *progression of the disease was arrested*. This finding prompted the hypothesis that, in addition to effects on axonal and synaptic neurotransmission, effects could also be exerted on the mechanisms responsible for demyelination. Moreover, we cannot rule out that ELF fields of a few mT, i.e. with a magnetic flux 10^9 times higher than that of the fields used by Sandyk, may arrest disease progression even more rapidly by inducing a Marinozzi effect in the plaque cells,.

In 1998, Richards et Al [120] also expressed the hope that electromagnetic fields might find application in the therapy of MS, both to manage symptoms and to achieve long-term effects by eliciting beneficial changes in the immune system and in nerve regeneration. Before such objectives can be practically and efficaciously reached, however, several important questions remain to be answered.

Can the Marinozzi Effect Occur in Vivo?

The first worrisome question is whether the Marinozzi effect can occur not only on cells in vitro, but also in vivo, when the potential

target cells (lymphocytes, macrophages, microglia, dendritic cells) are situated in the nervous tissue, and therefore in physical conditions and spatial relationships that are very different from those of cell cultures. A potentially fruitful approach to this question could involve ascertaining the induction of the Marinozzi effect in, for example, experimental autoimmune encephalopathy in the rat, an area of research recommended by Adams et Al [19]. Should such an experimental approach be unavailable, an alternative might be to apply a magnetic field to lymphoid Raji cells immersed in a brain-equivalent phantom like that used by Olson et Al [121] in their electroacoustic research.

Can the Marinozzi Effect Occur on Microglia Cells and Dendritic Cells?

The Marinozzi effect has been demonstrated in vitro on lymphoid cells [81,82,87,88] and other types of cells [84,86,89,90,91], but not on microglia cells or dendritic cells. The latter two cell types are carpeted with long, thin protrusions (figures 5, 6), which should respond to a magnetic field in the same way as microvilli. Indeed, in the studies conducted by Teodori et Al [91] on human glioblastoma cells, which are carpeted with long villi, exposure to a static magnetic field led to the disappearance of the villi. It is important to clarify this issue experimentally, in that "eliminating microglia-mediated inflammation is regarded as an important strategy in the therapy of neurodegenerative diseases" [18]. Moreover, with regard to dendritic cells, which are also present in the MS plaque, "therapeutics directed at dendritic cells could potentially be engineered for the treatment of MS" [20].

What Are the Most Likely Functional Consequences of the Marinozzi Effect?

In view of its profound membrane changes, the Marinozzi effect surely modifies the *ion channels* and *receptor proteins* by distorting the complex quaternary structure that determines their functional specificity. According to Rosen [122], most of the effects elicited by moderate static magnetic fields can be explained in terms of the deformation of the ion channels contained in the phospholipid bilayer. This bilayer is made up of single phospholipids, which are assembled in an orderly manner. Owing to the anisotropy of diamagnetic susceptibility of these phospholipids, which is enormously potentiated by the physical phenomenon of *cooperativity* [123,124], the bilayer is thought to transfer the deformation induced by the magnetic field to the ion channels, thereby modifying their activation kinetics. This has been demonstrated with regard to the calcium and sodium channels, though it is acknowledged that some channels are more susceptible than others to membrane deformation.

The action of static magnetic fields (27-37 μT) and time-varying ELF fields of 7-72 Hz (13-114 μT) on the ion channels was directly demonstrated by Baureus-Koch et Al [125], who used radioactive 45 Ca^{++} as a tracer in a biological system consisting of highly purified plasma membrane vesicles. All the more so, magneto-induced membrane deformations will impact negatively on mechanical-gated ion channels, thereby altering their electro-dynamics [126]. The *membrane receptors*, proteins free to move in the semi-fluid lipid bilayer, are subject to the action of ELF magnetic fields. Sun et Al [127] found that receptors for epidermal growth factor (EGF) and for tumour necrosis factor (TNF) exposed to an ELF field of 50 Hz at 0.4 mT underwent *clustering* within five minutes. However, when a static magnetic field was superimposed on the ELF field, clustering no longer occurred. This is an elegant demonstration of the sensitivity of these membrane micro-organelles to the different forms of magnetic field. Finally, it is plausible that the Marinozzi effect can slow down or inhibit the *migration of lymphocytes*, thus reducing their concentration at the inflammation site. Moreover, profound membrane alterations could hinder *macrophagic activity, antigen presentation* and *intercellular recognition*. This latter effect was demonstrated by Dini et Al (90) in the interaction between apoptotic lymphocytes that had been exposed to a static magnetic field and liver macrophages.

Is the Marinozzi Effect Reversible and, If So, at What Relaxation Rate?

The Marinozzi effect does not seem to influence the vitality of the cells it affects [81,82,86]. Moreover, its occurrence stems from a more or less intense and prolonged magneto-mechanical action on the F-actin filaments, which are diamagnetically anisotropic. It therefore seems very likely that the effect is transitory and reversible. The reversibility of the Marinozzi effect is indirectly supported by some magneto-biology studies. In 1988, Akimova and Navikova [128] found that a single four-hour exposure of rat and rabbit neocortex to a weak ELF field (500 μT and 3.12 Hz) caused ultrastructural changes only in the glial cells. However, five four-hour exposures gave rise to changes in both glial and neuronal cells. This study highlighted the differences in magneto-sensitivity between glial and neuronal cells as a function of the time factor. In a 2006 study, Salerno et Al [129] exposed T-cells for two hours to a static magnetic field of 0.5 T and of 0.5 mT generated by an MR unit, and then activated them by means of an appropriate mitogen. A general decrease in many of their specific activities (production of γ-interferon, cell proliferation, CD25 expression, concentration of free cytosolic Ca^{++}) was observed. While these effects were still statistically significant 24 hours after exposure, they were no longer so after a prolonged culture time - a clear sign of the *transitory nature* of the magnetic bioeffects induced in T-cells. From these experimental observations it emerges that the *relaxation rate* of the Marinozzi effect membrane changes will need to be ascertained and

carefully analysed. Indeed, should these fields be used in the therapy of MS, the relaxation rate would condition the choice of *intervals between treatment sessions*.

What Are the Effects on Oligodendrocytes and Myelin Synthesis?

Another issue that needs to be clarified concerns the effects of pulsed ELF magnetic fields of a few mT on the myelin-forming oligodendrocytes and on their precursors, the OPC stem cells. Indeed, it is thought [10,11] that areas of chronic demyelination develop as a result of the concomitant loss of oligodendrocytes and their precursors. It is therefore very important to ascertain whether ELF fields can act on these cells by stimulating their ability to synthesise myelin. It has already been demonstrated that ELF fields can stimulate the *regeneration of peripheral nerves* [130,131]. These studies used pulsed magnetic fields of 0.9-1.8 mT at 15 Hz for six hours/day, or of 0.3 mT at 2 Hz for four hours/day. The important regenerative effects obtained on variously injured nerves suggest a stimulatory effect on the Schwann cells (the peripheral equivalent of oligodendrocytes). Another interesting line of research can therefore be added to the fourth category of treatments, i.e. those aimed at repairing CNS damage (see section 3).

At this point, an obvious question arises: "How can the same ELF fields stimulate the functioning of oligodendrocytes, while on other cells they induce the Marinozzi effect, which is characterised by catabiotic features?" The answer may lie in the fact that the surface of the oligodendrocytes is smooth, unlike that of the presenting and effector cells, which are subject to the Marinozzi effect; a certain differential action may therefore occur (see figure 1).

On the Dose and Time Factors

Before the proposed clinical applications can be undertaken, the various components of the dose and time factors (frequency in Hz, magnetic flux density in mT, the duration of single sessions, the interval between sessions, total number of sessions, etc) will need to be analysed and discussed. The conditions indicated by Marinozzi et Al [84] as the most efficacious (1h exposure to a pulsed 100 Hz wave at 7 mT) certainly constitute a good starting point. However, they will need to be reconsidered after carrying out the investigations suggested in section 7.1.

In magneto-biology, we can identify different values of magnetic flux which produce a *null effect, stimulatory effects or inhibitory effects*. Thun-Battersby et Al [132] analysed the effects on B-lymphocytes, T-lymphocytes, NK cells, macrophages and granulocytes in *in vivo* experiments on Sprague-Dawley rats continuously exposed to 50 Hz ELF fields at 0.1 mT for periods ranging from three days to 13 weeks. They failed to demonstrate a significant effect of either short or prolonged exposure on the various end-points, including cell proliferation and apoptosis. By contrast, Vasiliev et Al [133], in their *in vivo* experiments on guinea pigs exposed to a 50 Hz ELF field at 20 mT for six hours, documented a fall in antibody production. Moreover, Podkolzin and Donzov [134], who experimented in vivo on mice previously immunised with rat erythrocytes and exposed for only four minutes to 10-50 Hz ELF fields at *20-100 mT*, observed not only a depression of antibody secretion but also its frequency-dependence, with a very narrow resonance peak (0.1 Hz) around the 21.1 Hz frequency.

Between these two extremes – a complete lack of immunological effect with fields of 0.1 mT [132] and depression of antibody production with fields of 20-100 mT [134] – a study by Frahm et Al [135] demonstrated the *in vitro* stimulation of mouse macrophages with 50 Hz ELF fields at *1 mT*.

All of these experimental observations indicate that the magnetic flux density (0.1 mT, 10 mT, 100 mT) has a certain importance. Consequently, when choosing "doses" aimed at producing a sure Marinozzi effect, together with some immunosuppressive effects, the values adopted should be closer to 10 mT than to 1 mT. Obviously, however, such choices will be guided by the results of the preliminary, specific in vivo studies suggested in section 7.1.

The *dose* factor cannot be separated from the *time* factor, the importance of which has also been demonstrated in magneto-biology. Toroptsev et Al [136], for instance, showed that exposing guinea pigs to a magnetic field of 50 Hz and 20 mT for 6.5 h caused severe lesions (haemorrhage, lung emphysema and tumefaction of the testicles). By contrast, studies by Gilinskaja and Zobina [137] in humans demonstrated that a low-frequency magnetic field at 20 mT for ten minutes on alternate days was able to speed up the repair of various lesions, reduce blood pressure and stop haemorrhages. Moreover, a paper by Mix et Al [138] reported that a single treatment with pulsed magnetic fields led to an increase in granulocyte phagocytosis in 20 patients, whereas 20 consecutive treatments led to a reduction in phagocytosis, without modifying the number of phagocytosing cells.

Some Technical Notes

From the technical standpoint, bearing in mind the concept of "electromagnetic immunomodulatory *boost*", pulsed ELF fields of 100 Hz and 7 mT (according to the original technique of Marinozzi et Al) [84] should be generated either by a small coil, to treat the brain alone, or by a large and sufficiently long coil to include the whole CNS. With a small coil, the Marinozzi effect will involve only the target cells inside the brain tissue, whereas with a larger and longer coil able to include the trunk down to L1-L2, the action will also be extended to the spinal cord. In both cases, a local (brain) or locoregional (brain and spinal cord) immunomodulatory effect will be exerted. However, all the cells of the immune system that

are carpeted with microvilli and distributed throughout the various organs and tissues (lymphocytes, macrophages dendritic cells) would be exposed to the immunomodulatory effect of the magnetic field. Thus, a general, or at least very extensive, effect might be added to the local effects: this *is not in conflict* with the objective of achieving an electromagnetic immunomodulatory boost with respect to the immunomodulatory action of drug therapy.

Conclusions

Sandyk amply demonstrated the efficacy of pulsed ELF magnetic fields of a *few pT* in alleviating the symptoms of multiple sclerosis (section 4) through their action on axonal and synaptic neurotransmission. By contrast, the current proposal aims to use pulsed ELF magnetic fields of a few mT aims to modify the autoimmune pathology of the disease by eliciting profound membrane changes (Marinozzi effect) in the MS plaque cells.

To achieve this objective, much more experimental work will need to be done. The recommended lines of research detailed in the Discussion section would involve:

- ascertaining that the Marinozzi effect also occurs *in vivo*;
- studying the Marinozzi effect on microglia and dendritic cells;
- ascertaining the duration of the Marinozzi effect and its relaxation rate;
- conducting simultaneous studies by means of cytochemical methods and modern ultramicroscopic imaging techniques to establish which functions are altered in the cells that undergo the Marinozzi effect; as yet, this is known only in part;
- ascertaining any stimulatory effect that ELF magnetic fields may have on oligodendrocytes and their precursors.

As ELF magnetic fields of a few mT do not produce thermal effects, it may be possible to incorporate their use into the therapy of MS, even in the long-term. In this way, they would be used as an adjunct both to immunomodulatory drugs, for which they would provide an electromagnetic *boost* at the local level, and to ELF fields of a few pT, the targets of which are different. The improvement in neurotransmission (Sandyk technique) and the local or locoregional immunomodulatory action that may be achieved through the technique proposed in this paper could act in concert – and not in competition – with drug therapy to improve the outcome of MS patients.

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WEAK, BUT COMPLEX PULSED MAGNETIC FIELDS MAY REDUCE DEPRESSION FOLLOWING TRAUMATIC BRAIN INJURY[1]

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Summary.—Many patients who display psychological depression following a traumatic brain injury do not respond completely to antidepressant drugs. We hypothesized that this type of depression is strongly correlated with subclinical, complex partial seizure-activity within the hippocampal-amygdaloid region that continues for months to years after apparent neurological and behavioral "recovery." Four depressed patients who had sustained traumatic brain injuries and who exhibited mild to moderate brain impairment according to standardized tests received 30 min. of weak (1 μT) burst-firing magnetic fields across the temporal lobes once per week for 5 weeks. There was a significant improvement of depression and reduction of phobias while physical symptoms and other complaints were not changed.

Symptoms attributed to psychological depression are common sequelae to traumatic brain injury (Persinger, 1993b, 1994). Although one traditional response by medical practitioners is to prescribe antidepressant medication, the rationale and the anticipation of successful treatment are derived from clinical trials that involved patients who were depressed in response to psychosocial factors rather than to trauma-induced etiologies. The symptoms of postconcussional and posttraumatic depression may result from neuroelectrical and neurochemical anomalies that differ from the synaptic changes associated with the more typical age-related, sociopsychologically induced depressions.

During the last ten years, the Laurentian Neuroscience Group (e.g., Persinger & Makarec, 1993) as well as R. J. Roberts and associates (Roberts, Gorman, Lee, Hines, Richardson, Riggle, & Varney, 1992) have pursued the hypothesis that a spectrum of complex partial epileptic-like symptoms are frequent consequences of mild to moderate brain trauma. The rationale is derived from the general premise that inhibitory interneurons are extremely vulnerable to the consequences of mechanical energy propagated through the skull into brain space. Typical consequences would include increases in (1) the release of corticotrophin releasing factor, (2) anomalous activity within the hippocampal-amygdaloid complex, and (3) the numbers of disruptions within the neuroelectrical processes that maintain reciprocal inhibition

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between hemispheres. These periods of anomalous, interhemispheric intercalations would be associated subjectively with "sudden depression or panic," an aversive "sense of a presence," or experiences that do not appear to originate from "the self" (Persinger, 1994).

Extreme and protracted paroxysmal but subclinical activity within the hippocampal-amygdaloid formation would be associated with a complete failure of long-term consolidation of memory. The consequences would be exhibited as an interval of posttraumatic amnesia (PTA) for the period following the brain trauma. Intermittent, briefer episodes following resolution of the clinically obvious posttraumatic dysmnesia would contribute to patients' experiences of "memory blanks" and to the reports by the spouses of the patients' anterograde dysmnesia. These dysmnesic episodes may persist for years after the brain trauma and would be exacerbated following behavioral events that release the cascade of corticotrophin releasing factor (an epileptogenic peptide), corticotrophin (from the pituitary), and cortisol.

About one-third of patients with diagnosed complex partial epilepsy also exhibit depression, although the etiology is attributed to other stimuli (Robertson, Trimble, & Townsend, 1987). Recent metabolic measures have shown that many depressed patients exhibit a significant increase in metabolic activity within the amygdaloid-hippocampal region and a decrease in activity within the temporal and (left) prefrontal cortices and traditional antiepileptic compounds such as carbamazepine (Tegretol) and valproic acid (Depakene) have been employed to treat refractory depression (Joffe & Calabrese, 1994). These observations are consistent with the hypothesis that a significant portion of patients who display depression and complex partial epilepsy (with a limbic focus) share similar neuroelectrical or neurochemical processes. Our working model is that the majority of patients who report and display persistent symptoms of depression following a closed head injury and correlative mild to moderate brain impairment also exhibit this type of limbic lability.

Application of magnetic fields that penetrate into the brain has been suggested as a possible method by which the neurons mediating depressive syndromes could be addressed (Zyss, 1994). Researchers reasoning from the principles of classical physics contend that relatively strong (1 Tesla) field strengths are required. For example, George, Wasserman, Williams, Callahan, Ketter, Basser, Hallet, and Post (1995) assumed that, if the left prefrontal cortex is dysfunctional in depression, then repetitive transcranial magnetic stimulation over this area should activate those neurons and improve the symptoms. These authors found that administration of magnetic fields to the left prefrontal cortex each morning for at least five days to six medication-resistant patients with primary mood disorders, significantly decreased symptoms of depression. The mean and standard deviations for the Hamil-

ton Depression scale were 23.8 and 4.2 before the treatment and 17.5 and 8.4 after the treatment (estimated η^2 would be about 70%); however, only two (of the six) patients exhibited clinical improvement.

Other researchers (Jacobson, 1994; Persinger, Richards, & Koren, 1994; Richards, Persinger, & Koren, 1996; Sandyk, 1994a, 1994b, 1995) contended that the *information* content of the applied signal is more critical than the magnitude. From this perspective the functional neural networks mediating neurocognitive processes are more optimally described by models of neuroelectromagnetic resonance or "narrow-band" processes within neural patterns or nets that respond to information (John, 1990). Although the conflict between methodologies that emphasize "meaning" of the stimulus versus the "magnitude" of the stimulus is frequent in science, the positive contributions of one technology to effective treatment do not necessarily imply the negation of the other.

Disruption of the microneuroelectrical processes that slowly escalate into sustained subclinical, complex partial conditions may require minimal energies if the parameters of the applied field employ disruptive properties and they are initiated before the numbers of recruited neurons achieve a critical mass. Recently Bureau and Persinger (1995) reported that the threshold for overt limbic seizures was elevated if the rats were exposed for approximately 1 ksec. to a 50 μT (microTesla) pulsed field once every two to three days. When human beings are exposed to similar pulsed, complex fields but at lower intensities (1 μT), they report most of the experiences (Persinger, 1993a; Ruttan, Persinger, & Koren, 1990) associated with neurosurgical stimulation (Bancaud, Brunet-Bourgin, Chauvel, & Halgren, 1994). Four weekly, 30-min. exposures to these field parameters did not produce adverse effects in normal volunteers (Gillis & Persinger, 1993).

The purpose of the present study was to test the feasibility of externally applying weak, complex magnetic fields through the brain to help reduce psychological depression in patients who still exhibit mild to moderate brain impairment after a neurologically verified, acquired brain injury. We reasoned that, like aspirin which does not influence core body temperature if a person is euthermic but clearly elicits an antipyretic effect if the person displays a fever, the neuroprocesses of this type of postconcussional depression would be particularly sensitive to weak, complex applied magnetic fields. If the effects of these weak, complex electromagnetic fields were considered to have any potential clinical utility, it should be evident with only four subjects who are exposed once per week for only five weeks. If a significant effect was not obvious during this period, then the feasibility of ultimately supplementing or replacing pharmacological treatments with this technology would be minimal.

Two male (ages 34 and 37 years) and two female (ages 40 and 45 years)

patients who had completed neuropsychological assessments were referred to the first author. All of these patients had sustained a closed head injury (2 within 12 months, 1 within 3 years, and 1 about 6 years before treatment began) and displayed mild to moderate brain impairment as defined by the Halstead-Reitan Index, toe graphaesthesia (Persinger & Richards, 1995), and a standardized aggregate of 31 subtests (Persinger, 1995). They also showed impaired dichotic word-listening performance and abnormally elevated scores for a scale from which one may infer complex partial epileptic-like signs (Roberts, *et al.*, 1992). All four patients had reported persistent or frequently intermittent (often with sudden onset) depression, as diagnosed by their physicians, that had not responded to any traditional antidepressant medications.

Because the limits of all conventional medical and psychological interventions had been approached, the patients were considered eligible as candidates for the experimental treatment. Each patient volunteered for the treatment after the potential risks and benefits had been described fully. The consent form indicated that there was some evidence that (1) the application of these fields has been associated with pleasantness (Persinger, *et al.*, 1994; Richards, *et al.*, 1993), (2) there was no clear scientific evidence that brief exposures to these fields had any long-term adverse effects, (3) they could withdraw from the experiment at any time, (4) they could call the second experimenter at any time if questions or symptoms emerged, and (5) the treatment would be given without cost to the patient.

Once per week for five successive weeks each patient met the experimenter within the same room. After a brief social exchange, the patient completed the Beck Depression Inventory (Beck & Steer, 1987), the Symptom Check List-90 (Derogatis, 1994), and the Wahler Physical Symptoms Checklist (Wahler, 1973). The rationale for completion of the inventories at the beginning of each session was to ascertain the persistence of any effect between treatments rather than after treatments. The patient then sat in a comfortable chair within an acoustic chamber. As described elsewhere (Richards, *et al.*, 1993), a complex, burst-firing magnetic field (1 microT) was applied along and through the temporoparietal regions through pairs of four solenoids embedded in small, 10-cm by 7-cm containers attached over the scalp by adhesive strips. The field was delivered once every 4 sec. by a computer. At the end of the 30-min. session, the experimenter discussed any concerns the patient may have had during the previous week and restructured or offered suggestions for any emerging problems. The entire interaction required about one hour.

Although placebo or sham-field conditions would be optimal from an experimental perspective, the concern for the patients (as indicated by their standardized scores on the measures of depression and anxiety) dictated that

all patients receive the treatment and be (indirectly) monitored by both experimenters. We assumed that the problem of placebo effects could be partially accommodated by requiring an extremely large effect size that would be evident with only four patients. An effect size of at least 50% (equivalent to an $r > .70$) of the variance in change of symptoms over a 5-wk. period should exceed the size of an effect from placebos, particularly if the change in scores were stable. Although some researchers would prefer random allocation to sham-field conditions, we concluded that treating all of the subjects and then assessing the strength of the treatment upon scores for depression would satisfy both experimental and humanist considerations.

All analyses involved SPSS on a VAX 4000 computer. A two-way analysis of variance with one level repeated (the five sessions) and one not repeated (sex) were completed for the mean total scores on the Beck Depression Inventory, the Physical Symptoms scores, and for scores on each of the nine scales (compulsion, phobia, hostility, depression, anxiety, paranoia, interpersonal sensitivity, psychasthenia, and psychoticism) from the Symptom Check List-90. There were no significant differences between sexes ($F_{1,2} < 1.00$, $p > .05$) or interactions between sex and sessions.

There were only two significant changes over time (all dfs = 4,8). There was a significant decrease in Beck Depression scores ($F = 4.22$, $p < .04$; partial $\eta^2 = 67\%$) over the five weeks. The means and standard deviations for the Beck Depression scores were 33 ($SD = 9$), 27 ($SD = 7$), 20 ($SD = 10$), 21 ($SD = 8$), and 17 ($SD = 9$), respectively. *Post hoc* paired t tests indicated that the effect was due to the attenuated depression between the first two sessions and the last three sessions. There was also a decrease in the magnitude of phobias ($F = 6.43$, $p < .01$; $\eta^2 = 76\%$). The means and standard deviations were 72 ($SD = 11$), 66 ($SD = 12$), 64 ($SD = 15$), 59 ($SD = 17$), and 56 ($SD = 16$) over the five weeks. There were neither main effects for gender nor interactions between gender and the within-subject measures for these variables. None of the other measures exhibited a significant decrease over time.

The results of this study indicate that four successively assigned patients who displayed persistent depression and complex partial epileptic-like indicators after a closed head injury (mild to moderate brain impairment) and who were refractory to (antidepressant) medication responded positively to a procedure that involved the brief 30-min., weekly application of burst-firing magnetic fields over the temporal lobes. The specificity of the effect is suggested by the change primarily for scores on the Beck Depression Inventory and the scale from which one may infer phobias (frequent symptoms, particularly after motor vehicle incidents). Although the mean scores for the depression scale of the Symptom Check List-90 did not achieve statistical significance, the attenuation of these scores over time approached this criterion

($F = 3.01$, $.10 > p > .05$; $\eta^2 = 60\%$) and also showed a mean decrease of 1.5 standard deviations (change in T score of 15 standardized units).

There are several limitations to this study. In addition to the absence of sham-field controls, we did not assess the continuity of the effect one week and ten weeks after the treatment had been completed. The role of the experimenter and the effects of the posttreatment discussion concerning personal issues during the previous week cannot be quantitatively removed from the effect. [One patient sent an unsolicited letter which stated an appreciation for the treatment, a reduction of the depression and thanks to the first author.] However, both quantitative (unpublished data, $n = 11$ patients) and qualitative evaluations of this population of patients have indicated that this magnitude of improvement does not normally occur within five weeks, even with casual contact about once per week from health professionals. An ABAB design, although experimentally elegant, was not considered ethically appropriate because (1) repeated stimulations were considered to be critical to the elicitation of the effect and (2) variability in the continuity of the attenuation of the depressive, aversive symptoms was considered counterproductive to the maintenance of the more adaptive behaviors emerging during the treatment.

Despite these limitations, the employment of weak, complex fields which may interfere with the limbic electrical activity that generates depression could be a supplementary procedure to traditional techniques. Electroconvulsive shock (ECS) has been considered an effective treatment for severe depressions that are refractory to pharmacological intervention. With this technique, current densities from highly redundant (symmetrical) wave forms are applied to one or both temporal lobes to induce paroxysmal discharges. The neuromechanism for the antidepressive consequences of massive surges of current across the temporal lobes (similar intensities of current applied across the brain stem would be fatal) is not clear (George, *et al.*, 1995). If the functional consequence of this massive current induction is to inhibit the disinhibited limbic structures, then the efficacy of this process for some patients who exhibit depression could be rationalized.

We suspect that the large electric currents associated with electroconvulsive shock are effective indirectly because they may damage the nonmyelinated varicosities of fibers from the locus ceruleus (noradrenaline), the ventral tegmentum, including the substantia nigra compacta (dopamine), and the median raphe (serotonin). When the cell bodies of these aggregates respond (to the insult) by reactive synaptogenesis and reconstruction of their processes, there is an epiphenomenal increase in the synthesis of neurotransmitters, unocclusion of the receptors inactivated by the depression process, and reactive protrusions of the axonal varicosities. Such changes would attenuate the interference with postsynaptic sequestering of these transmitters

whose behavioral manifestation had been psychological depression. The mood would improve.

There may be other less invasive technologies that can be as effective as electroconvulsive shock without its side effects or risks. There is now converging theoretical (Jacobson, 1994) and empirical evidence (Richards, *et al.*, 1993, 1996) that very weak, applied complex magnetic fields whose wave structure and pulse patterns are congruent with groups of neurons within specific regions of brain volume can improve neurocognitive function (e.g., Sandyk, 1994a, 1994b, 1995) in human beings. There may also be effects that facilitate the therapeutic process. For example, one consequence of application of complex fields to the right hemisphere of normal volunteers is the enhancement of poststimulation suggestibility (Tiller & Persinger, 1994). The magnitude of this effect would be sufficient potentially to augment the effects of brief, posttreatment cognitive restructuring upon patients' subsequent behaviors by increasing the reward value of the more adaptable attributions, perceptions, and solutions to personal problems.

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Specific Patterns of Weak (1 microTesla) Transcerebral Complex Magnetic Fields Differentially Affect Depression, Fatigue, and Confusion in Normal Volunteers

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Normal young adults were exposed for 20 min once per week for a total of 3 sessions to 1 of 7 configurations of weak (1 microTesla) magnetic fields or to a sham field. The fields were spatially rotated and applied through the brain at the level of the temporoparietal lobes. The Profile of Mood States was taken before and after each session. Before, during, and after the treatments, heart rate, plethysmographic activity, and skin conductance were measured by computer. The results indicated that the burst-firing pattern previously demonstrated to be effective for clinical depression, improved mood and vigour compared to the sham-field or other treatments. Subjects who were exposed to a burst-firing pattern, a complex-sequenced pattern, and a pattern whose electrical equivalents stimulate long-term potential in hippocampus slices also exhibited less psychometric fatigue after the sessions compared to subjects who received the sham field or random-sequenced fields. These results replicate previous studies and indicate that rationally designed complex patterns of magnetic fields may simulate pharmacological treatments.

Keywords Moods; Magnetic fields; Sensitive populations; Patterns.

Introduction

The essential principle of modern neuroscience is that all experiences are generated by the dynamic matrix of electromagnetic and chemical patterns that exist within the brain at any given time. Pharmacological agents are effective because they can be sequestered by a variety of different classes or subclasses of receptors within the cerebral volume and consequently modify neuropatterns by influencing the movement of ions through cell membranes or the activation of cytoplasmic proteins that modify these movements. Martin et al. (2004) proposed that the appropriate spatial complexity and temporal structure of weak magnetic fields applied through the brain can both simulate and augment the effects of pharmaceutical agents.

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Weak, complex magnetic fields generated by geomagnetic activity or by active weather systems (Persinger, 1980) have been reported to affect autonomic function and to influence mood (Persinger, 1975, 1987) and their analogs in non human animals (Galic and Persinger, 2004). Ehrmann et al. (1976) reported that nociceptive experiences could be attenuated and affect or pleasantness could be improved during brief exposures to symmetrical pulsed magnetic fields whose frequencies varied randomly between 1 and 20 Hz. As personal computers and their software were developed, the possibility of generating an unlimited number of complex patterns that might simulate the myriads of pharmacological activities was realized.

The application of a weak (1 microTesla) burst-firing magnetic field, whose temporal structure had been extracted from the pattern of amygdaloid neurons from an epileptic's brain, has been inferred to reduce pain in rats (Fleming et al., 1994). Some patients who had experienced chronic pain subsequent to a closed head injury (Baker and Price, 2003) reported a permanent attenuation of this experience following two or three weekly transcerebral exposures to this pattern. Maintained elevations in pain thresholds were also observed in rats with seizure-induced damage following 30 min, weekly exposures to these fields (Martin and Persinger, 2005).

A similar magnetic field pattern improved depression (Baker-Price and Persinger, 1997, 2003) in patients who had sustained closed head injuries. The temporal schedule of the treatment employed by Baker-Price and Persinger (1997) was derived from the clinical tradition of weekly visits rather than empirical results. Recently Tsang et al. (2002) exposed normal volunteers for three sessions either once per week or for three successive days and also found that weekly sessions produced significant decreases in psychometric depression.

The present study was designed to answer specific questions that would be relevant before substantial resources were committed to labor-intensive and costly clinical trials. There were three primary questions. First, are there other rationally designed patterns of magnetic fields that can positively affective mood in "normal" volunteers? Second, are the changes in psychometric indices of depression also associated with appropriate alterations in physiological variables? Third, are there any conspicuous adverse effects associated with applications of fields whose temporal structure approaches the 20 ms interval ostensibly associated with the re-entrant processes (Edelman, 1990; Llinas and Persinger, 1991) producing human consciousness?

To answer these questions, we measured heart rate, galvanic skin response, and plethysmographic activity before, during, and after each treatment session once a week for three weeks. This protocol was employed rather than daily treatment because both human (Tsang et al., 2002) and rodent research (Martin et al., 2003) showed weekly treatments may be more effective than repeated daily treatments. Before and after each session the subjects were administered the Profile of Mood State (POMS). It contains measures by which psychologists infer confusion, fatigue, vigor, anger, depression, and tension. During each session the subjects were exposed consistently to one of seven different patterns or to a sham-field condition. Our assumption was that if the effects from any of these fields were sufficiently powerful to be of any clinical utility, differences should be evident with only four subjects per group. Such differences at $p < .05$ would be equivalent to effect sizes of about 50%, which is sufficient for any potential clinical utility.

Method

Subjects

A total of 16 men and 16 women, who were enrolled in university courses, volunteered as subjects. Their ages ranged between 20 and 25 years. Reinforcement for participation involved either bonus marks for university courses or a small monetary reward after they completed the experiments.

Procedure

Each subject was tested singly. The subjects signed a consent form that indicated they may or may not be exposed to weak complex magnetic fields and they would be required to return for three sessions. They were then assigned randomly to one of the eight conditions. This condition was applied once per week in the same setting between 12 and 19 h local time during the summer of 2001.

The Profile of Mood States was administered before each session. The subject was then blind folded and fit with earmuffs that reduced the background sound level to less than 30 db. A pair of modified headphones for which the muffs has been removed and replaced with square plastic containers was placed over the temporal regions (just above the ears). Each box contained four sets of solenoids similar to the ones employed by Baker-Price and Persinger (1996). One solenoid on the left and right side of the brain were functionally connected so there were four sets of solenoids. Consequently, the magnetic field was generated through the brain at the level of the temporoparietal lobes.

While the subject sat quietly for 30 min, the following measurements were recorded automatically by computer: heart rate, plethysmographic activity, and galvanic skin response. Without the subject's knowledge for the specific timing, the session was divided into five components: 5 min of baseline (to allow habituation to the sensory deprivation), 20 min of field treatment, and 5 min of post-field baseline. After the goggles and ear piece were removed the Profile of Mood States was readministered.

The magnetic fields were generated by a 286 computer by transforming columns of numbers between 0 and 255 to the equivalent voltages where any value below 127 was negative polarity and any value above 127 was positive polarity. The shape of the pattern was determined by the value between 0 and 255 for each line of code and the numbers of lines. The real-time presentation of the pattern was affected by the point duration defined as the time in ms each point was presented, and the interstimulus duration defined as the time between the presentation of each pattern.

The values were delivered to a custom made d-to-a (digital to analog) converter which transformed the voltage into electric current within each pair of solenoids. They generated the magnetic fields. More detailed descriptions of this equipment have been published elsewhere (Persinger et al., 2000; Richards et al., 1993). The speed of rotation of the delivery of the patterns to each of the four pairs of solenoids was the third parameter. In all but one of the patterns the rotation was 0.5 Hz which means that a given pair of solenoids received the signals from the computer for 0.5 s (2 s for complete cycle).

Seven different patterns of fields and a sham condition were employed for comparison. The parameters of the fields are shown in Table 1. The specific characteristics for the burst firing pattern were selected because of their demonstrated

Table 1
The interstimulus interval (in ms), pixel duration, and frequency of rotation of the fields through the solenoids (Hz) for the different treatments

Pattern	Interstimulus interval	Pixel duration	Spatial frequency
1. Burst-firing	3000	3	0.5
2. Burst-firing (10 min)	3000	3	0.5
Long-Term Potentiation (10 min)	3000	3	0.5
3. Long-Term Potentiation	4000	1	0.5
4. Long-Term Potentiation	20	1	0.5
5. Complex sequence	3000	3	0.05
6. Random	1	3	0.5
7. Random	3000	3	0.5

efficacy for reducing thermal and electric current-induced pain in rodents (Fleming et al., 1994; Martin and Persinger, 2005) and depression in clinical patients (Baker-Price and Persinger, 1997, 2003). To test if a pattern that imitates the effects of long-term potentiation (LTP) when applied directly as electrical currents into hippocampal slices (Rose et al., 1998) could facilitate the effects of burst firing, a tandem sequence of 10 min of burst firing was followed by 10 min of LTP stimulation.

This long-term potentiation pattern, which was one pulse followed 170 ms later by four quick pulses, a natural pattern of hippocampal neurons, was the basis for the third and fourth treatments. This "signal," composed of 1 ms point durations for each point (value between 127 and 255) was presented once every 4,000 ms, was selected because of its impact upon the types of memories strongly coupled to hippocampal activity in humans (Richards et al., 1996) and rats (McKay et al., 2000). The interstimulus duration for the fourth treatment was to test if a 20 ms interlude applied transcerebrally across the hemispheres would be as effective as when the onset of a similarly patterned magnetic field was accelerated in 20 ms increments but applied sequentially around the skull (Persinger et al., 2002).

The complex sequenced field was composed of 50 different patterns. These various patterns, all of which have been shown to be physiologically effective, were separated by the LTP pattern. Each of the 50 patterns was presented for 750 ms with point durations of 3 ms such that the total time to complete one sequence was 30 s. This was followed by a period of 3 s with no field. The specific shapes that composed the complex-sequenced pattern have been published previously (Persinger et al., 2001) and were constructed to affect gene expression in the developing rat. In our clinical practice, we found this pattern produced remarkable improvement, after only two sessions, of mood in three patients who had sustained serious head injuries and who had been experiencing chronic depression and inability to work for years. Because of the protracted duration (30 s) of this specific pattern the rotation of the presentation of the signals to each solenoid was slowed to 5 s (one complete rotation every 20 s).

Finally, to discern the importance of specific temporal structures vs random variations, a single random sequence was repeated continuously or once every 3,000 ms. The sequence had been extracted from a random number generator. On the

basis of these different combinations we reasoned that we might more effectively discern if length, specific pattern, interstimulus interval, or point duration dominated any particular effects.

For each subject and each session, the mean values for heart rate, skin conductance (galvanic skin response) between the index and third finger of the left hand, and peripheral blood flow through the left thumb (plethysmograph) were obtained for 2 min intervals during the baseline, during the first and last 3 min of the treatment interval, and during the first and last 2 min intervals of the post treatment period.

Skin conductance was measured by two pieces of stainless steel metal (Lafayette standard GSR sensors). Changes in galvanic skin conductance, measured in nano-Siemens, were amplified and filtered through a custom-constructed device. The resulting digital displays clearly discriminated between startle vs relaxation. The plethysmographic device employed the standard sensor (photocell and light source) from Lafayette Instruments. Changes in light intensity due to arterial pulses of the thumb were filtered and amplified before the information was delivered to the Pentium-level computer. The measurements reflected the change in blood volume within the thumb.

A heart rate monitor (LifeGear), often employed for monitoring athlete's performance, measured heart rate. It consisted of a radio transmitter worn around the chest and secured by an elastic strip. The transmitter weighed 4 oz and was powered by a 3 V lithium battery. The radio signals were converted into digital pulses that were accurately validated as beats per min. The software within the 486 computer that collected the data recorded 150 samples from each channel sequentially.

To minimize individual differences, ratios for each of the three measures were completed for the average of the two treatment samples divided by the baseline value and the postbaseline measure divided by the baseline value for each of the three sessions. For the psychometric tests, the means for the scores for each scale of the emotions profile were divided by the pre-test measures for these scales for each session. This allowed the discrimination of any acute effect from the treatment immediately following each session. In addition, the pre-test measures for the second and third sessions were divided by the measure for the first pre-test session in order to discern any potential long-term, cumulative effects that were not acutely associated with the treatments. All analyses involved SPSS software on a VAX 4000 computer.

Results

The means and standard deviations during the first pre-treatment period for all subjects, regardless of treatment, for the three physiological measures and the six scales from the Profile in Mood States are shown in Table 2. The latter are presented as T-scores with a standardized average $=$ 50 and the standardized standard deviation $=$ 10. Because the repeated measure analysis for sessions revealed main effects between types of fields as the most powerful and statistically significant effects, only the simplified results of the overall effects (mean of the three sessions) are reported. For clarity, these means were analyzed as one-way analyses of variance.

One-way analysis of variance (all dfs $=$ 7,24) indicated statistically significant differences between the eight treatment groups for relative changes in psychometric scores before and after for all three weekly sessions for depression ($F = 2.05$, $.05 < p < .10$; eta-squared $=$ .50), fatigue ($F = 2.56$, $p < .05$; eta-squared $=$ .52), tension ($F = 2.58$, $p < .05$; eta-squared $=$ .53) and confusion ($F = 2.50$, $p < .05$; eta-squared $=$ .48).

Table 2
Means and standard deviations for the physiological measures and scales (T-scores) from the Profile of Mood States for the baseline for all subjects

	M	SD
Measure		
Skin Conductance (nanoSieman)	5646	2579
Plethysmograph	583	97
Heart rate (beats per min)	69	7
Mood profile		
Confusion	39.8	6.8
Fatigue	42.9	6.0
Vigor	50.8	8.2
Anger	42.1	6.4
Depression	42.3	6.4
Tension	39.9	5.6

Table 3
The averages of the means and standard errors of the mean for the relative changes in scores for depression, fatigue, and confusion after the session relative to before the session for three weekly sessions for normal subjects whose brains were exposed to different patterns (Table 1) of weak (1 microT) magnetic fields

	Depression		Fatigue		Confusion	
Field pattern	M	SEM	M	SEM	M	SEM
Sham	1.00[a]	.01	1.08[a]	.05	0.93[a]	.03
Random-continuous	0.97[a]	.03	1.02[a]	.03	0.90[a]	.02
Random-3,000 ms	0.92[b]	.02	0.97	.02	0.90[a]	.03
LTP-20 ms	0.95	.02	1.09[a]	.03	1.15[b]	.06
LTP-4,000 ms	0.97[a]	.02	0.91[b]	.05	0.95[a]	.02
Burst-3,000 ms	0.88[b]	.05	0.89[b]	.02	0.84[a]	.03
Burst/LTP-3,000 ms	0.93	.03	0.96	.03	0.89[a]	.03
Complex sequenced	0.95	.03	0.90	.04	0.86[a]	.02

a vs b in a column, $p < .05$.

There were no statistically significant treatment differences for the other components (vigor, anger, tension) for the POMS.

The averages of the means and standard errors of the mean for the relative changes in scores after the session relative to before the session for the three weekly sessions for depression, fatigue and confusion are shown in Table 3. *Post hoc* analyses (Student-Neumann-Keuls, $p < .10$) indicated that most of the explained variance was due to the reduction in depression scores after the treatment for the group that received the burst-firing and random patterns that were presented once every 3 s compared to the sham-field treated group.

Post hoc analyses indicated that the groups who received the burst firing pattern, the LTP pattern presented once every 4 s, or the complex sequence field reported significantly less fatigue than the groups that had been exposed to the LTP pulse once every 20 ms, the sham-field, or the random pattern presented for 20 min. *Post hoc* analysis showed that the group who had been exposed to the LTP pattern with interstimulus intervals of 20 ms also reported greater confusion following the 20 min of stimulation compared to any of the other treatment groups. There were no statistically significant group differences for changes in either heart rate, galvanic skin response, or plethysmographic readings before and after the treatments.

Discussion

Whereas Transcranial Magnetic Stimulation (TMS) involves very intense (Tesla range) simple, pulsed magnetic fields applied topically over the brain, treatment with transcerebral extremely weak, temporally complex magnetic field involves placing the subject's head between the functional poles of pairs of solenoids that generate intensities within the microTesla range. Baker-Price and Persinger (1996, 2003) reported that weekly applications of weak, complex burst-firing magnetic field once every 3-s across the temporal lobes was associated with a marked reduction in psychometric depression as well as a reduction in clinical presentation of depression in patients that had sustained mild closed head injuries without loss of consciousness. The effect size for or the amount of variance of the psychometric measures explained by the treatment was comparable to those produced by TMS that employs fields strengths a million more intense.

Tsang et al. (2002) showed that improvement of psychometric depression was greatest when treatments were given once per week for three weeks relative to once per day for three days. In the present studies, healthy young volunteers were also recruited as subjects. The psychometric dimension associated with depression was significantly more reduced than the other types of moods for subjects who were exposed to the same pattern (burst-firing presented once every 3 s) that had been effective for depressed patients. However, we also found that a random pattern presented once every 3 s also produced an acute reduction in psychometric depression in these normal volunteers.

These results suggest that a class of patterns generated once every 3 sec may be effective. Martin et al. (2003) found that the analgesic effects (for rats) of a burst-firing field presented once every 4 s (Fleming et al., 1994) could be simulated by a ''chaotic'' magnetic field pattern derived from the May algorithm whose structure was different with each presentation. However, a frequency-modulated field presented once every 4 s was not effective. We hypothesize that, like molecular analogs, only specific patterns presented with discrete interstimulus intervals produced specific effects. In the present study the LTP pattern presented once every 4 s was not associated with any significant changes in psychometric depression.

Subjects who were exposed to either the burst-firing pattern presented once every 3 s, the LTP pulse once every 3 s or the complex sequence pattern showed less fatigue from sitting in the experimental setting compared to subjects exposed to the other treatments. These patterns represented the simplest (LTP) to the most complex configurations and indicate that something specific to the information associated with them rather than simple artifacts of intensity might have been responsible for

the effects. It may be relevant that the complex sequenced magnetic field was composed of both the LTP and burst-firing patterns.

The increased scores for a scale by which "confusion" is inferred for subjects who were exposed to the LTP pattern with interstimulus durations of 20 ms may have theoretical value for the study of consciousness. "We had selected this value for the duration between the points' (the numbers between 0 and 255) conversions into a magnetic field…." because we hypothesized a resonance interference with the averaged re-entry process described by Edelman (1989) and observed empirically by Persinger et al. (2003) and replicated by Booth et al. (2005). We reasoned the "interference" would be analogous to "confusion" that occurs when information presented to each ear is slightly delayed. Confusion or "tension" would be a factor congruent with a mild disruption in this process.

Although the effect sizes for the field treatments were within the mild to moderate range and were not as large as the effect sizes Baker-Price and Persinger (1996) observed for patients with clinical depression, the importance of these observations should not be underestimated. First, the standard deviations for the POMs scores for the groups of normal subjects in this study were less than 10 and suggested a restricted range in variability. Groups who display more normal ranges in variability, typical of the general population, or elevated scores, typical of individuals with mood disorders not sufficient to seek psychiatric treatment, may have been more psychometrically responsive to the these treatments.

Secondly, Transcranial Magnetic Stimulation also produced very weak effects upon subjective mood rating scales in normal volunteers (Wasserman and Lisanby, 2001). These pulsed fields are much more intense and appear to depend upon the process of electric current induction sufficient to induce perceptible contraction of scalp muscles. The primary direction of the mood changes following TMS have been mildly positive or anxiolytic. Like the antipyretic agent acetyl salicylic acid that lowers body temperature in people with fevers but does not lower temperature in euthermic people, the potency of both TMS and our procedure may require the application to the appropriate population of patients with depression or chronic pain.

Contributions of secondary reinforcement or conditioning might also occur. Stevens (2001) reported that the presentation of 50 microTesla 20 Hz magnetic fields, about 50 times the strength employed in this study, increased the value of the affective rating of concurrently viewed images compared to images viewed during sham-field presentations. If this effect is generalizable to our field configurations than the setting, context, or personnel present during the pleasantness associated with the field might become secondary reinforcers. Repeated presentation of the subject or patient to these settings, even without the field, might be associated with positive experiences. They are considered important contributions for the patient's compliance with treatment.

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ORIGINAL ARTICLE

Alterations of human electroencephalographic activity caused by multiple extremely low frequency magnetic field exposures

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Abstract In the past, many studies have claimed that extremely low frequency (ELF) magnetic field (MF) exposures could alter the human electroencephalographic (EEG) activity. This study aims at extending our ELF pilot study to investigate whether MF exposures at ELF in series from 50, 16.66, 13, 10, 8.33 to 4 Hz could alter relative power within the corresponding EEG bands. 33 human subjects were tested under a double-blind and counter-balanced conditions. The multiple repeated three-way analysis of variance (ANOVA) mixed design (within and between-subject) analysis was employed followed by post-hoc *t*-tests and Bonferroni alpha-correction. The results from this study have shown that narrow alpha1 (7.5–9.5 Hz) and alpha2 (9–11 Hz) bands, associated with 8.33 and 10 Hz MF exposures, were significantly ($p < 0.0005$) lower than control over the temporal and parietal regions within the 10–16 min of first MF exposure session and the MF exposures were significantly higher than control of the second session MF exposure (60–65 min from the commencement of testing). Also, it was found that the beta1 (12–14 Hz) band exhibited a significant increase from before to after 13-Hz first MF exposure session at frontal region. The final outcome of our result has shown that it is possible to alter the human EEG activity of alpha and beta bands when exposed to MF at frequencies corresponding to those same bands, depending on the order and period of MF conditions. This type of EEG synchronisation of driving alpha and beta EEG by alpha and beta sinusoidal MF stimulation, demonstrated in this study, could possibly be applied as therapeutic treatment(s) of particular neurophysiological abnormalities such as sleep and psychiatric disorders.

Keywords EEG · ELF · Magnetic field · Stimulation · ANOVA

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1 Introduction

In most bioelectromagnetics studies which examined the effects of extremely low frequency (ELF) magnetic fields (MF) upon the human electroencephalographic (EEG) activity, there have been inconsistencies in findings between experiments [4], due to differences in the experimental protocols, electromagnetic field characteristics, pulse shape, spatial characteristics, frequency, period of exposure and organism itself. The ELF research is still surrounded by a fair share of controversy within the scientific and general communities, despite extensive research during the past several decades in this area.

Over the last two decades, several ELF studies have claimed that MF exposure characteristics could alter the human EEG activity, as follows: a sinusoidal 60-min intermittent MF exposure of 45 Hz and 1.26 mT [14]; a 2-s MF exposure of 60 Hz/20–100 μT [2]; 2-s epochs exposed to MF exposure of 10 Hz/40 μT and 1.5 Hz/20 μT [3]; a 2-s light and MF exposures to 1.5 Hz and 10 Hz/80 μT [15]; a 16 Hz/28.3 μT MF exposure [19]; an intermittent 16.7-Hz MF exposure [12]; a 2-s 60 Hz/100 μT MF exposure followed by 5-s control [16]; a 90-min MF exposure 50 Hz/80 μT [11]; and a 8.3 Hz–12.2 Hz/5 μT sinusoidal MF exposure frequencies corresponding to recorded frontal EEG signals [20]. Other studies have investigated the effects of ELF pulsed electromagnetic fields (PEMF) on EEG activity after the applied *Thomas pattern* signal

(0–500 Hz) pulses in 853-ms segments (18 pulses) at various periods of 110, 220, 330 ms at ±200 μT [4, 5, 21]; 'during' an ELF PEMF exposure [6]. The EEG responses to these MF exposures are described and discussed in Results' section of this article.

The authors' pilot studies examined the effects of sinusoidal 8.33 Hz/174 μT ELF MF [7, 8]. These results have led the authors to further investigate the EEG activity alterations due to MF exposures at frequencies associated with EEG bands. For example, authors' pilot study [8] has revealed a marginal significant decrease in MF exposure compared to control, found in the alpha1 EEG band (7.5–9.5 Hz) at the vertex head position, where MF exposure was applied at the alpha1 frequency of 8.33 Hz/174 μT.

This study aims at extending our ELF pilot study to investigate whether MF exposures at ELF in series from 50, 16.66, 13, 10, 8.33 to 4 Hz could alter relative power within its corresponding EEG bands.

2 Materials and methods

2.1 Subjects

The experiments were conducted on 33 healthy subjects (24 male and 9 female) with mean age of 30 years, SD 11 years, range 20–59 years. The RMIT University's ethics committee approved the study, and all the subjects gave written informed consent prior to the experiment.

2.2 Magnetic field exposure system

A pair of standard circular Helmholtz coils have been designed by the authors, having a driving current of 140 mA, total coil impedance 71 Ω, average radius of 65 cm, copper wire of 0.8 mm in diameter and 250 turns each (Fig. 1) [9]. A signal generator effective in producing high quality sine waveforms of high stability/accuracy ELF signals was designed and developed using EXAR XR-2206 monolithic IC together with an audio amplifier with the gain of 10 to deliver sufficient current level to the coils. The magnetic flux density was verified by direct measurement using 'Wandel and Goltermann' EFA-200 EMF Analyser. The linearly polarised field was perpendicular to the Earth's North–South MF at magnetic flux density of 20 μT (rms). The Helmholtz coils were designed and constructed to ensure the matching of source impedance with the coil reactance, exact series inductance and mutual inductance between the coils of the pair. Uniformity levels from the inner to the outer region were 0.01, 0.1 and 1% with respect to the centre value. According to magnetic flux density measurements acquired, the uniformity of the inner level, 0.01%, was 15 cm (x- and y-axis) and the outer level 1% was 40 cm (x-axis) and 50 cm (y-axis) [9]. The measured ambient or geomagnetic field inside the RF-shielded room was approximately 200 nT at the ELF range 6–11 Hz.

2.3 Electroencephalogram

The EEG data acquisition equipment used throughout testing was the Mindset MS-1000 (Nolan Computer Systems Inc., USA) recording system. Neuroscan 19 channel cap (Compumedics Neuroscan Limited, USA) electrodes were used with referential montage of 16 channels. The left brain hemisphere electrodes: Fp1, F7, F3, T7, C3, P7, P3 and O1 were all referenced to M1 (left mastoid), while the right brain hemisphere electrodes: Fp2, F8, F4, T8, C4, P8, P4 and O2 were referenced to right mastoid M2.

2.4 Experimental procedure

During the EEG recording sessions, subjects were asked to lie down between the coils in sagittal plane direction perpendicular to the coil axis and in the supine position. The entire experiment was performed in a darkened, sound

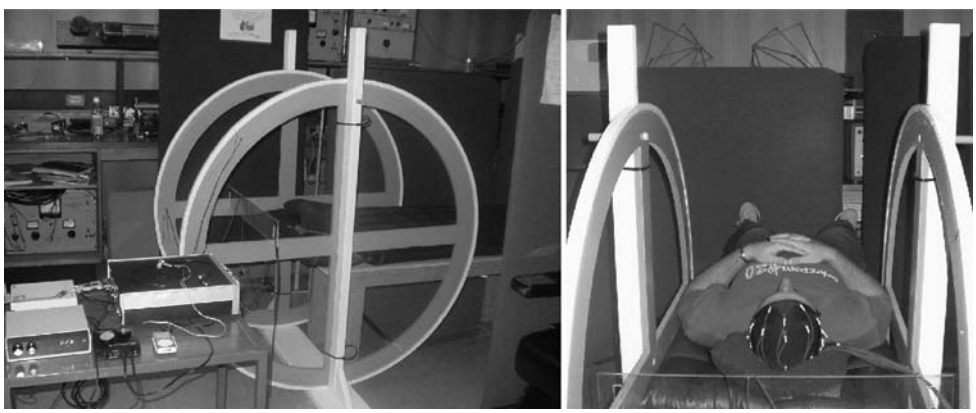

Fig. 1 The design of magnetic field exposure system consisting of Helmholtz coils and a signal generator

proof and RF-shielded room to prevent erroneous recordings due to the standing waves and power line interference.

The baseline EEG was recorded prior to any stimulation for one minute. Each stimulation (50, 16.66, 13, 10, 8.33 and 4 Hz) lasted for 2 min followed by 1 min EEG recording, as shown in Fig. 2. The total duration of an experiment was 19 min. The same procedure was repeated for the 'no MF' or control sessions. The order of control and exposure sessions was determined randomly according to the subject's ID number. Subjects with odd ID numbers were first tested with control condition (no MF exposure) followed by MF exposure after a 30-min break. Double-blind and counter-balanced condition was exercised. This condition was highly considered in the analysis as a factor that might reveal that if the first session was an MF exposure, the EEG activity results during the second MF control session could still be influenced or dependent on the results of the first MF exposure session.

The MF study protocol consisted of:

- first EEG baseline recording, followed by
- first MF exposure/control at 50 Hz; continued by second EEG recording (after 50 Hz);
- second MF exposure/control at 16.66 Hz; third EEG recording of after 16.66 Hz;
- third MF exposure/control at 13 Hz; fourth EEG recording (after 13 Hz);
- fourth MF exposure/control at 10 Hz; fifth EEG recording (after 10 Hz);
- fifth MF exposure/control at 8.33 Hz; sixth EEG recording (after 8.33 Hz);
- sixth MF exposure/control at 4 Hz; and seventh EEG recording (after 4 Hz).

The narrow EEG band intervals were as theta (3–5 Hz), alpha1 (7.5–9.5 Hz), alpha2 (9–11 Hz), beta1 (12–14 Hz), beta2 (15.5–17.5 Hz) and gamma (49–51 Hz). The theta and gamma band data was excluded from this particular analysis. We compared the EEG activity 'before' and 'after' stimulation for each frequency stimulation and band. The traditional EEG band definitions were not considered in this study. However, the EEG bands were custom defined around the stimulation frequency acting similar to a central frequency. For example, the stimulation of 8.33 Hz (close to 8.5 Hz) could investigate only within the theta band of ± 1 Hz, which explains the 7.5–9.5 Hz range. The same procedure is repeated for other stimulation frequencies. It is important to point out that the EEG bands were defined based on the stimulation frequency parameters and not vice versa.

The computed extracted parameters were: the total spectral power of each stimulation EEG data (i.e. before, 50, 16.66, 13, 10, 8.33 and 4 Hz); spectral power in the stimulated band, before/after; central band frequency before/after; and relative difference 'ratio' between the individual band and total spectral power before/after. The relative spectral power estimate of each narrow frequency band corresponds to its own stimulus frequency, as described in Fig. 2. For example:

- gamma EEG band (49–51 Hz) relative power was calculated within the first EEG baseline recording and second EEG recording (after 50 Hz);
- beta2 EEG band (15.5–17.5 Hz) relative power within second EEG recording (after 50 Hz) and third EEG recording of after 16.66 Hz;
- beta1 EEG band (12–14 Hz) relative power within third EEG recording (after 16.66 Hz) and fourth EEG recording of after 13 Hz;

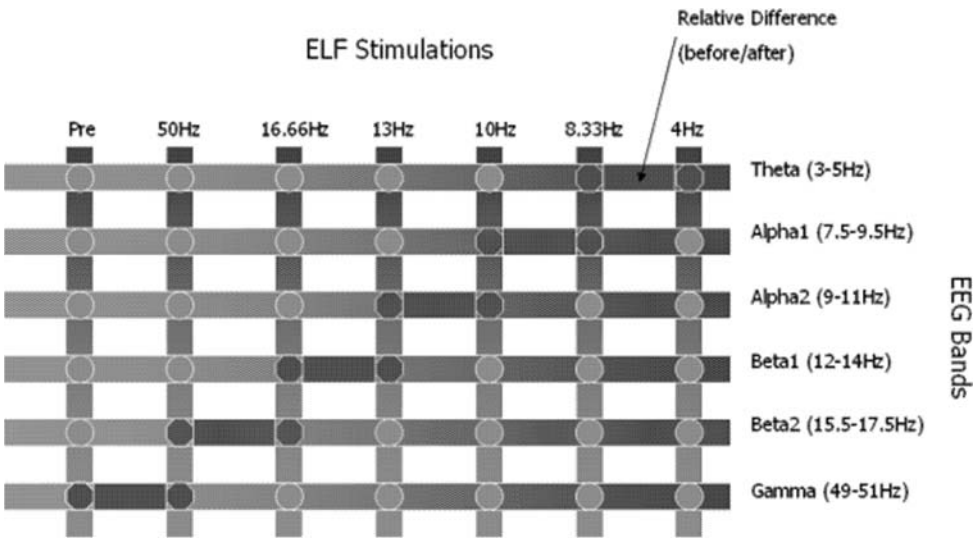

Fig. 2 The design of EEG measurement and MF condition protocol was instrumental in the design of the original data analysis method applied in this study. The analysis method consisted of computing the relative spectral power or the 'power ratio' between the individual band and total spectral band before and after MF condition. The relative spectral power estimate of each frequency band corresponds to its own stimulus frequency. The dark colour circles indicate the computed EEG data

- alpha2 EEG band (9–11 Hz) relative power within fourth EEG recording (after 13 Hz) and fifth EEG recording of after 10 Hz;
- alpha1 EEG band (7.5–9.5 Hz) relative power within fifth EEG recording (after 10 Hz) and sixth EEG recording of after 8.33 Hz;
- theta EEG band (3–5 Hz) relative power within sixth EEG recording (after 8.33 Hz) and seventh EEG recording of after 4 Hz.

The advantage of applying this particular method by only analysing the stimulus frequency EEG band was to simplify and reduce the number of multiple statistical tests in the analysis. Throughout the statistical analysis of this study, the gamma EEG band at MF stimulus of 50 Hz data was excluded from the analysis due to an interest in EEG frequency responses, less than 50 Hz.

2.5 Signal processing

All the collected EEG data was processed using MATLAB (Mathworks, USA) employing the Short Time Fourier Transform (STFT) function to compute the spectral analysis of all the 16 channels for all the subjects and extract parameters to be used in the statistical analysis. The EEG power was calculated by integrating the area under the curve at specific frequency band intervals. The EEG power spectra were computed with the resolution of 0.5 Hz with 60 epochs (1 s for each epoch) for 60-s recording with sampling frequency of 256 Hz.

3 Results

All-subject processed data were statistically analysed using statistical SPSS software tool (SPSS, Statistical Packages for Social Sciences, version 14, SPSS, Inc., Chicago, IL, USA). Initially, multiple repeated three-way analysis of variance (ANOVA) mixed design (within and between-subject) tests, with a significant level set at 0.05, were conducted. The factorial designs were used to evaluate the possible existence of the main effect such as: within-subject 'condition' (exposure/control) and 'order' (before/after); and between-subject 'session' (first/second); and its combined effects or interaction between factors, such as: 'condition × order', 'condition × session' and 'order × sessions'. Following the multiple ANOVA tests, the post-hoc analysis was conducted using multiple paired samples two-tailed *t*-tests. The first test conducted was for the first session of MF exposure consisting of 16 subjects (df = 15). The second test was the second session MF control (df = 15), the third test was the first session MF control (df = 16) and the fourth test was the second session MF exposure (df = 16). The following two sub-sections will describe the results, initially describing the exposure followed by control 'order' and control followed by the exposure 'order'.

3.1 MF exposure followed by MF control results

In alpha1 band, under 8.33 Hz, second MF control session (no field) *t*-test results revealed a significant increase from before to after at T7 ($t_{15} = -2.397$, $p < 0.030$) (Fig. 3). ANOVA test revealed a significant difference for the interaction between exposure/control and sessions factors (T7) $F_{1,31} = 5.992$, $p < 0.020$ (Table 1). In alpha2 band 10 Hz MF second control session (P3), there was a significant decrease from before [mean (M) = 0.1789, standard error (SE) = 0.0201] to after ($M = 0.1573$, SE = 0.0140), $t_{15} = 3.081$, $p < 0.008$) (Fig. 4). At P4 alpha2 band, the significant decrease from before ($M = 0.1861$, SE = 0.0223) to after ($M = 0.1510$, SE = 0.0134), $t_{15} = 2.812$, $p < 0.013$ was also evident. Also, at alpha2 band, in the occipital regions, the significant decrease at O1 from before ($M = 0.1399$, SE = 0.0156) and after ($M = 0.1243$, SE = 0.0111), $t_{15} = 2.256$, $p < 0.039$; and at O2 from before ($M = 0.1383$, SE = 0.0137) and after ($M = 0.1203$, SE = 0.0104), $t_{15} = 3.283$, $p < 0.005$ was revealed. There was a large decrease in relative difference from before to after by 12% (P3), 18.4% (P4), 11.2% (O1) and 13% (O2) than at any other electrode and stimulation. The three-way ANOVA revealed a significant interaction between exposure/control and sessions for P3 electrode $F_{1,31} = 11.918$, $p < 0.002$ and the main factor before/after $F_{1,31} = 5.230$, $p < 0.029$. At P4 electrode, a significant difference between exposure/control and sessions was $F_{1,31} = 14.827$, $p < 0.001$ and before/after $F_{1,31} = 4.406$, $p < 0.044$; O1 revealed $F_{1,31} = 9.346$, $p < 0.005$ (exposure/control and sessions); and O2 $F_{1,31} = 13.071$, $p < 0.001$ (Table 1). The *t*-test results for 13-Hz stimulation in beta1 band revealed no significant differences at any electrode, as shown in Fig. 5. For the first MF exposure session, the *t*-test results revealed a significant increase at Fp1, Fp2, F7, F3 and C3 for 13-Hz stimulation in beta1 band. At F7 before, $t_{15} = -2.798$, $p < 0.014$; F3 before $t_{15} = -2.659$, $p < 0.018$; and C3 before $t_{15} = -2.391$, $p < 0.030$. There was an increase from before to after by 10.1% (Fp1), 8% (Fp2), 8.4% (F7), 10.8% (F3) and 9.3% (C3). The ANOVA results revealed significant differences between before and after main factors at Fp1 $F_{1,31} = 12.852$, $p < 0.001$; Fp2 $F_{1,31} = 7.058$, $p < 0.012$; F7 $F_{1,31} = 5.730$, $p < 0.0001$ (Table 1). In first MF exposure beta1 band (13 Hz), ANOVA's significant results for before and after main factor, were very similar to *t*-test's results (Fig. 5; Table 1).

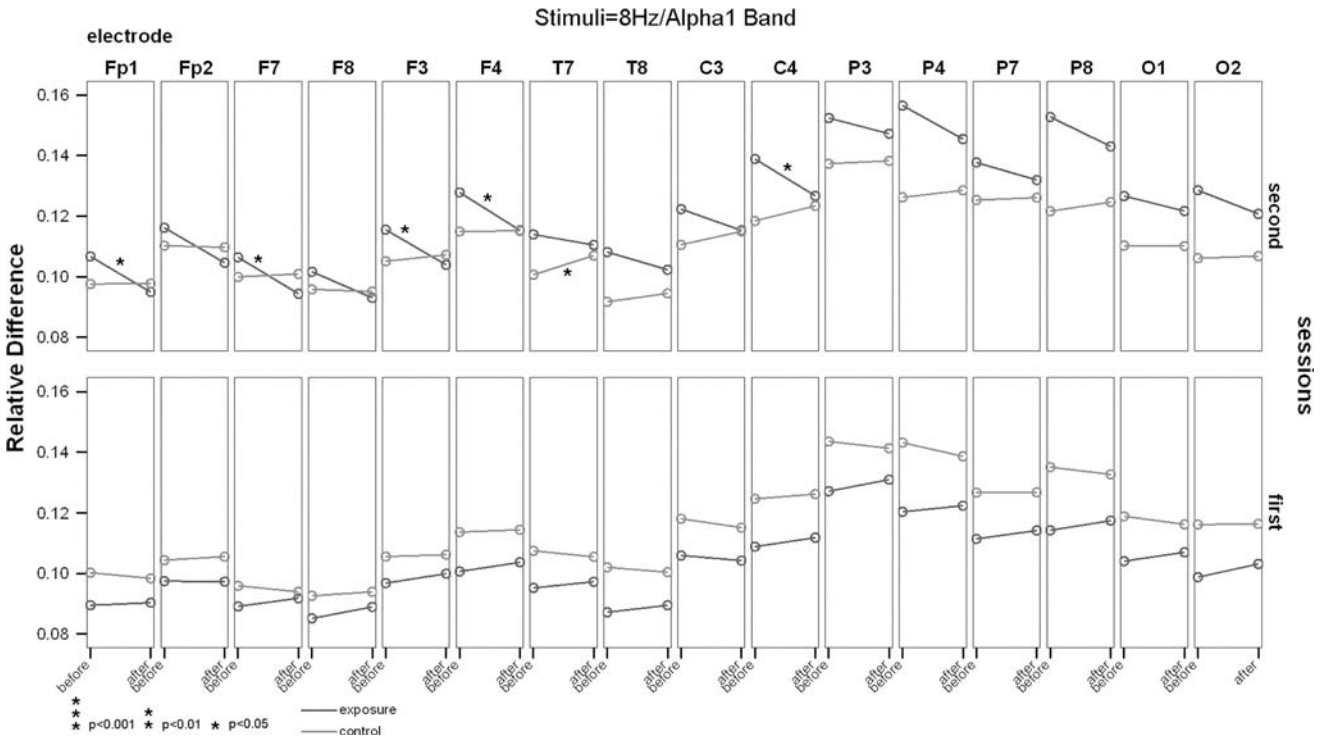

Fig. 3 The mean relative difference (*y*-axis) within alpha1 EEG band is represented as 'before' and 'after' (*x*-axis) MF condition (exposure/control) over 16 EEG electrodes (*columns*) at 8.33 Hz stimulus. The MF condition was represented by MF exposure (*darker colour line*) and MF control (*lighter colour line*) at first and second sessions (*rows*). The post-hoc analysis (multiple *t*-tests) was described by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. The significant ($p < 0.05$) decrease from before to after MF exposure, in relative alpha1 band power, was mainly exhibited over the frontal regions during the second session

3.2 MF control followed by MF exposure results

For the second MF exposure session, the *t*-tests results for 8.33 Hz stimulation in alpha1 band revealed a significant decrease from before to after at electrodes Fp1, F7, F3, F4 and C4: F7 $t_{16} = 2.120$, $p < 0.050$; F3 $t_{16} = 2.862$, $p < 0.011$; F4 $t_{16} = 2.682$, $p < 0.016$; and C4 $t_{16} = 2.872$, $p < 0.011$ (Fig. 3). There was a decrease in relative difference from before to after by 11.1% (Fp1), 11.3% (F7), 10% (F3), 9.8% (F4) and 8.8% (C4). The ANOVA results (Table 1) indicated a significant difference at: F7 $F_{1,31} = 6.485$, $p < 0.016$ (exposure/control and sessions) and $F_{1,31} = 4.485, p < 0.042$ (before/after and sessions); F3 $F_{1,31} = 4.524$, $p < 0.041$ (exposure/control and sessions) and $F_{1,31} = 4.297, p < 0.047$ (before/after and sessions); F4 $F_{1,31} = 11.554, p < 0.002$ (exposure/control and sessions); and C4 $F_{1,31} = 5.121$, $p < 0.031$ (exposure/control and sessions) and $F_{1,31} = 6.035$, $p < 0.020$ (before/after and sessions). For the second MF exposure session, the *t*-test results revealed a significant increase from before to after 10-Hz exposure in alpha2 band at F4, $t_{16} = -2.130$, $p < 0.049$, as shown in Fig. 4. ANOVA revealed a significant difference for the interaction between exposure/control and session's factor, $F_{1,31} = 11.043$, $p < 0.002$ (Table 1). For 13-Hz stimulation, there was no significant difference (Fig. 5).

3.3 Bonferroni alpha-correction for multiple ANOVA and post-hoc *t*-tests

Alpha-adjusted procedure is a frequently used method to control the inflated *type I error* due to repeated measures. The post-hoc analysis was performed using Bonferroni's corrected alpha rate, which needed to be conducted due to multiple *t* and ANOVA tests. Initially, for the multiple *t*-tests, there were 16 electrodes, five bands/stimulations, two conditions (exposure/control) and two sessions (first/second). As a result, the alpha rate of $p < 0.05$ was corrected to $p < 0.05/320 = 0.000156$. No significant differences were observed as a result of this correction.

On the other hand, the ANOVA tests conducted for 16 electrodes and five bands/stimulations, resulted in the corrected alpha rate of $p < 0.05/80 = 0.000625$. Clearly, the ANOVA's new alpha rate was higher than the *t*-test's by a factor of 4. Under this alpha rate correction (Table 1), the significant difference was revealed at: beta1 (13 Hz) band/simulation F7 electrode, $F_{1,31} = 15.73, p < 0.0005$ for main 'before/after' effect factor; alpha2 (10 Hz) T8 $F_{1,31} = 16.81$ $p < 0.0005$ and P7 $F_{1,31} = 17.25$ $p < 0.0005$ for 'condition × sessions' factor; alpha1 (8.33 Hz) P7 $F_{1,31} = 16.40$ $p < 0.0005$ and P8 $F_{1,31} = 15.00$ $p < 0.0005$ for 'condition × sessions' factor.

Table 1 The multiple repeated three-way ANOVA mixed design (within and between-subject) test results

Three-way ANOVA	Beta2 (16.66 Hz)		Beta1 (13 Hz)		Alpha2 (10 Hz)		Alpha1 (8.33 Hz)		Theta (4 Hz)	
$F_{1,31}$	F	p	F	p	F	p	F	p	F	p
Fp1	$4.585^{b\cdot c}$	$0.040^{b\cdot c}$	12.85^{a}	0.001^{a}	$11.12^{b\cdot c}$	$0.002^{b\cdot c}$	$5.667^{b\cdot c}$	$0.024^{b\cdot c}$		
Fp2	$5.287^{b\cdot c}$	$0.028^{b\cdot c}$	7.058^{a}	0.012^{a}	$10.18^{b\cdot c}$	$0.003^{b\cdot c}$	$8.400^{b\cdot c}$	$0.007^{b\cdot c}$		
	$5.529^{a\cdot c}$	$0.025^{a\cdot c}$								
F7			**15.73**^{a}	**0.0005**^{a}	$11.14^{b\cdot c}$	$0.002^{b\cdot c}$	$6.485^{b\cdot c}$	$0.016^{b\cdot c}$	$10.86^{b\cdot c}$	$0.002^{b\cdot c}$
							$4.485^{a\cdot c}$	$0.042^{a\cdot c}$	5.066^{a}	0.032^{a}
F8	4.781^{b}	0.036^{b}			$10.98^{b\cdot c}$	$0.002^{b\cdot c}$	$5.565^{b\cdot c}$	$0.025^{b\cdot c}$	$7.788^{b\cdot c}$	$0.009^{b\cdot c}$
F3			8.140^{a}	0.008^{a}	$8.727^{b\cdot c}$	$0.006^{b\cdot c}$	$4.524^{b\cdot c}$	$0.041^{b\cdot c}$	$10.99^{b\cdot c}$	$0.002^{b\cdot c}$
			$5.146^{b\cdot a}$	$0.030^{b\cdot a}$			$4.297^{a\cdot c}$	$0.047^{a\cdot c}$	5.944^{a}	0.021^{a}
F4	$8.728^{b\cdot c}$	$0.006^{b\cdot c}$	$7.960^{b\cdot a}$	$0.008^{b\cdot a}$	$11.04^{b\cdot c}$	$0.002^{b\cdot c}$	$11.55^{b\cdot c}$	$0.002^{b\cdot c}$	$8.751^{b\cdot c}$	$0.006^{b\cdot c}$
									5.448^{a}	0.026^{a}
T7			$4.449^{b\cdot a}$	$0.043^{b\cdot a}$	$1.564^{b\cdot c}$	$0.002^{b\cdot c}$	$5.992^{b\cdot c}$	$0.020^{b\cdot c}$	$9.390^{b\cdot c}$	$0.004^{b\cdot c}$
					4.473^{c}	0.043^{c}				
T8					**16.81**$^{b\cdot c}$	**0.0005**$^{b\cdot c}$			$6.975^{b\cdot c}$	$0.013^{b\cdot c}$
									$4.157^{b\cdot a}$	$0.050^{b\cdot a}$
C3	4.300^{a}	0.047^{a}			$7.898^{b\cdot c}$	$0.008^{b\cdot c}$			$13.75^{b\cdot c}$	$0.001^{b\cdot c}$
					4.278^{c}	0.047^{c}				
C4			$4.176^{b\cdot a}$	$0.050^{b\cdot a}$	$13.64^{b\cdot c}$	$0.001^{b\cdot c}$	$5.121^{b\cdot c}$	$0.031^{b\cdot c}$	$8.825^{b\cdot c}$	$0.006^{b\cdot c}$
							$6.035^{a\cdot c}$	$0.020^{a\cdot c}$		
P3					$11.92^{b\cdot c}$	$0.002^{b\cdot c}$	$7.381^{b\cdot c}$	$0.011^{b\cdot c}$	$9.911^{b\cdot c}$	$0.004^{b\cdot c}$
					5.230^{a}	0.029^{a}			5.222^{a}	0.029^{a}
P4					$14.83^{b\cdot c}$	$0.001^{b\cdot c}$	$6.606^{b\cdot c}$	$0.015^{b\cdot c}$	$10.45^{b\cdot c}$	$0.003^{b\cdot c}$
					4.406^{a}	0.044^{a}				
P7	4.156^{c}	0.050^{c}			**17.25**$^{b\cdot c}$	**0.0005**$^{b\cdot c}$	**16.40**$^{b\cdot c}$	**0.0005**$^{b\cdot c}$	$10.93^{b\cdot c}$	$0.002^{b\cdot c}$
									$5.688^{b\cdot a}$	$0.023^{b\cdot a}$
P8					$13.41^{b\cdot c}$	$0.001^{b\cdot c}$	**15.00**$^{b\cdot c}$	**0.0005**$^{b\cdot c}$	$8.632^{b\cdot c}$	$0.006^{b\cdot c}$
					4.229^{a}	0.048^{a}				
O1					$9.346^{b\cdot c}$	$0.005^{b\cdot c}$	$6.837^{b\cdot c}$	$0.014^{b\cdot c}$	$8.614^{b\cdot c}$	$0.006^{b\cdot c}$
O2	$5.918^{b\cdot c}$	$0.021^{b\cdot c}$			$13.07^{b\cdot c}$	$0.001^{b\cdot c}$	$9.671^{b\cdot c}$	$0.004^{b\cdot c}$	$8.492^{b\cdot c}$	$0.007^{b\cdot c}$
									$4.679^{b\cdot a}$	$0.038^{b\cdot a}$

The ANOVA main effect and interaction between factors were represented as: before/after^{a}; condition^{b}; sessions^{c}; before/after × sessions$^{a\cdot c}$; condition × before/after$^{b\cdot a}$; and condition × sessions$^{b\cdot c}$. For all the results, the degree of freedom was adjusted by Greenhouse and Geisser's epsilon when appropriate. The tests that showed no significant ($p > 0.05$) differences are denoted by empty spaces. The ANOVA values highlighted in bold show the Bonferroni's alpha-corrected significant differences ($p < 0.000625$)

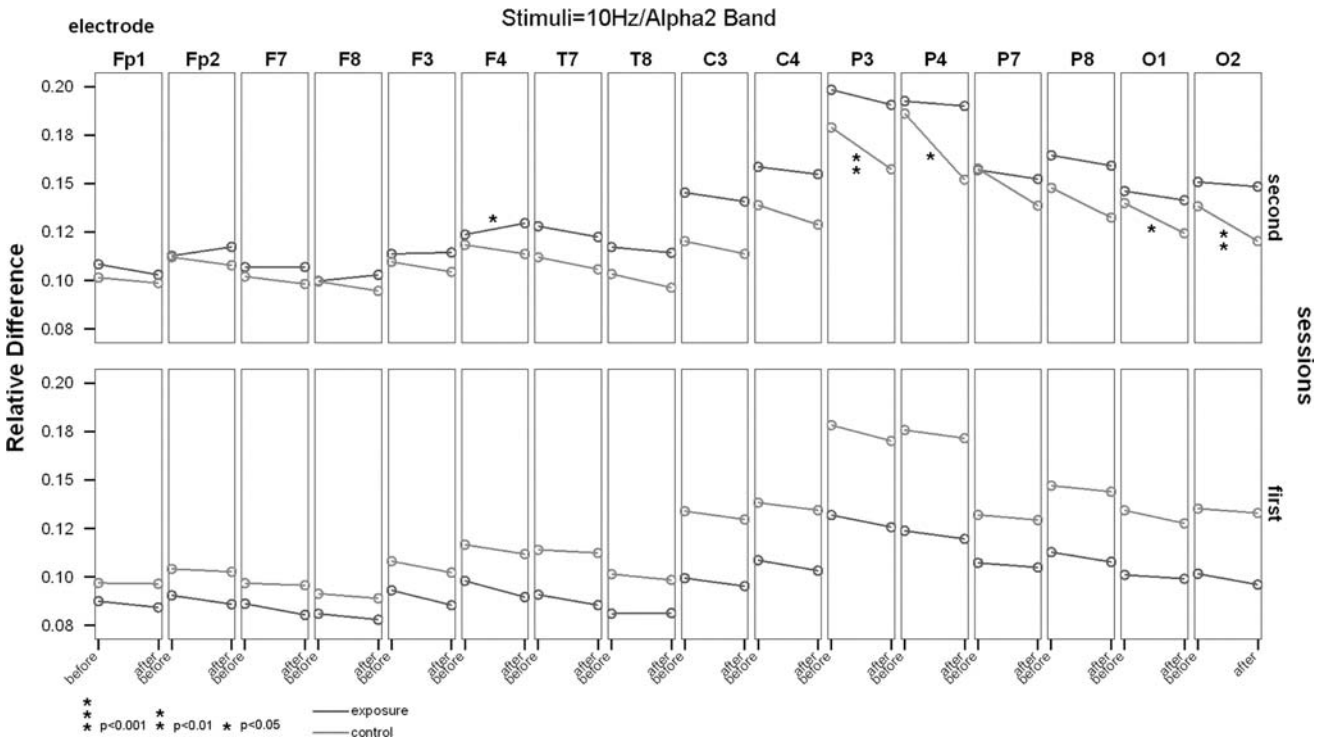

Fig. 4 The mean relative difference (*y*-axis) within alpha2 EEG band is represented as 'before' and 'after' (*x*-axis) MF condition (exposure/control) over 16 EEG electrodes (*columns*) at 10-Hz stimulus. The MF condition was represented by MF exposure (*darker colour line*) and MF control (*lighter colour line*) at first and second sessions (*rows*). The post-hoc analysis (multiple *t*-tests) was described by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. The significant ($p < 0.05$ and 0.01) decrease from before to after MF control, in relative alpha2 band power, was mainly exhibited over the parietal and occipital regions during the second session

4 Discussion

The statistical tests have been conducted to find any possible alteration of human EEG responses due to ELF MF exposures. The Bonferroni's corrected alpha rate on multiple ANOVA tests was able to indicate the significant differences of the main effect test factors and its combined effects or interaction between factors, such as MF exposure/control versus sessions (first/second). In response to alpha-corrected ANOVA results, it has been revealed that the relative power in alpha1 (7.5–9.5 Hz) and alpha2 (9–11 Hz) bands, associated with 8.33 and 10 Hz MF exposures, were significantly ($p < 0.0005$) lower than at MF control over the temporal and parietal regions within the 10–16 min of first session MF exposure. In addition, at the MF exposures, the relative powers in alpha1 and alpha2 bands were significantly higher than at MF control of the second session MF exposure (60–65 min from the commencement of testing).

It is unknown why the relative power in alpha (7.5–11 Hz) bands was 'suppressed' or decreased, associated with 8.33 and 10 Hz stimulus throughout the first 10–16 min MF exposure period. Its behaviour was consistent with our pilot study and other studies. The results from our previous pilot study revealed a 'marginal' significant decrease in MF exposure compared to MF control, found in the alpha1 EEG band (7.5–9.5 Hz) at the vertex head position, where MF stimulation was applied at the alpha1 frequency of 174 μT/8.33 Hz [8]. The consistent results with our study were also reported in another study with a decrease in the alpha (8–13 Hz) EEG activity at the occipital region after 2-s MF exposure of 0–60 Hz/20–100 μT [2]. Another study revealed a decrease in global field power and no indication in any frontal alpha asymmetry [ratio of right (F4) and left (F3) frontal powers] [20]. Our future analysis could perhaps employ similar method such as the EEG hemispheric asymmetry of anterior–posterior (A–P) regions and inter/intra hemispheric coherence. This type of analysis method was undertaken by other authors to investigate the photic stimulation responses on EEG activity [17]. The recent double-blind counter-balanced study (20 subjects), among the first to assess EEG activity changes during a weak ELF PEMF exposure, revealed that the alpha (8–13 Hz) EEG band was significantly lower over the occipital region after the first 5 min of MF, related to order of MF-sham versus sham-MF condition [6]. Our study has also shown that alpha1 and alpha2 bands were also significantly lower, but over the temporal and parietal regions (instead of occipital) during

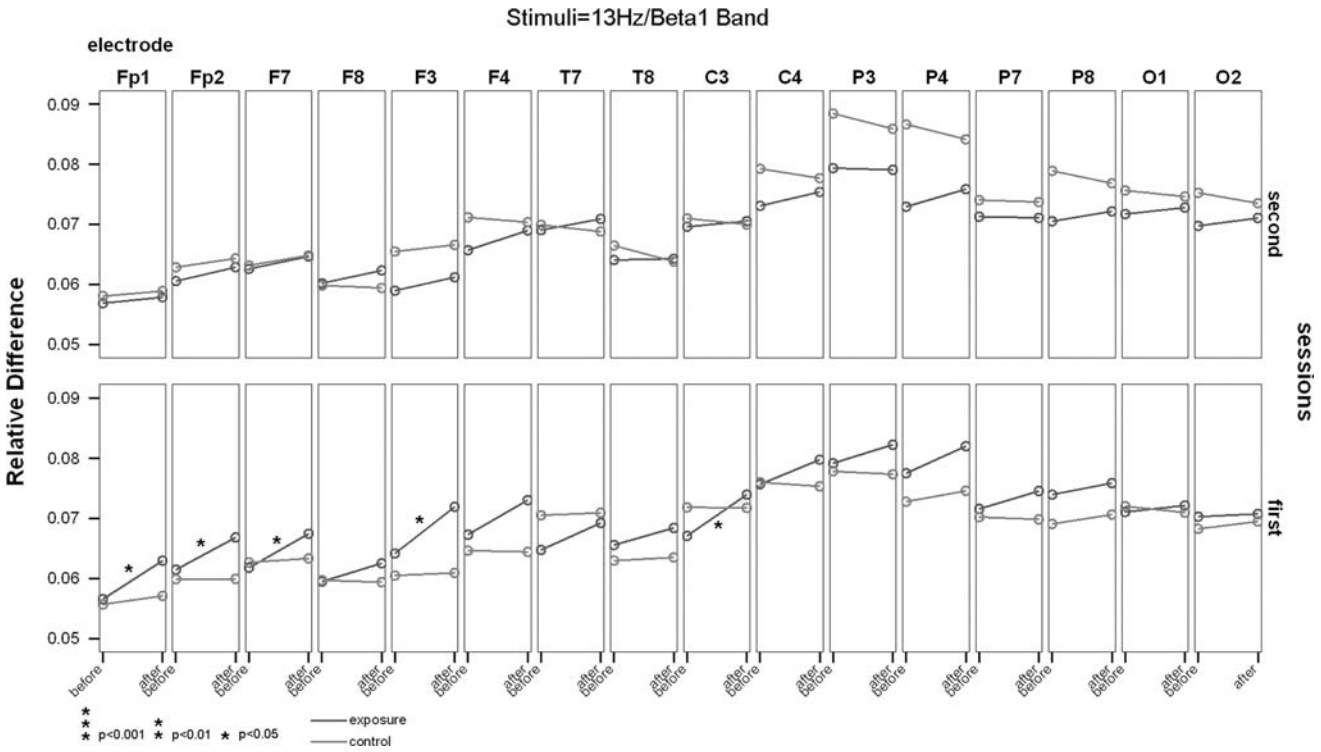

Fig. 5 The mean relative difference (*y*-axis) within beta1 EEG band is represented as 'before' and 'after' (*x*-axis) MF condition (exposure/control) over 16 EEG electrodes (*columns*) at 13 Hz stimulus. The MF condition was represented by MF exposure (*darker colour line*) and MF control (*lighter colour line*) at first and second sessions (*rows*). The post-hoc analysis (multiple *t*-tests) was described by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. The significant ($p < 0.05$) increase from before to after MF exposure, in relative beta1 band power, was mainly exhibited over the frontal regions during the first session

the first session (after 10–16 min). Some of the evident differences between ours and [6] Cook et al.'s protocol and analysis were: PEMF (0–500 Hz) versus ELF (4–16.66 Hz); Cook's alpha band (8–13 Hz) was much wider in frequency range compared to our narrow alpha1, alpha2 and beta1 bands, resulting in frequency range from 7.5 to 14 Hz; and ANOVA measures were analysed by four sets of EEG electrodes (O1, Oz, O2; P3, Pz, P4; CPz, C3, Cz, C4; FCz, F3, Fz, F4) versus 16 individual EEG electrodes in this study. The 15-min duration and the 'session' counter-balanced design (MF exposure/control) was very similar in both studies. For our future statistical analysis, we should adopt the 4–5 sets of EEG electrodes which could definitely increase the altered-alpha rate value, and therefore improve the significant difference of our results.

It is also unknown why the relative powers in alpha1 and alpha2 bands, associated with 8.33 and 10 Hz stimulus, were significantly higher than at MF control of the second session MF exposure. These results were contradictory with our pilot study [8], but consistent with many other previous studies [4, 5] on EEG responses, which revealed that the alpha activity was significantly higher over the occipital region, and marginally higher over the parietal electrodes at 15-min post exposure. The other consistencies in the alpha band increase at MF exposure compared to MF control were also reported in the following studies: a decrease in the delta (1–3 Hz) and theta (4–7 Hz) (frontal/central/parietal regions) and an increase in the alpha (7–13 Hz) and beta (14–25 Hz) in the respective occipital and frontal regions (45-Hz MF) [14]; an increase in the alpha (10 Hz) EEG activity at the central region after 10-min MF exposure of 10 Hz/40 μT and 1.5 Hz/20 μT [3]; an increases in the spectral power mainly at higher than 10 Hz EEG frequencies at the central, parietal and occipital regions due to 2-s light and EMF exposures to 80 μT/1.5 Hz and 10 Hz [15]; and a significant increase in the alpha (8–13 Hz) EEG activity over occipital region due to a 90 min MF exposure 50 Hz/80 μT [11].

Considering that our ANOVA tests findings were not able to determine whether there was a significant increase or decrease (Fig. 6) from before to after MF exposure or control at the specific session, the post-hoc analysis was conducted using multiple paired samples two-tailed *t*-tests and later employing the Bonferroni's corrected alpha rate. From the combination of the ANOVA and *t*-test results, it was found that the beta1 (12–14 Hz) band exhibited a significant increase from before to after 13-Hz first MF exposure session at left frontal region (Figs. 5, 6; Table 1). This particular finding was also consistent with previous

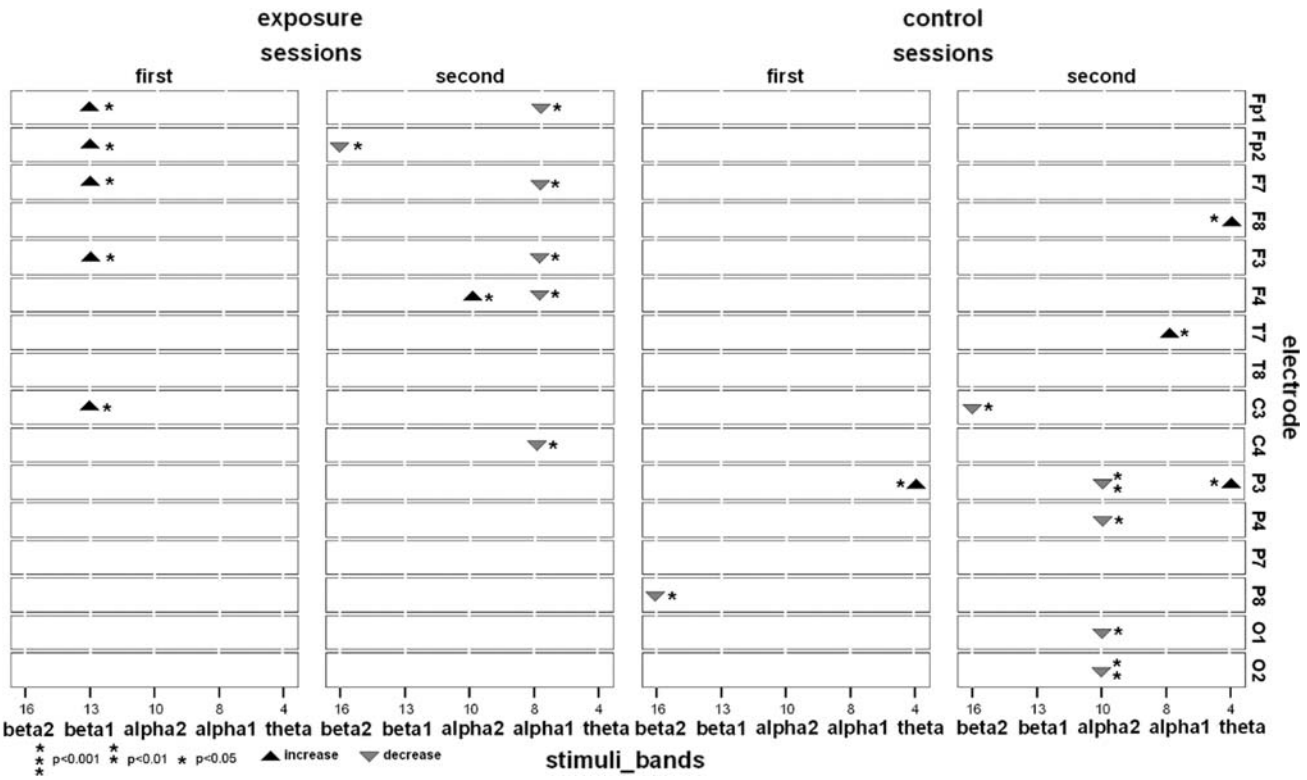

Fig. 6 A graphical representation of the relative increase (*darker colour pointer facing up*) and decrease (*lighter colour pointer facing down*) at each MF stimulus and EEG band (*x*-axis) over the 16 EEG electrodes (*y*-axis). The MF condition was represented by MF exposure (first two columns on the left-hand side) and MF control (last two columns on the right-hand side) at first and second sessions (*columns*). The post-hoc analysis (multiple *t*-tests) was described by * $p < 0.05$, ** $p < 0.01$ and *** $p < 0.001$. The significant ($p < 0.05$) increase from before to after MF exposure during the first session was exhibited in relative beta1 band power over the frontal regions. On the other hand, the significant decrease from before to after MF exposure (second session) was revealed over frontal region at alpha1 band and MF control (second session) over the parietal and occipital regions at alpha2 band

studies, in particular [14] Lyskov et al. study where an increase in beta band (14–25 Hz) was evident in the frontal region during the 45-Hz MF exposure [13] and Hausser et al. study which revealed a significant increases in the beta (12.5–25 Hz) EEG bands over the occipital region after the 20-min, 3-Hz MF exposure.

The Bonferroni's corrected alpha rate *t*-tests did not reveal any significant differences due to an extremely low alpha rate of $p < 0.000156$. However, it did reveal a significant ($p < 0.05$) increase from before to after MF exposure during the first session in relative beta1 band power over the frontal regions (Figs. 5, 6). The significant decrease from before to after MF exposure (second session) was also revealed over the frontal region at alpha1 band and from before to after MF control (second session) over the parietal and occipital regions at alpha2 band (Fig. 6).

Our beta1 and alpha2 band findings indicate that humans could exhibit significant changes in the EEG during the MF exposure, such as by having the natural ability to detect ELF MFs [16]. The beta2 frequency (16.66 Hz) used in this study was also used as the frequency within the beta1 band of the EEG in [19] Sastre et al. study which claimed that beta1 band exhibits a close temporal association with REM sleep and has a reciprocal relationship with delta activity during NREM sleep. Griefahm et al. study also utilised 16.7-Hz MF exposure [12]. However, this study on the effects of 16.66 Hz on beta2 EEG band failed to present any evident and significant changes.

In vitro experimental study has observed in 'real-time' the intracellular synchronised bioelectric activity in neurons, from the brain ganglia of the snail *Helix aspersa*, under (1–50 Hz/15 mT) [1]. This study demonstrated the ability of low frequency sinusoidal weak MF to promote synchronisation in the bioelectric activity in neurons. The results revealed that the decrease of neuron firing frequency and synchronisation can be observed with an increasing static and ELF MF. In vivo studies, similar EEG synchronisation has been demonstrated using the non-invasive direct stimulation of the brain via pulsed MF, known as transcranial magnetic stimulation (TMS) [17]. However, there has not been enough research on EEG

synchronisation from sinusoidal 'weaker' MF stimulation which has been demonstrated in this study.

Over the years, the research examining the effects of MF on human performance and physiology has produced inconsistent results [22]. Our 'lack' of significant findings due to Bonferroni's adjustment could be based on Podd et al. study [18] which reported negative findings that 0.2-Hz MF affected simple reaction time in humans, and whereas a 0.1-Hz field did not. They discussed the various issues which could improve the significance of their results. One of those issues raised was the degree of statistical power. In order to increase the statistical power is to increase the number of subjects which in our study was minimal. Also, adjusting the probability of a type I error (alpha level) or by reducing the number of relevant experimental design factors could increase the statistical power. Thus, any other future research studies including ours must give serious thought to minimise the error of variance and maximise statistical power.

Thus, an increased sample size, adopted EEG hemispheric asymmetry method and alternative alpha-adjustment tests could improve on the significant differences of these findings in the future studies.

5 Conclusion

The results from this study have shown a two-folding outcome which is equally consistent and inconsistent with our pilot and other studies, conducted over the last several years. In order to investigate whether weak MF exposures at ELF in series from 50, 16.66, 13, 10, 8.33 to 4 Hz could alter relative power within the corresponding EEG bands, this study's consistency with our pilot study has revealed that the relative power in alpha bands, associated with 8.33 and 10 Hz MF exposures was significantly lower than at MF control over the temporal and parietal regions within the first session of MF exposure. However, at the second session of MF exposure, the relative power in alpha bands was significantly higher than at MF control. Also, it was found that the beta1 (12–14 Hz) band exhibited a significant increase from before to after 13-Hz first MF exposure session at frontal region.

The final outcome of our result has shown that it is possible to alter the human EEG activity of alpha and beta bands when exposed to MF at associated EEG frequency bands, depending on the order and period of MF conditions. This type of EEG synchronisation of driving alpha and beta EEG by alpha and beta sinusoidal MF stimulation, demonstrated in this study, could possibly be applied as therapeutic treatment(s) of particular neurophysiological abnormalities such as sleep and psychiatric disorders. In future, authors expect that the EEG synchronisation can effectively be demonstrated by either flickering of lights [10] and/or MF at ELF.

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Multiple human electrophysiological responses to extremely low frequency pulsed electromagnetic field exposures: a pilot study

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Abstract. This pilot study was conducted to investigate whether multiple electrophysiological activities could be altered when exposed to extremely low frequency pulsed electromagnetic field (PEMF) over a consecutive 5 day period. Our results revealed substantial changes in electroencephalographic hemispheric asymmetry, observed at the theta band posterior predominance, an increase in the low frequency heart rate variability, changes in the surface skin temperature and skin bioelectric impedance fluctuations in its frequency spectrum. Due to the small sample size these results reveal no solid and conclusive evidence in the possible alterations in electrophysiological responses due to PEMF.

Key words: electrophysiological response, electroencephalography, heart rate variability, photoplethysmography, bioelectric impedance analysis.

1. INTRODUCTION

In the past few decades, the responses of human and animal electrophysiological signal activity to non-ionising extremely low frequency (ELF) pulsed electromagnetic field radiation have been studied. Various studies have reported that humans and animals are particularly sensitive to ELF alternative or ELF pulsed/modulated sensory stimulation [1]. A number of studies have investigated the therapeutic effects of ELF PEMF in the following applications: relieving insomnia [2], accelerated bone repair and reducing pain [3–4] and dental sensory and cutaneous pain [5].

Several studies have reported on the influence of the ELF PEMF exposure to the heart rate variability (HRV) alterations (the low frequency (LF) and high frequency (HF) ratio) [6–8], on the 16 Hz/28.3 μT MF exposure to HRV [9], on an

intermittent 16.7 Hz MF exposure to the heart rate (HR) [10] and on parameters derived from the ECG, such as heart rate, duration of P and QRS waves, duration of PR and QT intervals and corrected QT (QTc) [11]. Other studies have investigated the effects of ELF PEMF on the EEG activity after the applied frequency modulated 'Thomas pattern' (0–500 Hz pulses in 853 ms segments (18 pulses), varying periods of 110, 220, 330 ms at ±200 μT) [1,12,13] during a weak ELF PEMF exposure [14]. Another study investigated the effect of the magnetic field on the skin conductance [15].

The authors' pilot studies examined the effects of sinusoidal 8.33 Hz/174 μT ELF MF [16–17] and ELF PEMF (0–100 Hz) on the human EEG activity [18–19]. These results have led the authors to further investigation of the electrophysiological activity alterations due to PEMF exposures. The aim of this pilot study is to investigate whether there are any alterations in the multiple electrophysiological activities due to ELF PEMF exposures over a consecutive 5 day period. The multiple electrophysiological parameters consisted of electroencephalographic (EEG), photoplethysmographic (PPG), heart rate and heart rate variability values, derived from electrocardiographic (ECG), skin bioelectric impedance analysis (BIA) and skin temperature (surface) measurements. Statistical analysis for any significant differences was not carried out due to small sample size.

2. METHODS

2.1. Subjects

Five healthy subjects (3 females and 2 males) were recruited to participate (mean age 30, SD 7.8 and range 24–42) for 5 consecutive working days. RMIT University ethics committee approved the study and all subjects gave written consent prior to the experiment.

2.2. Experimental Protocol and the ELF PEMF exposure system

The experiment was of double-blinding counter-balanced type. In total there were 25 recording sessions. At each session (day) the experimental protocol was designed to record the biosignals before (baseline) and after (post) PEMF exposure during the control and exposure conditions. The experiment was conducted between 10 am and 3 pm. The ELF PEMF stimulations were carried out with a Bioresonance® BRS-500 system (Medec Limited, Australia). The BRS magnetic field exposure system consists of the programmed control unit, an applicator mattress and pillow. In order to secure double-blind conditions, a switch board was constructed to control whether the magnetic field exposure was active. The mattress (60 × 140 cm in size) was used as a magnetic field exposure system; it consisted of 3 coil pairs, embedded within the mattress and spaced strategically in order to apply the exposure to the whole body area, as shown in

Fig. 1. The magnetic flux density (B_{rms}) was verified by direct measurement using "Wandel and Goltermann" EFA-200 EMF Analyser and external B-field probe with a diameter of 3 cm with measurement accuracy of 6%. The applied magnetic field direction was perpendicular to the body and mattress. The commercial mattress contained three pairs of coils of different size resulting in generating different magnetic flux densities. The 10 min exposure generated the maximum magnetic flux densities of 8.3, 2 and 1 μT at the subject's back of the neck, head/chest and stomach/hip/leg/feet region, respectively. The signal, generated from the control unit to create PEMF, consisted of 'saw-tooth-like' waveform (0–100 Hz) bundles and delays, each lasting 20 msec (Fig. 2).

All the biosignals were recorded for 60 sec after the ELF PEMF exposure. In order to shield the subjects and electrodes from the electric fields in the laboratory, the recording took place in a dim, 20 °C temperature-controlled room, inside a Faraday cage ($1.95 \times 1.83 \times 2.67$ m), constructed of mesh wire (2.5×2.5 cm) and steel frames. Inside the cage, subjects were laid in a comfortable semi-reclining Metron® chair.

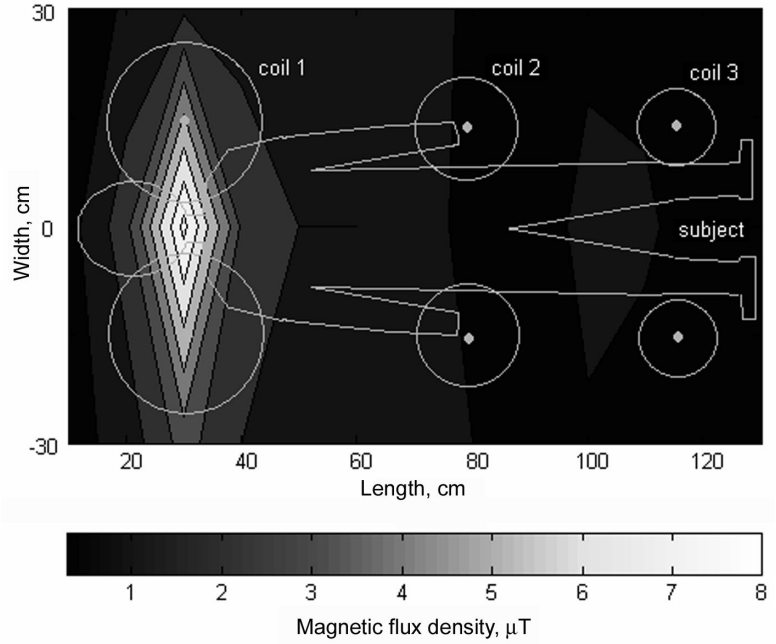

Fig. 1. The applicator mattress consisted of three pairs of coils. The first coil pair, 20 cm in diameter each, was spaced 10 cm apart at the top side of the mattress, where the subject's head was positioned. The second coil pair, 13 cm in diameter each, was spaced at 23 cm from the first pair, approximately positioned in the middle of the mattress. The third coil pair, 10 cm in diameter, was spaced at 28 cm from the second coil pair (at the bottom of the mattress).

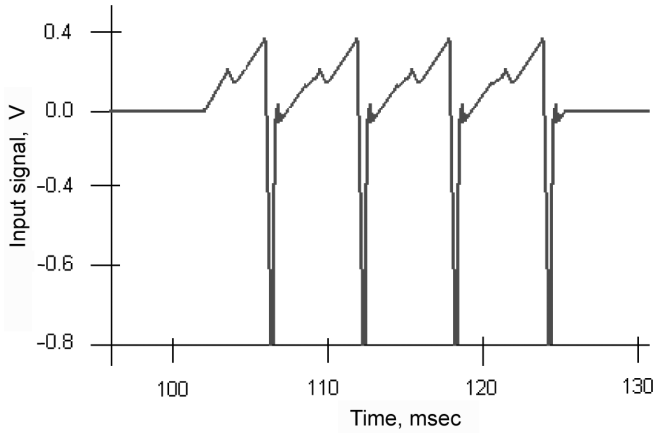

Fig. 2. The signal, generated from the control unit to create PEMF, consisted of 'saw-tooth like' waveform (0–100 Hz) bundles and delays, each lasting 20 msec. The signals pulsate, directed plus or minus in one direction with 0.4 V maximum, followed by a fast fall to –0.8 V.

2.3. EEG recording montage and signal processing

EEG was recorded using Mindset EEG data acquisition system and standard 10/20 International system Neuroscan 19-electrode EEG cap. The referential montage of 16 channels was used throughout this investigation. The left brain hemisphere electrodes Fp1, F7, F3, T7, C3, P7, P3 and O1 were all referenced to M1 or A1 (left mastoid) while the right brain hemisphere electrodes Fp2, F8, F4, T8, C4, P8, P4 and O2 were referenced to the right mastoid (M2 or A2). The EEG signals were sampled at the rate of 256 Hz.

Initially we analysed the data to derive the hemispheric asymmetry of left (L), right (R), anterior (A) and posterior (P) hemisphere. Four sets of EEG sites were evaluated as follows: R – over the right sites F8, F4, T8, C4, P8, P4 and O2; L – over the left sites F7, F3, T7, C3, P7, P3 and O1; A – F3, F4, F7, F8, T7, T8; and P – C3, C4, P3, P4, P7, P8, O1, O2. Fp1 and Fp2 were excluded from the analysis. Subject's eyes remained closed throughout the testing.

The EEG frequency bands were extracted using Wavelet Packet Decomposition (WPD). The WPD originates from Wavelet Transforms (WT) and offers greater flexibility in defining the frequency bands of decomposition than is given by a standard discrete wavelet transform (DWT). The WPD are generated by a filtering scheme similar to that used in a conventional DWT and are closely related to filter banks, often used to divide the EEG signal into finer set of band-pass signals. The WPD permits the functions to be further split into two or more subbands. In this study, each EEG band was decomposed by Daubechies [18–20] order 6 wavelet (db6). The calculation of the wavelet packet decomposition coefficients was made using selected nodes of the wavelet packet analysis tree. Delta band (0.5–4 Hz) was created from the following combination: delta1 (9.1 node) 0.5–1 Hz; delta2 (8.1 node) 1–2 Hz; delta3 (7.1 node) 2–4 Hz. The other

bands were computed as theta band (6.1) 4–8 Hz, alpha band (5.1 node) 8–16 Hz, beta band (4.1 node) 16–32 Hz and total band (combination of all bands) 0.5–32 Hz. Entropy was used as a measure of information comprised in a given amount of signals [21]. The Shannon entropy reflects the degree of order/disorder by comparing probability distributions of the EEG signals. The Shannon entropy (E_{WT}) was computed for each band over the 60 sec interval: $E_{WT} = -\sum P_i \log(P_i)$, where P_i is the wavelet energy. The relative entropy of each band was derived as $E_{WT\_REL.} = E_{WT\_BAND} / E_{WT\_TOTAL}$. Likewise, the ratio 'after $(E_{WT\_REL.})/$ before $(E_{WT\_REL.})/$' was computed both for the PEMF exposure and control conditions.

2.4. HRV recording and signal processing

The ECG signals were recorded using BIOPAC Inc. data acquisition device, which consisted of a MP100A system with ECG100C amplifier. The ECG electrodes (Skintact®, Ag/AgCl) were attached to subjects in the Lead-I configuration. All the signals were filtered by an analogue 50 Hz notch filter and transmitted to a PC's Acqknowledge 3.7 software for processing. Matlab program was also written to compute the HRV. The steps used in this program consisted of computing the R-R intervals (msec) from detection of maximums of QRS, R peaks and their time occurrence and the mean and standard deviation of R-R intervals, generated from Lead-I ECG signals sampled at 250 Hz. The R-R intervals were visually checked for consistency and accuracy. A mean value (DC) subtraction was performed on the time series. The spectral power was computed using the Thompson Multitaper spectral estimation algorithm. The Thomson algorithm comprises a bank of optimal bandpass filters, derived from a set of sequences known as orthogonal tapers (windows in the frequency domain) specified from Discrete Prolate Spheroid Sequences (DPSS) also known as Slepian sequences [22]. The total spectral estimates were obtained in the 0–0.5 Hz range where the power was computed as power/frequency (dB/Hz). The very low frequency (VLF), low frequency, high frequency and total frequency bands were examined at the following frequency ranges: 0–0.04, 0.04–0.15, 0.15–0.4 and 0–0.4 Hz. The power within the frequency bands was calculated by integrating the area under the spectral curve. The LF and HF parameters were converted to normalized units (n.u.) as

$$LF_{\text{n.u.}} = LF/(Total\_power - VLF) \times 100\%,$$

$$HF_{\text{n.u.}} = HF/(Total\_power - VLF) \times 100\%.$$

The LF/HF ratio was further derived from the LF and HF n.u.

2.5. PPG recording and signal processing

The PPG signals were recorded using Biopac PPG100C amplifier module. The peak measurement indicated the point of maximal blood density. The PPG

transducer TSD200 was attached to subject's right hand index finger using stretchable Velcro strap. The spectral density of the PPG signals, sampled at 100 Hz, were computed using the same Thompson Multitaper spectral estimation algorithm for derivation of HRV, mentioned previously. Three frequency bands were computed to estimate the blood power density. The total frequency, VLF, LF and HF (without the total frequency for normalized estimate) bands were taken as 0.003–1.125, 0.01–0.04 and 0.125–8 Hz, respectively.

2.6. Heart rate, skin temperature and bioelectric impedance analysis

The heart rate (HR) was recorded using Biopac OXY100C pulse oxymeter module via UIM100C, which was primarily used to measure the blood oxygen saturation level in a non-invasive manner. The oxygen saturation level was not measured in this study, but its TSD123 series SpO2 finger transducer (photo-diode) was used for HR recordings only. The skin (surface) temperature was recorded using Biopac SKT100C skin temperature differential amplifier module at sampling frequency of 100 Hz. The TSD202D surface temperature thermistor probe was secured to subject's right-hand finger using the Velcro strap. The scaling was set to corresponding 5 °F/V, adjusted on the amplifier module.

The application of the bioelectric impedance analysis (BIA) method is safe, non-invasive, rapid, inexpensive, easy to use, and amenable for laboratory, clinical and field assessment of the human body composition [[23]]. BIA consists of the local injection of a small alternating current into the human body and the measurement of induced complex impedance (*Z*). Our study has used the 'whole-body' multiple-frequency bioelectric impedance analysis (MF-BIA) [[24]], using 1260 Impedance/Gain-phase Analyzer, interfaced to PC with Solarton 1294 Impedance Interface via GPIB. The 4-terminal measurement configurations were used in the recordings, where the current or signal inducing electrodes were placed on the mid-dorsum of the hand midway between voltage electrodes and the proximal metacarpal-phalangeal joint line, and similarly on the appropriate foot. The voltage sensing electrodes, 3M™ Red Dot 2330 (Ag/AgCl) series resting electrodes with tab style connector, were applied on the mid-dorsum of the wrist with the distal bony prominence of the radius and ulna as land-marks, and on the mid-anterior aspect of the ankle with the medial and lateral malleoli as location landmarks. The signal frequency sweep, 1 Hz–1 MHz/100 mVrms source was applied for each recording of the bioimpedance. The magnitude of impedance, capacitance and admittance, together with phase impedance data was collected using 'Solartron Smart' software and exported to Microsoft Excel and Mathwork Matlab software for further processing.

3. RESULTS

All-subject data was processed using Matlab™ (Mathworks, USA) software tool and processed results were statistically analysed using statistical SPSS 14

(SPSS Inc.) software tool. No statistical elaboration of the data has been attempted in this investigation due to the small number of subjects.

3.1. HRV and heart rate results

The R-R interval results were represented as the relative difference ratios (exposure/before and control/before). Throughout the 5 days of testing, relative R-R interval results did not reveal any substantial difference, recorded between the exposure and control conditions (Fig. 3a). The minimal standard error (SE) difference was shown on day 4, (exposure 0.01 and control 0.02). The only evident difference between PEMF exposure and control was on days 3 and 5, with exposure being slightly smaller than control. However, the error difference was greatest on these days. The maximum mean and SE difference was exhibited on the day 3 (exposure 1.81 ± 0.84 and control 1.84 ± 0.83) and 5 (exposure 1.90 ± 0.91 and control 1.964 ± 0.97). The exposure and control conditions were all relatively larger than the 'before' condition (R-R interval > 1). In general, no substantial difference was found in the R-R interval results between the relative PEMF exposure and control conditions, as shown in Fig. 3a.

The heart rate results, represented by beats per minute (bpm) in Fig. 3b, revealed a two-fold characteristic. Firstly, on days 1 and 3, the PEMF exposure was substantially less than both 'before' and 'control' conditions (day 1 before 67.34 ± 3.81, exposure 64.87 ± 4.17 and control 67.47 ± 3.56, day 3 before 67.73 ± 2.85, exposure 64.12 ± 2.86 and control 67.12 ± 2.25). Secondly, on days 2, 4 and 5, the HR 'before' was the highest, followed by the exposure and control (least). Interestingly, the HR was also generally highest on day 2 (before 71.92 ± 4.65, exposure 69.39 ± 4.44 and control 66.07 ± 5.09) and gradually decreased throughout day 4 (before 67.39 ± 2.62, exposure 65.94 ± 3.79 and control 63.20 ± 3.26) and day 5 (before 64.91 ± 3.56, exposure 61.80 ± 4.07 and control 60.36 ± 4.52, as shown in Fig. 3b).

The LF HRV band was represented as the relative difference ratios (exposure/before and control/before) in Fig. 3c. The relative difference of exposure was higher in comparison to control on days 1, 3 and 4, with both conditions exhibiting gradual increase (day 1 exposure 1.01 ± 0.09 and control 0.93 ± 0.14, day 3 exposure 1.20 ± 0.38 and control 1.02 ± 0.26 and day 4 exposure 0.74 ± 0.15 and control 0.91 ± 0.11). Whereas the control was higher than exposure on days 2 and 5. At the HF HRV band, the control was higher than exposure condition for all the days except day 5 (exposure 1.32 ± 0.39 and control 1.07 ± 0.14, Fig. 3d).

In our pilot study, the LF/HF ratio revealed once again a two-fold characteristic: exposure substantially and identically higher than before and control on the days 3 (before 3.64 ± 2.50, exposure 5.85 ± 3.19 and control 1.05 ± 0.52) and 5 (before 3.33 ± 1.33, exposure 5.45 ± 3.50 and control 1.25 ± 0.56, Fig. 3e). The exposure was also slightly higher than before and control on day 1 and lower than control on days 2 (before 0.81 ± 0.25, exposure 1.60 ± 0.35 and control 2.01 ± 0.95) and 4 (before 1.03 ± 0.33, exposure 2.66 ± 1.07 and control 3.39 ± 2.09).

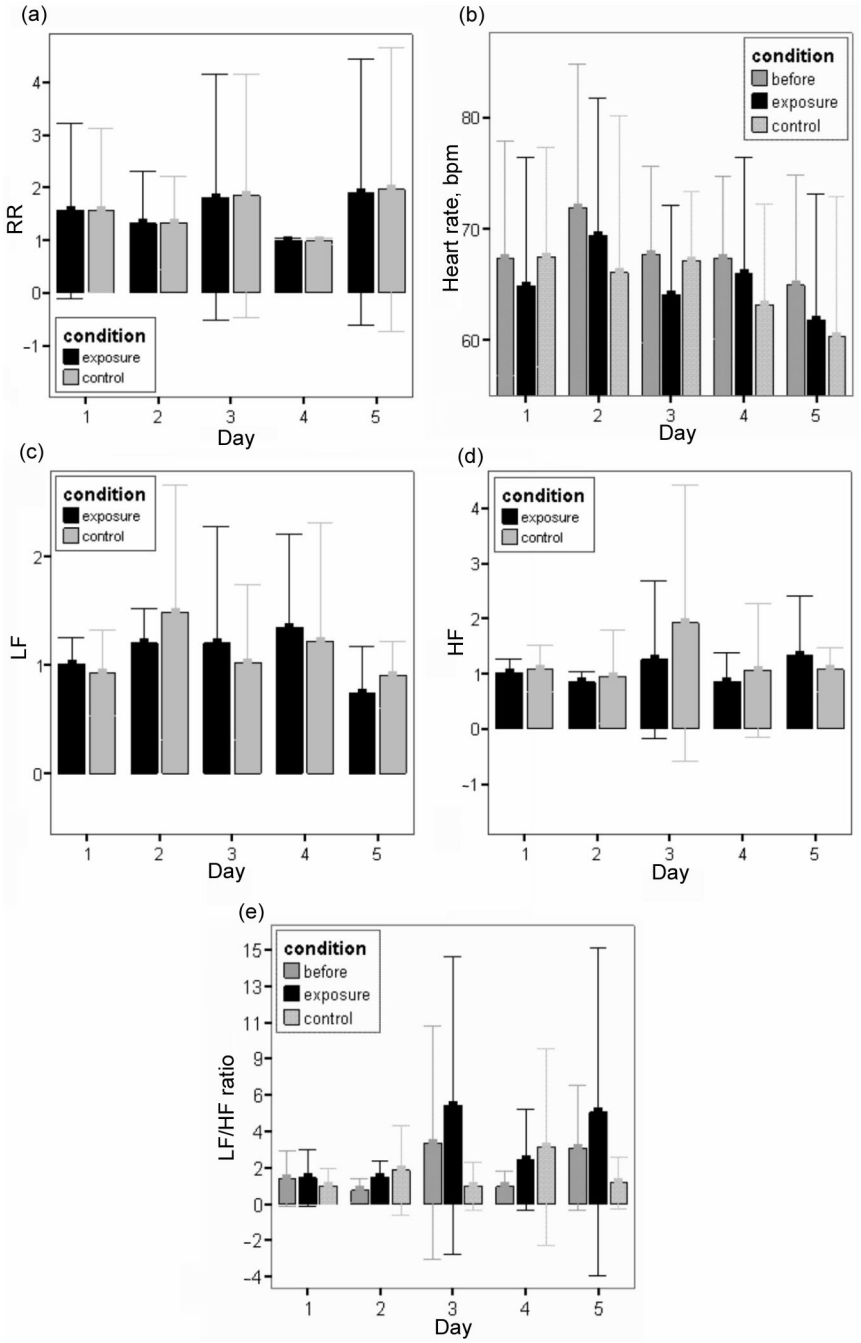

Fig. 3. The 95% confidence interval (*y*-axis) bar plots of ECG's RR (a), heart rate (b), HRV's low frequency band (c), HRV's high frequency band (d), and HRV's low/high frequency band ratio (e). The *x* axis represents the days of testing. The legends represent the PEMF conditions.

3.2. PPG results

The total PPG band results are shown in Fig. 4a. The most obvious difference between before, exposure and control was revealed on days 2 (before 1.38±0.16, exposure 1.24±0.065 and control 1.34±0.11) and 4 (before 1.32±0.08, exposure 1.22±0.035 and control 1.34±0.08). The PEMF exposure was substantially lower than before and control. Similar decrease in exposure in respect to before and control was exhibited at the HF PPG band (Fig. 4c) on days 3 (before 0.21±0.10, exposure 0.11±0.03 and control 0.21±0.09) and 4 (before 0.71±0.40, exposure 0.34±0.18 and control 0.45±0.15). The only time the exposure was slightly higher than both the before and control was on days 2 and 5 (before 1.116±0.002, exposure 1.119±0.003 and control 1.115±0.002) at the LF PPG band, as shown in Fig. 4b.

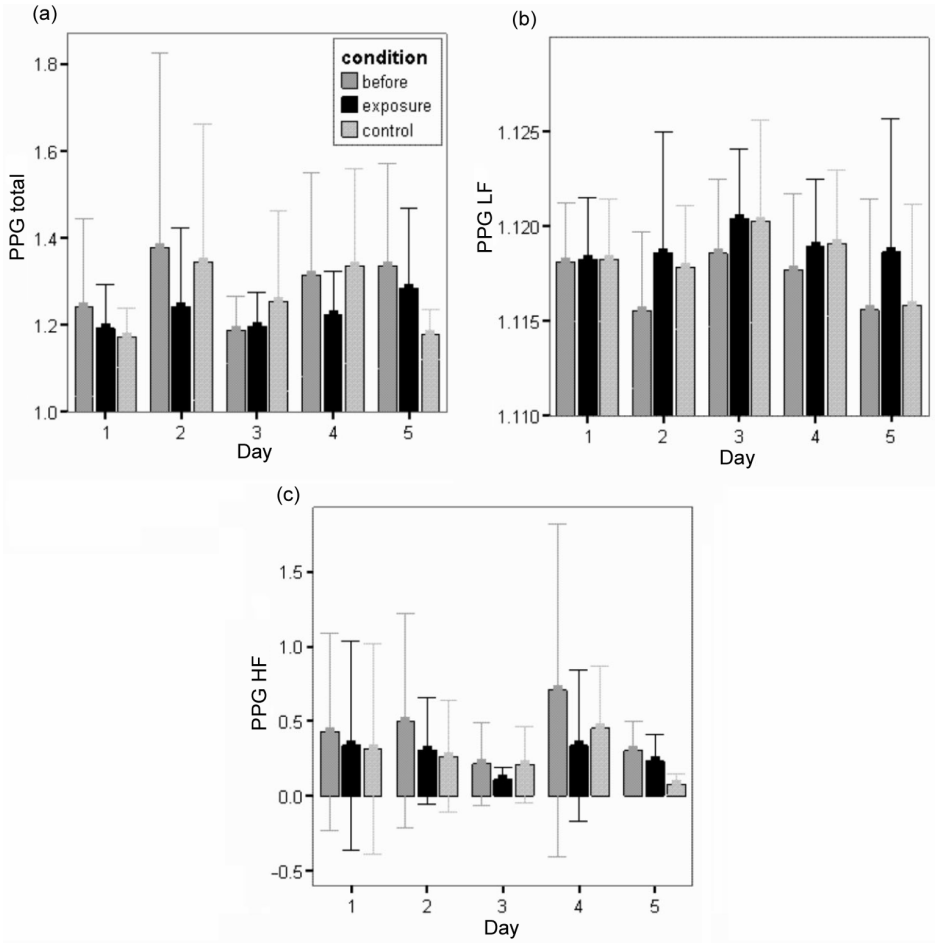

Fig. 4. The 95% confidence interval (*y* axis) bar plots of PPG's total frequency band (a), PPG's low frequency band (b) and HRV's high frequency band (c). The legend represents the PEMF conditions.

3.3. Skin temperature results

The skin temperature (ST) results are shown in Fig. 5a as the ratio of exposure/control (post) and before (pre). An interesting characteristic of this result is the fact that the control is higher than exposure on days 1, 2 and 3, and the exposure is higher than control on days 4 and 5. The pre-stimulation recording of 'before' was higher than both the exposure and control on the first and last day of testing. On the day 3 of testing, both exposure and control conditions

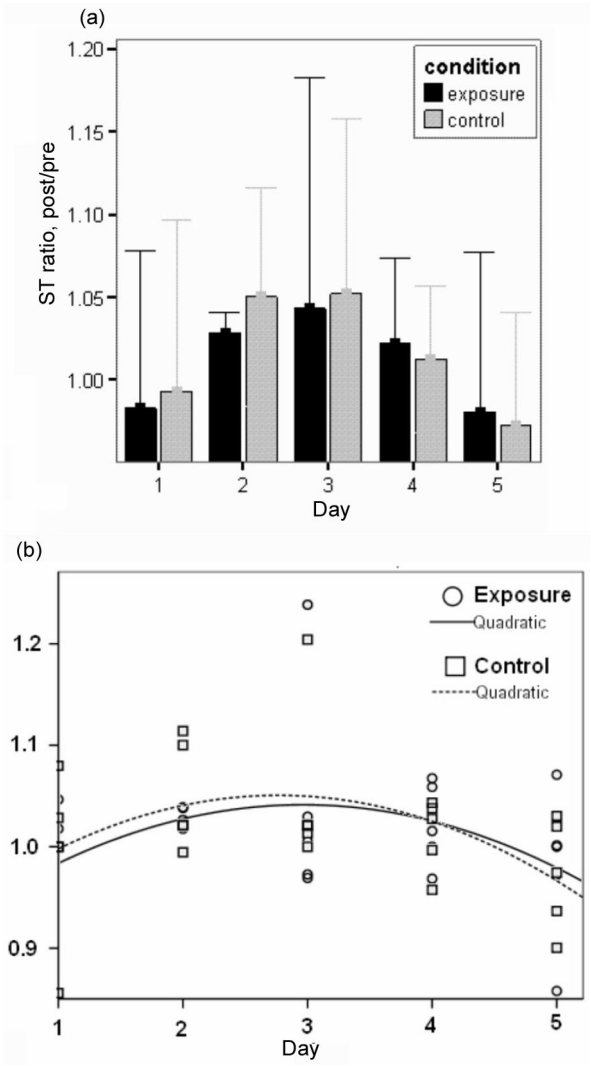

Fig. 5. The skin temperature results, relative difference between after and before (*y* axis), are represented by the 95% confidence interval bar (a) and quadratic curve estimation plot (b). The legend represents the PEMF conditions.

exhibited maximum relative difference, 1.05 and 1.04, respectively. The ST data was further described using quadratic curve estimation, represented by the following equations (Fig. 5b):

$$Y_{\text{contr}} = 0.921 + 0.093t - 0.017t^2,$$

$$Y_{\text{exp}} = 0.910 + 0.089t - 0.015t^2.$$

The intersection of the two curves occurs on the day 4 that describes the commencement of the increase in exposure over the control condition.

3.4. EEG results

The EEG hemispheric asymmetry of anterior-posterior (A-P) and right-left (R-L) was computed for delta, theta, alpha and beta bands (Fig. 6). The positive ratio indicated the predominance of anterior (A-P) and right (R-L) hemispheres, whereas the negative ratio indicated the posterior and left hemisphere predominance over the 5 day testing period. The most evident difference between the anterior and posterior hemisphere in Fig. 6a is shown at the theta band, where the exposure condition exhibited predominance at the posterior hemisphere throughout the 5 days. The results revealed similar characteristics at the control condition, except on days 3 (control 0.09 ± 0.14) and 4 (control -0.03 ± 0.05), which showed the anterior predominance. At the delta band, the main difference in A-P asymmetry occurs on the day 2, with exposure (anterior) and control (posterior). The alpha band exhibited a slight asymmetric A-P difference on the days 1 (exposure (anterior) and control (posterior)) and 2 (exposure (posterior) and control (anterior)). There were minimal A-P asymmetric changes in the beta band on the day 3, where the control condition showed the only posterior shift.

The right-left (R-L) hemispheric asymmetry was once again evident at the theta band. From the day 1 to day 5, there seems to be a dynamic transition from the right to the left hemisphere. For the exposure condition, this transition started earlier, from the day 2 (0.001 ± 0.05) to 3 (-0.02 ± 0.05), as shown in Fig. 6b. Whereas, for the control condition, the right to left hemisphere transition commenced later, from the day 4 (0.03 ± 0.04) to 5(-0.04 ± 0.04). The theta band clearly characterized the substantial difference in R-L hemispheric asymmetry at the exposure/control conditions. At the delta band, the only R-L asymmetric difference was revealed on the day 3: exposure (left) and control (right). The alpha band also exhibited a slight change in the R-L asymmetry on days 2, 3 and 5. There was no substantial difference in R-L asymmetry between the exposure and control conditions.

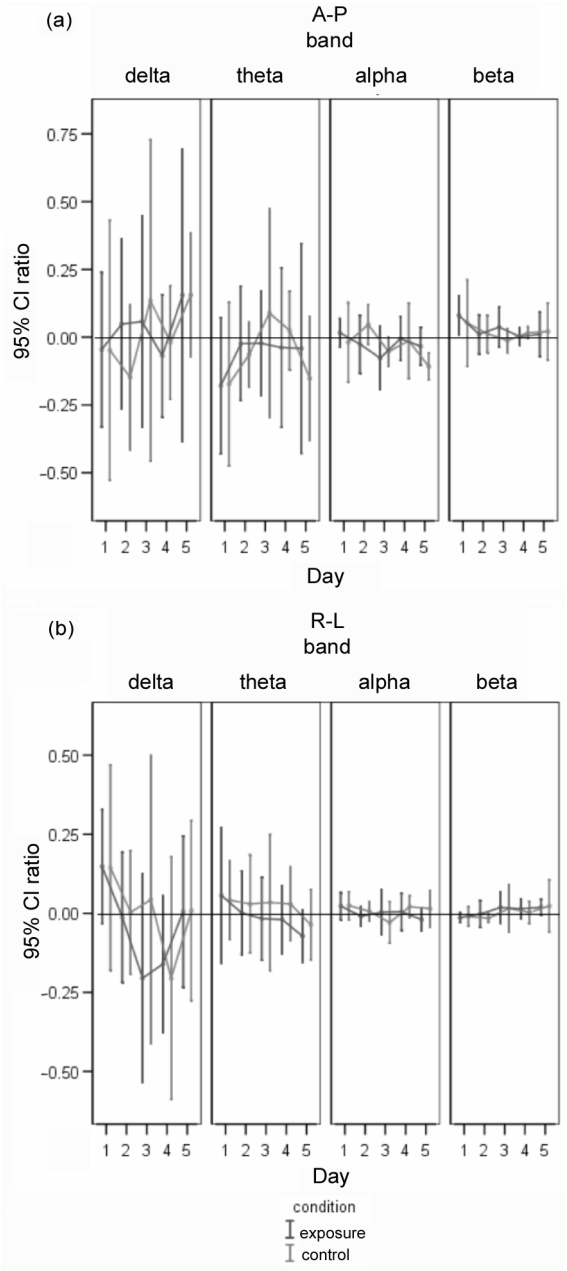

Fig. 6. The EEG hemispheric asymmetry of anterior-posterior (A-P) (a) and right-left (R-L) (b) is computed for delta, theta, alpha and beta bands by the 95% confidence interval line plots of relative difference (after/before) (*y* axis). The 5 days (*x* axis) comparison is shown between the PEMF exposure and control conditions (dark and light lines, respectively) by EEG relative entropy computations. The positive ratio indicates the predominance of anterior (A-P) and right (R-L) hemispheres, whereas the negative ratio indicates the posterior and left hemisphere predominance.

3.5. Skin bioelectric impedance results

The skin bioelectric impedance results of the relative PEMF exposure and control conditions are shown in Fig. 7. On the first day of testing, no substantial difference between the PEMF exposure and control conditions at 100 Hz, 10 kHz, 100 kHz and 1 MHz was observed. The exposure seemed to be smaller than control at the 10 Hz (exposure 1.16 ± 0.16 and control 0.74 ± 0.04) and 1 kHz (exposure 0.67 ± 0.03 and control 1.34 ± 0.17) segments. The 'post-stimulation' (exposure/control) was less than 'pre-stimulation' (before) from 100 Hz to 100 kHz (below the dashed line) and greater than 'before' at 10 Hz and 1 MHz. At the second day, control (1.77 ± 0.28) was substantially higher than exposure (1.05 ± 0.17) at 10 Hz with post-stimulation recording being greater than pre-stimulation (before). The exposure was less than control at 100 Hz, 1 kHz and 10 kHz and slightly greater than control at 100 kHz and 1 MHz. The most

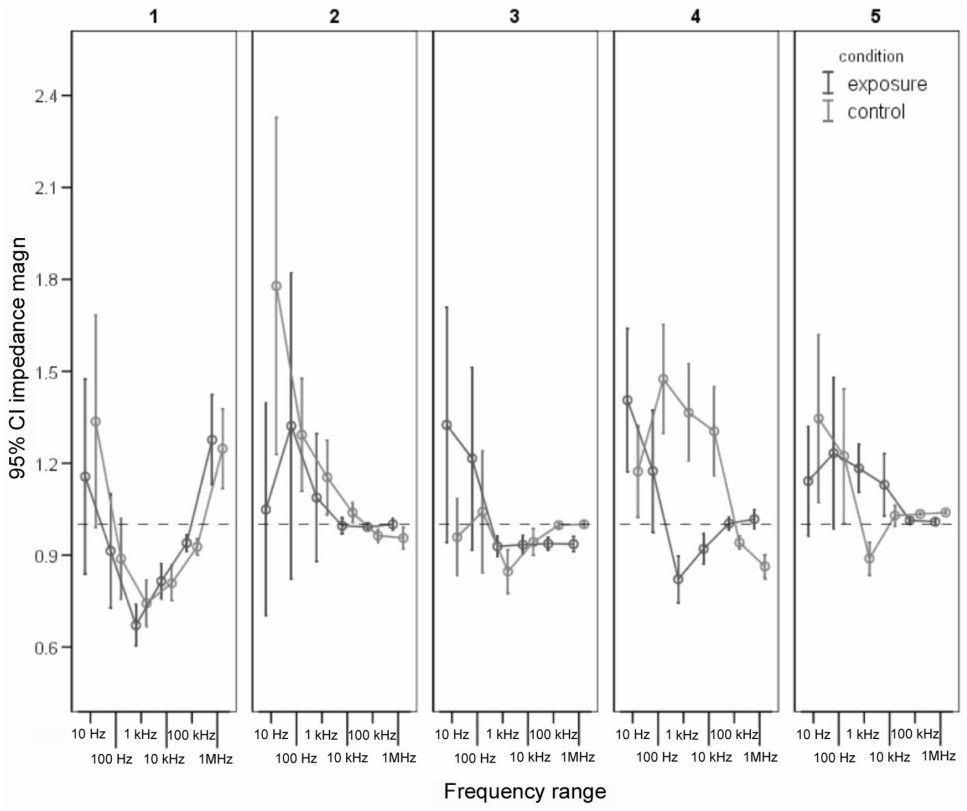

Fig. 7. The 95% confidence interval line plots of relative difference (after/before) of skin impedance magnitude (*y* axis). The dashed line passes through the relative value of 1 and represents the relative skin impedance increase (rel > 1) and (rel < 1) decrease from before to after. The comparison was shown between the PEMF exposure and control conditions (dark and light lines, respectively).

evident difference between the exposure and control conditions was seen on the day 3, greater exposure at 10 Hz (exposure 1.33±0.19 and control 0.96±0.06) and 100 Hz (exposure 1.22±0.15 and control 1.04±0.10), day 4, with substantially smaller exposure from 100 Hz (exposure 1.17±0.10 and control 1.47±0.09) to 10 kHz (exposure 0.92±0.02 and control 1.30±0.07) and day 5, smaller control at 1 kHz (exposure 1.18±0.04 and control 0.89±0.03) and smaller exposure at 10 Hz (exposure 1.14±0.09 and control 1.35±0.14). It was also evident from these results that at higher source frequencies (100 kHz and 1 MHz), the relative difference was minimal (approaching rel = 1) for both exposure and control on days 2 and 5.

4. DISCUSSION AND CONCLUSION

Considering that inadequate statistical tests were conducted due to the small sample size and multiple exposure intervals, our results may only represent the substantial differences with a large variation in high standard errors and therefore *reveal no solid and conclusive evidence in the possible alterations in electrophysiological responses due to PEMF*.

The substantial EEG hemispheric asymmetry alterations were observed at the theta band with the posterior predominance and gradual transition from the right to left hemisphere at the PEMF exposure period. The HRV results revealed a substantial increase in the LF band with inconsistent decrease in heart rate over the entire PEMF exposure period. A substantial increase in the vascular tone at LF PPG band was evident on certain days of PEMF exposure, in addition to a substantial decrease at total and HF PPG bands. The surface skin temperature at PEMF control was higher than exposure during the first half of testing and exposure was higher than control during the second half of testing. The measurements of the skin bioelectric impedance revealed an increase and decrease at PEMF relative exposure between 100 Hz and 10 kHz on days 4 and 5, respective.

Future studies might consider an inter-subject and intra-subject variance and perceived sensitivity to electromagnetic fields (electromagnetic hypersensitivity) [25–26]. Individual differences as well as sensitivity to EMF between subjects may cause high variability in a group. One possibility would be evaluation of alterations of individual subjects during at least a 10 weekday testing with an exclusion of the weekend, which would make it semi-consecutive, making a statistically liable test with at least 10 samples. Another option is to conduct a month-long testing (excluding weekends) on two (normal and hypersensitive) human subjects. If we were to extend our pilot study by pursuing this individual subject investigation, our pilot study protocol will need to be modified accordingly.

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Mitmesed inimese elektrofüsioloogilised reaktsioonid kiiritamisele ultramadalsagedusliku impulsselektromagnetväljaga: pilootuuring

Dean Cvetkovic, Qiang Fang ja Irena Cosic

Pilootuuring teostati muutuste hindamiseks mitmesugustes elektrofüsioloogilistes elutoimingutes, kui inimest mõjutati ultramadalsagedusliku impulsselektromagnetväljaga (UIEMV) 10 minutit päevas viie järjestikuse päeva jooksul. Iga päev mõõdeti viiel inimesel elektroentsefalograafilisi (EEG), elektrokardiograafilisi (EKG) ja fotopletüsmograafilisi signaale, naha bioelektrilist impedantsi ning temperatuuri. Uuringutes täheldati muutusi EEG teetariba signaalide poolkeradevahelises asümmeetrias, tõusu südame taktsageduse spektri madalsageduslikus osas, muutusi nahatemperatuuris ja bioimpedantsi fluktuatsioonides. Mõõtetulemuste hajuvus oli väga suur, seepärast ei pruugi need tõeselt peegeldada elektrofüsioloogilist reaktsiooni UIEMV toimele ega selle poolt tekitatud võimalike muutuste olemasolu.

Effect of Pulsed Electromagnetic Field on Healing of Mandibular Fracture: A Preliminary Clinical Study

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Mushira Dahaba, BDS, MSc, PhD‡

Purpose: The aim of the present study was to evaluate the effect of a pulsed electromagnetic field on the healing of mandibular fractures. Pulsed electromagnetic fields have been shown to accelerate healing of fractures of the long bones.

Patients and Methods: A total of 12 patients with mandibular fractures were selected for the present study. Each patient was treated by closed reduction using maxillomandibular fixation (MMF) and was assigned into 1 of 2 equal groups. The fracture sites of group A only were exposed to pulsed electromagnetic fields (PEMF) 2 hours daily for 12 days, after 2 weeks postoperatively the MMF was removed. For group B (control group), the MMF was removed at 4 weeks postoperatively. The effectiveness of the 2 treatment modalities was evaluated clinically and radiographically using computerized densitometry. The data were statistically analyzed.

Results: After releasing the MMF, a bimanual mobility test of the fractured segments showed stability of the segments in all cases. An insignificant difference was found between the mean bone density values of the 2 groups at all study intervals. In contrast, the percentage of changes in bone density of the 2 groups revealed that group A had insignificant decreases at the 15th postoperative day and a significant increase 30 days postoperatively compared with group B.

Conclusions: From the present limited series of patients, PEMF stimulation might have a beneficial effect on the healing of mandibular fractures treated with closed reduction. However, additional research, using randomized controlled trials, should be conducted to ascertain its effectiveness compared with other treatment modalities.

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Mandible fractures are the second most frequent of the facial bone fractures.[1,2] The treatment of mandibular fractures has evolved for thousands of years and will likely continue to change. The main objectives have been to restore the normal pain-free range of mandibular motion and the preinjury occlusion and contour of the mandible.[3] The basic treatment principles of mandibular fractures include reduction, fixation, immobilization, and supportive therapies.[3,4] These principles have been achieved using 1 of 2 methods. The first has involved wiring the teeth and jaws together for a period of 4 to 6 weeks to allow the broken jaw to heal (closed treatment). The second method has entailed surgical exposure of the fracture to allow the reduction and stabilization of the broken bone (open treatment).[3,4]

Closed treatment of mandibular fractures offers valuable advantages, including obviation of hospitalization, surgical morbidity, and the relatively high cost.[5-7] However, the need for a relatively long period of immobilization with the subsequent delay of rehabilitation has been its main disadvantage. Therefore, a reduction of the immobilization period by accelerating healing of the broken bone has been the topic of

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numerous studies.[8-12] A number of simple, noninvasive approaches have been used to enhance bone healing, including low-intensity pulsed ultrasound[8-10] and PEMF, with varying success.

The beneficial effect of PEMF stimulation on bone healing has been reported. In 1976, Bassett et al[13] demonstrated augmentation of bone repair by inductively coupled electromagnetic fields. In 1992, Greenough[14] reported that PEMF might affect tissue healing through a primary effect on vascular growth. This potential mechanism for stimulation of the healing process was supported by Roland et al.[15] Moreover, Smith et al[16] showed that local application of PEMF waveforms elicited significant arteriolar vasodilation in the rat muscle.

A number of reports have shown the clinical application of PEMF in stimulating osteogenesis in patients with fracture nonunion,[17,18] treating delayed healing of foot and ankle arthrodesis,[19] increasing spine fusion,[20-22] and treating femoral head osteonecrosis.[23] PEMF stimulation in limb-lengthening procedures enhanced callus formation and maturation at the distraction site, allowing earlier removal of the external fixation device.[24] However, its mechanism of osteogenesis enhancement has not been clear.[25] The use of PEMF to stimulate osteogenesis in patients with mandibular fracture has not yet been reported. Therefore, the aim of the present study was to investigate the effectiveness of PEMFs in enhancing the healing of mandibular fractures treated by closed reduction and a short period of maxillomandibular fixation (MMF).

The assessment of bone healing of mandibular fracture presents a problem for maxillofacial surgeons because of the few methods available. Computer-assisted densitometric image analysis (CADIA) has been used to quantify the variations in bone mineralization occurring in many pathologic conditions (eg, osteoporosis, osteomalacia, hyperparathyroidism).[26] This objective method offers excellent measurement reproduction and a high level of correlation between the values obtained and the loss or gain in bone mineral, as determined by atomic absorption spectroscopy after successive periods of bone demineralization.[27]

Patients and Methods

STUDY DESIGN

The present study was a prospective study of 12 patients with mandibular fracture selected from those attending the outpatient clinic of the oral and maxillofacial surgery department (Cairo University Faculty of Oral and Dental Medicine). The selection was determined using the following criteria:

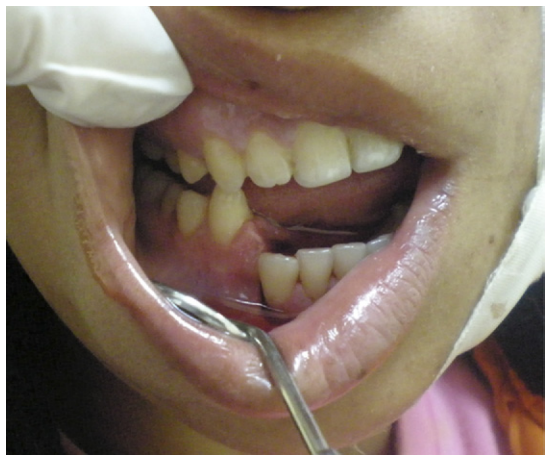

FIGURE 1. Photograph showing mandibular fracture at tooth-bearing area in patient B2.

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1. Mandibular fracture at a tooth-bearing area (Fig 1)
2. Sufficient occluding teeth present on either side of the fracture to allow MMF using an arch bar or eyelet wiring
3. No infection at the fracture site
4. No systemic problems that could affect normal bone healing
5. Patients who chose closed reduction after a discussion of the options regarding closed or open reduction

On initial presentation to the department, the patients were clinically and radiographically evaluated. The demographic data of the selected patients, etiology of the fracture, and the fracture location are listed in Table 1.

TREATMENT PHASE

Each patient received 75 mg diclofenac sodium (Voltaren) intramuscularly immediately preoperatively. After placing the patient under local anesthesia, the mandible fractures were manually reduced, and the patients were placed into MMF using arch bars and 24-gauge circumdental wires (Fig 2) or eyelet wiring (according to the condition of the teeth and patient cooperation). The teeth present in the fracture line were not removed, unless they were mobile or interfering with reduction of the fracture.

PATIENT GROUPS

The patients were given the option of conventional treatment or PEMF and were thus assigned to 1 of 2 equal groups: group A (patients A1 through A6), the fracture sites were exposed to PEMF for 2 hours daily for 12 days, after 2 weeks postoperatively the MMF was removed; and group B (patients B1 through B6),

Table 1. DEMOGRAPHIC DATA

Pt. No.	Age (yr)	Gender	Fracture Location	Fracture Etiology
A1	23	Female	Left parasymphysial/right ramus	Fall
A2	19	Male	Right body	Interpersonal violence
A3	22	Male	Left body	Interpersonal violence
A4	19	Male	Left body	Vehicular accident
A5	36	Male	Left body	Vehicular accident
A6	23	Male	Left body	Vehicular accident
B1	18	Male	Left body/right angle	Vehicular accident
B2	13	Female	Right parasymphysial/left angle	Vehicular accident
B3	59	Male	Symphysial	Vehicular accident
B4	27	Male	Left parasymphysial	Interpersonal violence
B5	43	Female	Left body	Interpersonal violence
B6	35	Male	Left body	Vehicular accident

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the fracture sites acted as the controls, with the MMF removed 4 weeks postoperatively.

POSTOPERATIVE CARE

All patients received 1,500 mg sulbactam (Unicatm) intramuscularly every 12 hours for 4 days, pain medication, and chlorhexidine mouth rinse.

The fracture sites in group A only were exposed to PEMF for 2 hours daily for 12 days using EM-probe Solo device (pulse duration 200 nanoseconds, rise time 8 nanoseconds; electromagnetic segment at 50 MHz and down to kilohertz range) (Fig 3). The pulse was carrier modulated at 72 Hz. All PEMF sessions were performed in the oral and maxillofacial department (Cairo University Faculty of Oral and Dental Medicine, Cairo, Egypt) by one of us (A.A.). PEMF was applied at the fracture site as illustrated in Figure 4.

MMF was maintained for 2 weeks in group A and for 4 weeks in group B, except for patient B3, who was 13 years old. For that patient, the MMF was removed at 22 days postoperatively. The patients maintained a liquid and pureed diet during the MMF period. They were instructed to continue a soft diet for 3 weeks after MMF removal.

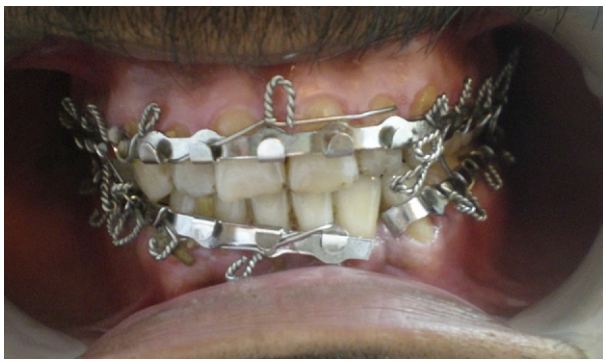

FIGURE 2. Photograph showing arch bar used for patient B4.

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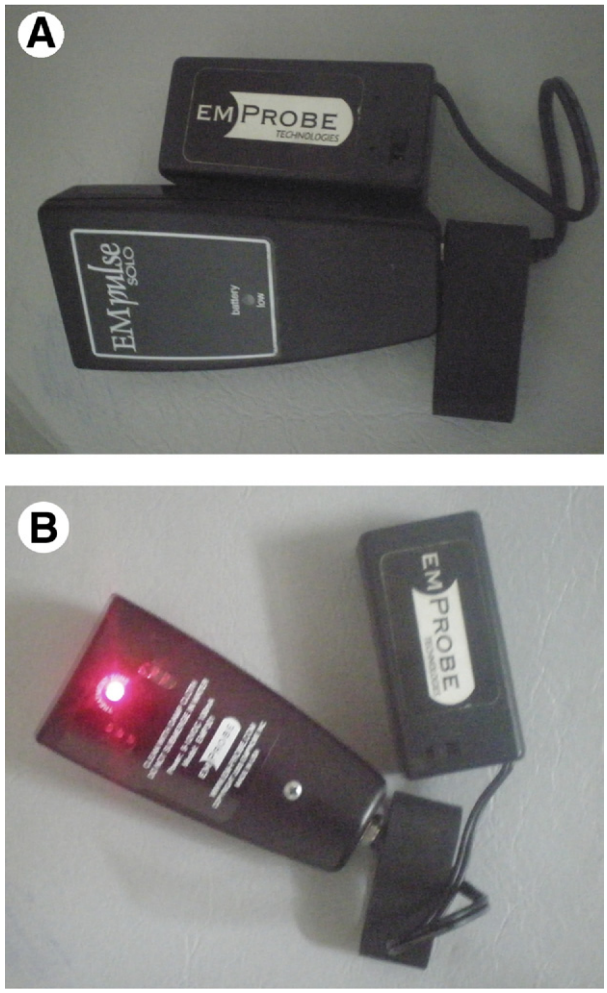

FIGURE 3. *A,B,* Photographs showing EM-Probe device used.

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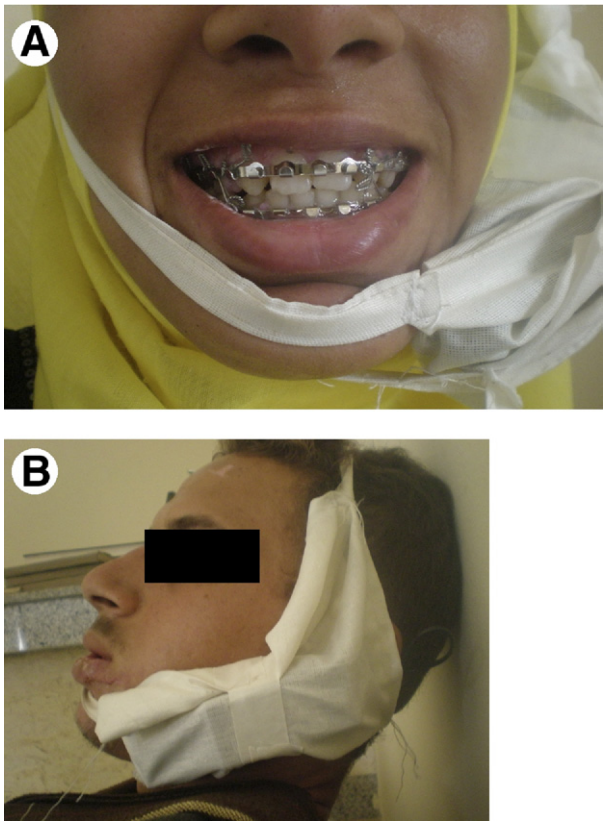

FIGURE 4. Photographs showing PEMF session using EM-Probe device. *A,* Patient A1; and *B,* Patient A4.

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POSTOPERATIVE FOLLOW-UP

All patients were given follow-up appointments for postoperative days 1, 6, 15, and 30. At each appointment, the patients were evaluated and data was collected.

CLINICAL EVALUATION

The evaluated parameters of outcome were occlusion, pain, segment mobility, and the sign and symptoms of infection. Occlusion was assessed by patient self-assessment. Pain was evaluated using a scale noting no pain, mild, moderate, or severe pain at the fracture site. The segment mobility was noted as absent or mobile. The signs and symptoms of infection included the presence of erythema, edema, or purulent drainage over the fracture site.

RADIOGRAPHIC EVALUATION

Three standardized digital panoramic radiographs were taken for each patient immediately and 15 and 30 days postoperatively. The bone density was measured and recorded on all radiographs. All collected data were tabulated and statistically analyzed.

All postoperative radiographs were taken using a direct digital panoramic machine (OT100 Instrumentarium Imaging, GE, Finland 2003) using the following exposure parameters: 85 kVp, 16 mA, and exposure of panoramic program set at 17.6 seconds. The same exposure parameters (which were electronically controlled according to preprogrammed procedures) were kept constant for the baseline and follow-up radiographs.

The digital images were manipulated using the ImageJ software (ImageJ is a public domain Java image processing program inspired by the National Institutes of Health). ImageJ can be used to measure the area mean and pixel value density; gray scale calibration is also available.[28] On each image, an analysis of the changes in the mean gray value was performed using the line measurement facility of the software used. The unit of measurement for bone density is pixels (mean gray value). Successive lines were drawn along the whole length of each of the investigated fracture lines. The densitometry values were obtained for each line expressed in gray levels from 0 to 255. Each of these values corresponded to the average density of the fracture area (Fig 5).

An analysis was performed by the same radiologist twice at 2 different sessions, with a 1-week interval in between in an attempt to eliminate intraobserver error. The data of the 2 trials were pooled, and the mean was included in additional statistical analysis. All collected data were then tabulated and statistically analyzed. The data are presented as the mean ± standard deviation. The data were explored for normality using the Kolmogorov-Smirnov test. Student *t* test was used to compare the differences between the 2 groups. The paired *t* test was used to study the changes by time within each group. The significance level was set at $P \leq .05$. Statistical analysis was performed using the Statistical Package for Social Sciences, version 16.0, for Windows (SPSS, Chicago, IL).

Results

All patients passed the 1-month follow-up period for inclusion in the present study. Of the 12 patients, 9 were males (75%) and 3 were females (25%). Their age range was 13 to 59 years (mean 28). The etiology of fractures in the present study was a motor vehicle accident in 7 (58.3%), interpersonal violence in 4 (33.3%), and an accidental fall in 1 patient (8.3%).

All patients had developed mild edema immediately postoperatively. The edema had started to resolve by the third postoperative day and had completely resolved by the end of the first postoperative week. The intraoral and/or extraoral wounds had healed by the end of the first postoperative week. Infection related

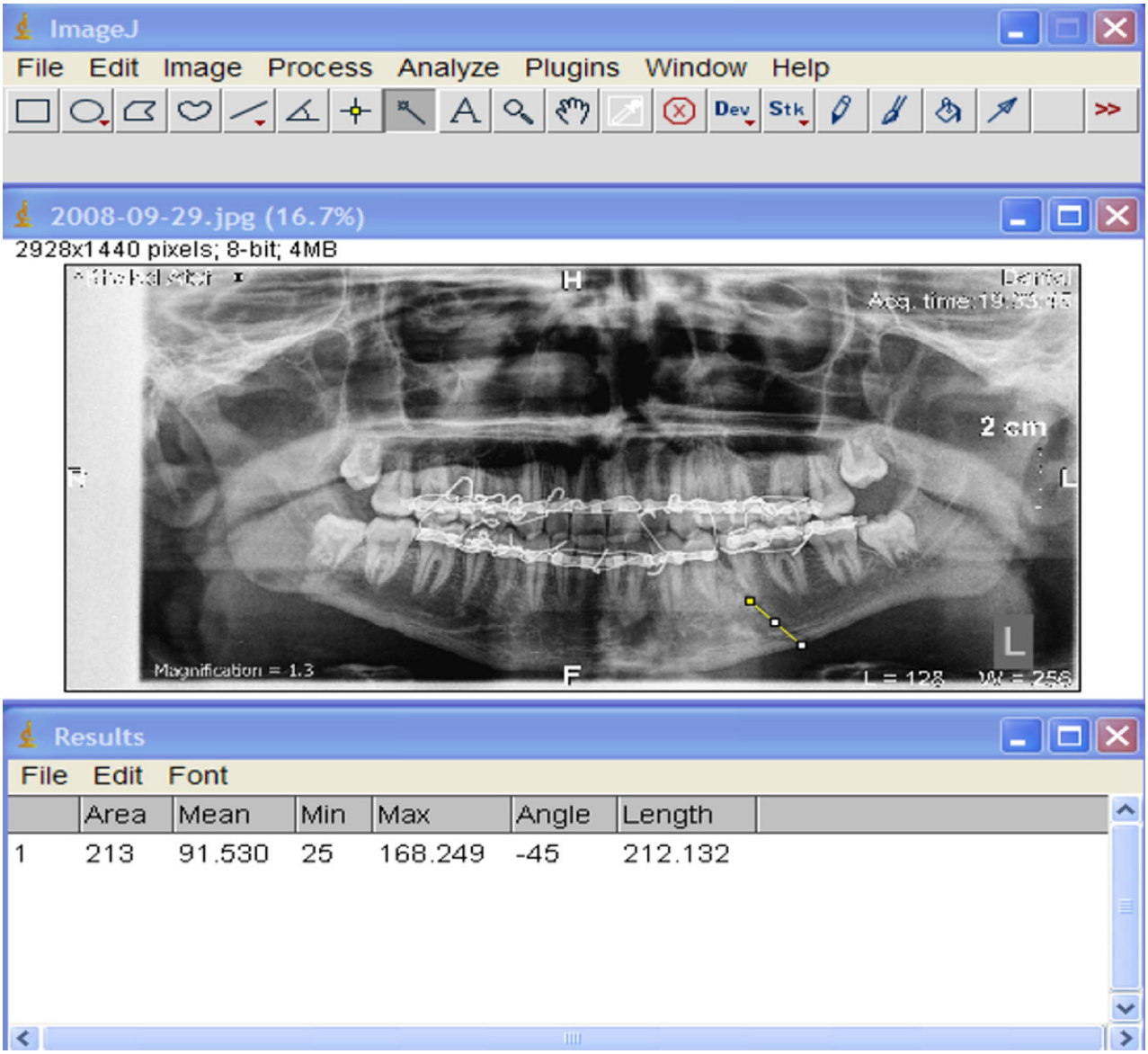

FIGURE 5. Panoramic radiograph of patient B1 demonstrating 1 of the lines drawn using ImageJ software. Similar lines were drawn parallel and 1-mm apart from this line until the whole fracture area had been covered.

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to the fracture line was observed in 2 patients at the second postoperative week. The infection was successfully treated with sulbactam (Unicatm) 1,500 mg intramuscularly every 12 hours for 4 days. In group A, PEMF was well tolerated by all patients. The pain intensity had decreased from severe to mild by the end of the first postoperative week. In contrast, the patients in group B had reached this grade by 2 weeks postoperatively. After releasing the MMF (after 2 weeks for group A and 4 weeks for group B), the bimanual mobility test of the fractured segments showed stability of the segments in all patients. The preinjury occlusion had been maintained in all patients.

RADIOGRAPHIC FINDINGS

The postoperative radiographs of all patients revealed good bony alignment of the bony segments (Fig 6). An insignificant difference was found between the mean bone density values of the 2 groups throughout the study period (Table 2 and Fig 7). However, the changes in bone density within the same period were dependent on the treatment modality used. This became obvious after the expression of the increase or decrease in the bone densities in percentages. At 15 days postoperatively, the mean density in the fracture sites had decreased by 2.3% on average in group A and by 6% on average in group B. At 30 days

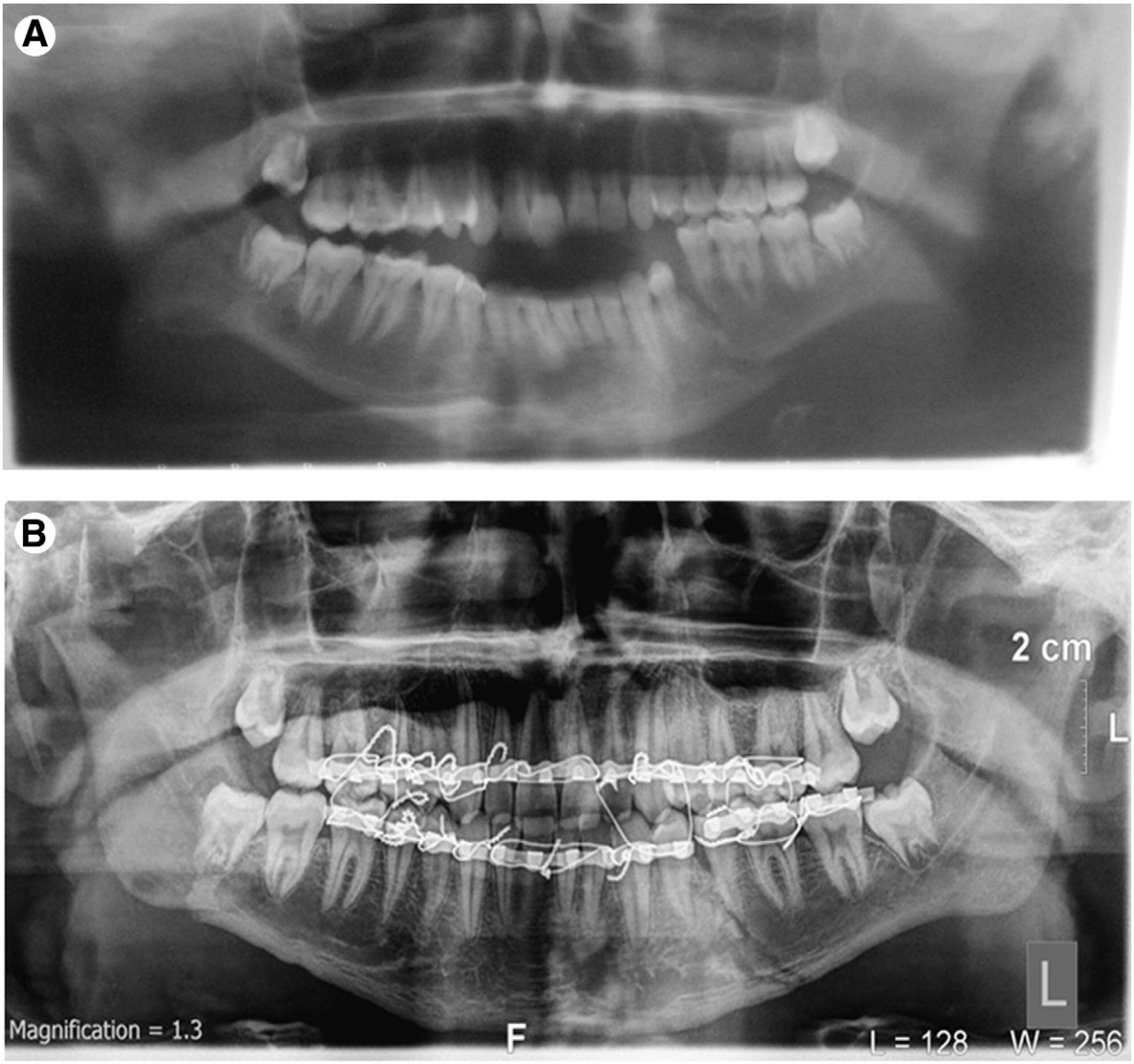

FIGURE 6. Panoramic radiographs showing good bony alignment of bony segments of patient B1. *A,* Preoperative radiograph and *B,* immediate postoperative radiograph.

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postoperatively, the mean density in the fracture sites in group A had increased by 10.2% compared with the density found at 15 days postoperatively. In contrast, the density in group B had increased by 1.9%. A comparison of the percentage of change in bone density between the 2 groups showed that group A had had an insignificant decrease at the 15th postoperative day and a significant increase at 30 days postoperatively compared with the values found for group B (Table 3 and Fig 8).

Group A showed significant differences between the study intervals except between the immediate postoperative examination and the 30-day postopera-

Table 2. RESULTS OF STUDENT *t* TEST FOR COMPARISON BETWEEN BONE DENSITIES OF 2 GROUPS

Period	Group A	Group B	*P* Value
Baseline	138.5 ± 32.4	124.5 ± 35.4	.492
15 d	135.4 ± 32.4	115.7 ± 34.5	.330
30 d	147.3 ± 28.5	118.5 ± 38.3	.171

Data presented as mean ± standard deviation.

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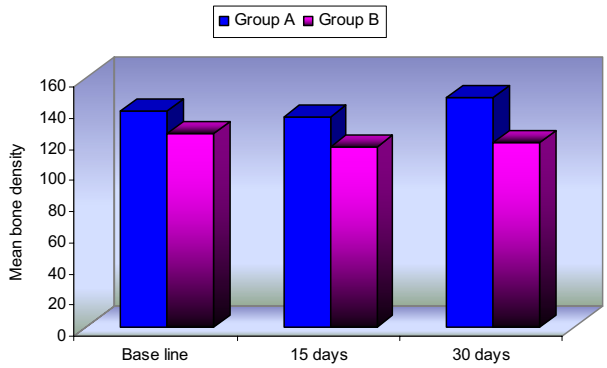

FIGURE 7. Mean bone density values of 2 groups at each study interval.

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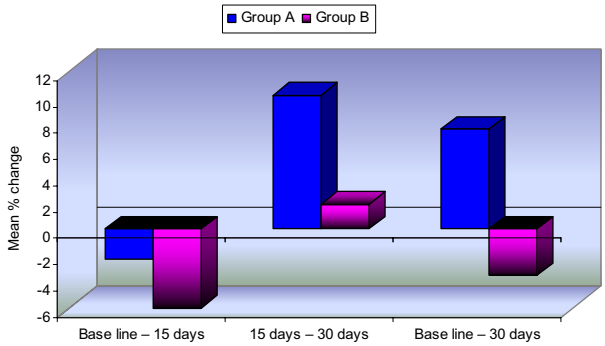

FIGURE 8. Percentage changes in bone density in 2 groups.

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tive examination (Table 4 and Fig 9). In contrast, insignificant differences were noted between the study intervals in group B (Table 5 and Fig 9).

Discussion

Numerous studies have focused on the outcome and morbidity associated with the treatment of mandibular fractures. However, some controversy remains regarding the optimal treatment modalities for these fractures. Closed reduction techniques have yielded a lower level of complications compared with open techniques; however, the need for a relatively long period of immobilization, with the subsequent delay of rehabilitation, has been their main drawback.[29-31] Traditionally, the duration of MMF used for adult mandibular fractures has been 6 to 8 weeks.[32] In the present study, all the patients in group B had clinical stability after removal of the MMF at 4 weeks postoperatively (100%). This finding is in general agreement with those of other studies.[33,34] Juniper and Awty[33] reported that 80% of mandibular fractures treated with open or closed reduction and MMF were clinically united by 4 weeks. Amaratunga[34] found that 75% of mandibular fractures were clinically stable by 4 weeks, that almost all fractures in children had healed within 2 weeks, and that a significant number of fractures in older patients needed 8 weeks to heal. Al-Belasy[35] found that the period required for the healing of mandibular fractures in the tooth-bearing area treated by MMF was 4.67 ± 0.72 weeks.

Amaratunga[34] suggested the application of a short 2-week period of immobilization, followed by splinting the lower jaw with an arch bar or acrylic splint, or a period of a soft diet as options available to the surgeon for the treatment of mandibular fracture. Al-Belasy[35] compared the use of a 2-week period of MMF followed by an arch bar splint wired to the lower jaw for an additional 4 weeks with a 6-week period of MMF for the treatment of mandibular fractures in the tooth-bearing area and found the period required for fracture healing was 4.93 ± 0.7 and 4.67 ± 0.72 weeks, respectively. In contrast to the modification of the stabilizing methods of closed treatment, the objective of the present study was to enhance bone healing with PEMF stimulation. It was impressive that the mandibular fractures treated with simultaneous PEMF stimulation and MMF for 2 weeks (group A) were clinically stable within 2 weeks. This could be explained by the conclusions from other orthopedic studies[36-39] that the use of PEMF accelerates bone regeneration, increases osteogenesis in vitro,[40] and maturation of callus in vivo.[41] However,

Table 3. RESULTS OF STUDENT *t* TEST FOR COMPARISON BETWEEN PERCENTAGE OF CHANGES IN BONE DENSITY

Period	Group A	Group B	*P* Value
Baseline to 15 d	−2.3 ± 1.4	−6 ± 2.7	.563
15 d to 30 d	10.2 ± 3.4	1.9 ± 1	.042*
Baseline to 30 d	7.6 ± 3.7	−3.5 ± 1.2	.033*

Data presented as mean % ± standard deviation.
*Significant at $P \leq .05$.

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Table 4. RESULTS OF PAIRED *t* TEST FOR CHANGES BY TIME WITHIN GROUP A

Period	Mean Difference	*P* Value
Baseline to 15 d	−3 ± 2.3	.026*
15 d to 30 d	11.8 ± 10.5	.040*
Baseline to 30 d	8.8 ± 10.4	.092

Data presented as mean ± standard deviation.
*Significant at $P \leq .05$.

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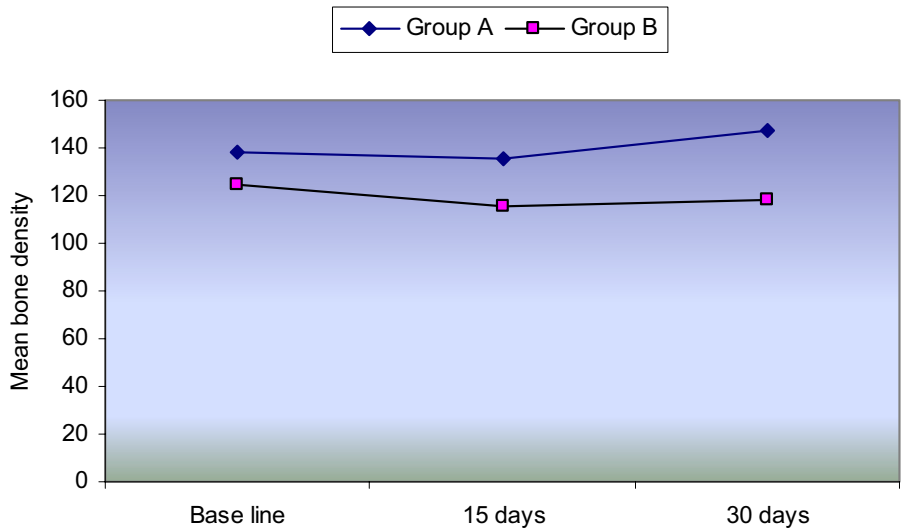

FIGURE 9. Changes in mean bone density by time within each group.

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our findings contradict those from Markov[42] that one should not expect that the magnetic field that is beneficial for superficial wounds would be as good for fracture healing.

In the present study, the reduction of pain intensity from severe to mild by the end of the first postoperative week was noted in all patients in group A. In contrast, the patients in group B reached this grade by 2 weeks postoperatively. This can be attributed to the analgesic and antinociceptive effects of PEMF. Numerous studies have reported on the effectiveness of PEMF in relieving pain with, however, contradictory results.[43-46] Lap et al[43] tested the value of PEMF therapy in the treatment of pain. They found that patients with chronic pain refractory to conventional conservative methods showed significant subjective pain improvement after application of PEMF for 20 minutes daily for 10 days.[43] Other investigators have also reported similar experiences.[44-46] However, Weintraub et al[47] found PEMF ineffective in reducing diabetic neuropathic pain. These inconsistent results could have resulted from the use of fields of varying strengths and frequencies.

Table 5. RESULTS OF PAIRED *t* TEST FOR CHANGES BY TIME WITHIN GROUP B

Period	Mean Difference	*P* Value
Baseline to 15 d	−8.8 ± 18.1	.287
15 d to 30 d	2.9 ± 6.7	.355
Baseline to 30 d	−5.9 ± 11.6	.570

Data presented as mean ± standard deviation.

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A wide variety of diagnostic methods have been used to assess the process of bone fracture healing. However, the necessity for noninvasive and repetitive methods has drawn the attention of many researchers toward the application of CADIA. Experimental studies found a significant correlation between CADIA and the quantitative histologic measurements of bone mineralization[48-51] and proved the validity of CADIA for detecting changes in bone density and mineralization, with highly reproducible measurements.[48-50] In the present study, the digital images were manipulated using ImageJ software, which has been used in published studies to measure the changes in the bone density of the mandible.[52-54] CADIA of both groups showed a decrease in the mean bone densities at 15 days postoperatively, corresponding to the initial stage of healing.[55] At 30 days postoperatively, an increase in these values was noted, corresponding to the second—the "soft" callus—stage of healing.[55] These findings were in general agreement with those from Razukevičius et al,[56] who reported that the greatest decrease in optical density was observed during the second week and that the mean optical densities in the fracture site started to increase, with the greatest increase in the optical densities in the fracture site registered at the sixth to eighth week after repositioning and fixation of the fracture fragments. In contrast, Villarreal et al,[57] in an evaluation of mandibular fracture repair after either MMF or rigid internal fixation using CADIA demonstrated an unexpected increase in density at 15 days and attributed it to soft tissue edema and swelling. At 30 days, a significantly greater decrease in optical density was observed in the MMF group that was attributed to the formation of soft fracture callus.[57]

In the present study, the most striking differences in the healing process between the 2 treatment modalities were noted using quantitative radiodensitometry. The changes in the mean bone density after fracture fragment repositioning and fixation were analogous in both groups, as evident by the lack of a significant difference between mean bone densities in the 2 groups throughout the study period. However, the percentage of changes in bone density in group A showed an insignificantly lower decrease by the 15th postoperative day. This could be explained by the observation of Cruess et al[58] that PEMF reduced bone loss (osteoclasis) in animals subjected to disuse osteoporosis. A significantly greater increase in the percentage of changes in bone density in group A was also noted at 30 days postoperatively. This might have been the result of enhanced osteogenesis, because PEMF has been shown to increase osteogenesis in vitro[40] and the maturation of callus in vivo.[41] Therefore, it seems that the percentage of changes in the bone densities within the same period were dependent on the treatment modality used. This finding confirms that of Razukevičius et al[56] in their comparative analysis of the effectiveness of the mandibular angle fracture treatment methods. They showed that the percentage of changes in bone densities rather than their mean values was dependent on the fracture fragment fixation method.

According to the findings from our limited series of patients, PEMF stimulation might have beneficial effects on the healing of mandibular fractures treated with closed reduction. However, additional research, using randomized controlled trials, should be conducted to ascertain its effectiveness compared with other treatment modalities.

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ORIGINAL ARTICLE

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Effectiveness of pulsed electromagnetic field therapy in lateral epicondylitis

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Abstract We aimed to investigate the efficacy of pulsed electromagnetic field (PEMF) in lateral epicondylitis comparing the modality with sham PEMF and local steroid injection. Sixty patients with lateral epicondylitis were randomly and equally distributed into three groups as follows: Group I received PEMF, Group II sham PEMF, and Group III a corticosteroid + anesthetic agent injection. Pain levels during rest, activity, nighttime, resisted wrist dorsiflexion, and forearm supination were investigated with visual analog scale (VAS). Pain threshold on elbow was determined with algometer. All patients were evaluated before treatment at the third week and the third month. VAS values during activity and pain levels during resisted wrist dorsiflexion were significantly lower in Group III than Group I at the third week. Group I patients had lower pain during rest, activity and nighttime than Group III at third month. PEMF seems to reduce lateral epicondylitis pain better than sham PEMF. Corticosteroid and anesthetic agent injections can be used in patients for rapid return to activities.

Keywords Electromagnetic fields therapy · Epicondylitis

Introduction

Lateral epicondylitis is defined as pain and tenderness related to the overuse injury of soft tissues around lateral epicondyle, which attach to this region [1, 2]. Extensor carpi radialis brevis muscle consists of a keel-shaped tendon with attachments to extensor carpi radialis longus, extensor digitorum communis, supinator muscles and attachments to the radial collateral ligament, the orbicular ligament, the capsule of the elbow joint, and the deep fascia [3]. Extensor carpi radialis brevis and extensor digitorum communis especially produce the largest increases while the superficial head of supinator produce a moderate increase in tensile force in the common extensor tendon. The excessive stress may damage the muscle attachment and cause inflammation and pain [4]. Lateral epicondylitis is a common problem effecting 1–2% of the general population [5]. Because it causes reduction in the grip strength, it restricts daily living activities. Epidemiological studies have shown that it leads to sick leave and decrease in work capacity [6]. Despite its self-limiting character, recurrence is possible in many patients. Thus, it should be treated promptly and effectively [7, 8].

Conservative treatment with its most important components, such as rest and activity modification, is reported to be the main therapeutic approach [1]. In addition, nonsteroidal anti-inflammatory drugs can be administered. Also, some physical therapy agents such as ultrasound, iontophoresis, extracorporeal shock wave therapy, laser, and acupuncture can be useful [9–13]. In cases of failure to respond to those initial treatment methods, local anesthetic and corticosteroid injections may be of use [8, 14, 15]. Deconditioning response of the forearm muscles to the pain in lateral epicondylitis can be treated by passive stretching and isometric strengthening of wrist extensor muscles followed by resistive painless exercises in rehabilitation programs for a more rapid return to a normal functional level of strength and flexibility [14, 16]. Despite the existence of many therapy choices, there is still no consensus on the effectiveness of these methods because randomized controlled studies are lacking [6, 7].

Pulsed electromagnetic field (PEMF) therapy can be used in medical practice and also musculoskeletal disorders as a therapeutic agent. There are two ways of PEMF application. The first method is the placement of opposing electrodes of the device in direct contact with the

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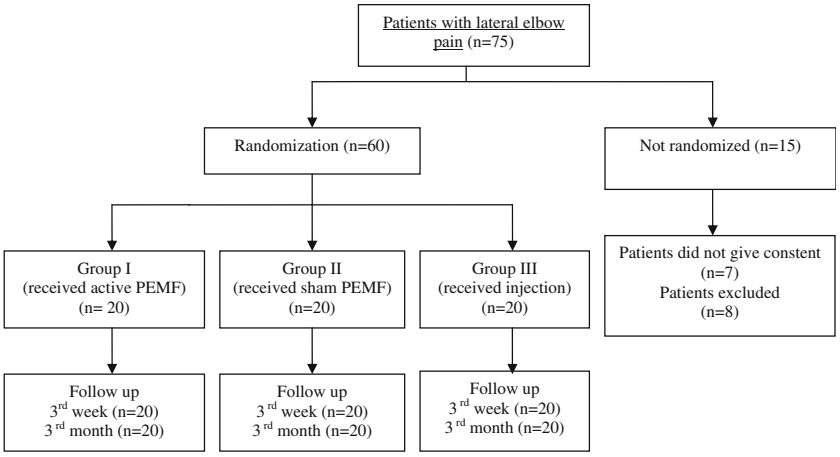
Table 1 Randomization and follow up



skin surface surrounding the tissue of interest and the second method is a PEMF stimulation, which the electrodes of the applicators of the device do not require direct contact with the skin [17]. It has numerous biologic effects on tissues. PEMF is used especially in bone fractures and many musculoskeletal system diseases such as osteoarthritis and rotator cuff tendonitis. Many studies have investigated and reported the benefits of PEMF on these indications [17–22]. However, only one study, to the best of our knowledge, exists which evaluated the success of PEMF on tennis elbow [23]. In fact, experimental studies on biological effects of PEMF are encouraging to use the modality in tennis elbow. The focal degeneration in the extensor wrist tendons that attach to lateral epicondyle and microruptures in collagen fibers due to overuse and repetitive microtraumas are responsible for the occurrence of tennis elbow [24]. No thermal effect can be observed in low frequency PEMF applications. It shows its effectiveness by changing the cell membrane potentials and ion transport. This leads to an anti-inflammatory effect by inhibiting the edema and enhancing microcirculation [25, 26]. Furthermore, some in vitro cellular studies have shown that low frequency PEMF can stimulate collagen production and maintain tendon alignment by induction of both collagen-producing cells and growth factor synthesis such as transforming growth factors-β [27, 28]. So, it seems logical to think that PEMF can decrease edema and induce the healing of collagen fibers in tennis elbow.

We, in this study, aimed to investigate the efficacy of PEMF comparing the modality with sham PEMF and a local steroid injection that is another therapeutic approach.

Materials and methods

This study was approved by a local ethics committee and all patients gave informed consent.

Patient selection Seventy-five patients who applied to the outpatient clinic of Trakya University Hospital, Physical Medicine and Rehabilitation Department with lateral elbow and forearm pain that lasted for more than 6 weeks were evaluated from January 2004 to December 2004. The diagnosis was made based on the tenderness in

Table 2 The comparison of demographic characteristics and evaluated parameters in the groups before treatment

Parameters	Group I (PEMF)	Group II (Sham PEMF)	Group III (Injection)	Significance
Age	46.76±6.24	51.47±8.23	47.80±6.05	NS[a]
Pain duration (month)	4.14±3.24	2.45±0.80	3.37±1.65	NS[b]
Rest pain (VAS)	3.43±2.56	3.39±2.08	4.02±2.05	NS[a]
Activity pain (VAS)	7.26±2.08	7.31±1.47	7.70±1.52	NS[a]
Night pain (VAS)	3.14±3.79	3.47±2.97	2.65±3.74	NS[b]
Pain during resisted wrist dorsiflexion (VAS)	5.98±2.31	6.53±1.94	5.10±1.67	NS[a]
Pain during resisted forearm supination (VAS)	2.59±3.18	4.34±1.47	3.42±2.85	NS[b]
Pain during resisted forearm pronation (VAS)	1.24±2.55	2.79±2.60	2.40±2.50	NS[b]
Algometric pain threshold (kg/cm^2)	3.08±1.09	2.63±0.49	3.04±0.86	NS[a]

Data presented are mean±SD
PEMF Pulsed electromagnetic field, *VAS* visual analog scale, *NS* nonsignificant ($p>0.05$)
[a]Performed by one-way ANOVA
[b]Performed by Kruskal–Wallis ANOVA

Table 3 The changes of outcome parameters within the groups after the interventions

	Group 1				Group 2				Group 3			
	BT	AT	3rd month	*p*	BT	AT	3rd month	*p*	BT	AT (3rd week)	3rd month	*p*
Rest pain (VAS)	3.43±2.56	1.05±1.69[a]	0.09±0.44[b]		3.39±2.08	1.95±1.75[a]	1.79±1.93[b]		4.02±2.05	0.50±0.69[a]	1.40± 2.09[b]	0.000
Activity pain (VAS)	7.26±2.08	3.88±1.90[a]	0.62±0.80[b]	0.000	7.31±1.47	4.42±2.45[a]	3.37±2.16[b]	0.000	7.70±1.52	1.75±1.62[a]	2.75±2.90[b]	0.000
Night pain (VAS)	3.14±3.79	1.52±2.06[a]	0.00±0.00[b]	0.000	3.47±2.97	1.37±2.14[a]	0.74±1.24[b]	0.000	2.65±3.74	0.45±1.36[a]	0.65±2.01[b]	0.011
Pain during resisted wrist dorsiflexion (VAS)	5.98±2.31	2.67±1.49[a]	0.86±0.85[b]	0.000	6.53±1.94	4.16±2.47[a]	3.42±2.09[b]	0.000	5.10±1.67	1.57±1.79[a]	1.75±2.05[b]	0.000
Pain during resisted forearm supination (VAS)	2.59±3.18	1.00±1.34[a]	0.24±0.70[b]	0.000	4.34±1.47	2.39±2.31[a]	1.53±1.74[b]	0.000	3.42±2.85	0.95±1.54[a]	1.4±2.54[b]	0.001
Algometric pain threshold (kg/cm^2)	3.08±1.09	3.61±1.23[a]	4.24±1.24[b]	0.000	2.63±0.49	2.97±0.67[a]	2.98±0.71[b]	0.001	3.04±0.86	3.98±1.29[a]	3.92±1.44[b]	0.000

Data presented are mean±SD. Friedmann ANOVA and Mann–Whitney U tests were used.
BT Before treatment, *AT* after treatment, *VAS* visual analog scale
[a]After treatment, values are significantly different than before treatment values.
[b]Values at third month are significantly different than before treatment values.

the origin of the extensor carpi radialis brevis muscle and increased tenderness of dorsiflexion of the wrist against resistance and of forearm supination. Differential diagnosis from cervical problems and radial tunnel syndrome was made with radiological and electrophysiological investigations in case of an existence of clinical suspicion. The exclusion criteria were as follows: (1) accompanying painful conditions, which may confuse the clinical picture such as upper extremity fracture, inflammatory arthritic conditions, carpal tunnel syndrome, thoracic outlet syndrome, cervical radiculopathy, and tendon ruptures; (2) accompanying medial epicondylitis; (3) contraindications for PEMF such as tuberculosis, pregnancy, cardiac pacemaker, and malignancy; and (4) contraindications for corticosteroid injection. Eight patients were excluded from the study and seven patients did not want to participate so 60 patients were included in the study.

Blinding and randomization The patients were evaluated during their initial visit by investigator A who is experienced in musculoskeletal pathologies and blind to the randomization process and the therapy applications. Investigator A sent the patients to investigator B, who was blind to the clinical status of the patients, for randomization and allocation to the therapy groups. Investigator B sent the patients to investigator C for therapy applications. All the patients were sent back to investigator A 3 weeks and 3 months after their first visit for the post therapy and evaluations after 3 months. So the baseline and subsequent evaluations were performed by an investigator who was blind to the therapies applied. The patients were randomly divided into three groups according to their application turns by investigator B. Group 1 consists of the first, fourth, seventh...and 58th patient, Group 2 the second, fifth, eighth...and 59th patient, and Group 3 the third, sixth, ninth...and the 60th patient. Table 1 shows the randomization and follow-up process of the patients.

Therapy protocols in the groups Active PEMF therapy was performed in Group 1 by a magnetotherapy device (BTL-09, manufactured by BTL Benesov, Czech Republic). The injured elbow of each patient was put in the middle portion of a big circle solenoid applicator in prone position. The dose and application time were selected according to the recommendations of the manufacturer. The total dose applied was 6 mT/session. This dose was completed by applying the PEMF in a frequency of 25 Hz and a frequency of 4.6 Hz, consecutively. A therapy session lasted for 30 min and 15 sessions were performed during 3 weeks (five sessions a week for 3 weeks).

Sham PEMF was used in Group 2. Their elbows were position the same way as with the Group 1 patients in the same applicator. However, the electric current producer was connected to another solenoid applicator (small solenoid). The patient sensed the same visual and auditory stimuli like the patient taking the active therapy, but was not exposed to the real magnetic field.

Local corticosteroid injection was administered to the most painful area with pressure around lateral epicondyle

Table 4 The group comparisons suggesting significant differences between the groups after therapy and at third month

	After treatment (or at 3rd week for injection group)	At 3rd month
Rest pain (VAS)[a]	Group 2 > Group 3	Group 1 < Group 2
		Group 1 < Group 3
Activity pain (VAS)[a]	Group 2 > Group 3	Group 1 < Group 2
	Group 1 > Group 3	Group 1 < Group 3
Night pain (VAS)[a]	NS	Group 1 < Group 3
Pain during resisted wrist dorsiflexion (VAS)[b]	Group 1 > Group 3	Group 1 < Group 2
	Group 2 > Group 3	Group 2 > Group 3
	Group 1 < Group 2	
Pain during resisted forearm supination (VAS)[a]	Group 1 < Group 2	Group 1 < Group 2
	Group 2 > Group 3	
Algometric pain threshold (kg/cm^2)[b]	Group 2 < Group 3	Group 1 > Group 2
		Group 2 < Group 3

VAS Visual analog scale and *NS* nonsignificant

for only once in Group 3 patients. Injected material consisted 1 cc of methylprednisolone acetate (40 mg) and 1 cc of prilocaine hydrochloride (20 mg).

All the patients in three groups were advised to rest, modify the daily living activities during 3 months, and were prescribed with volar static wrist splint. Paracetamol intake was permitted in case of pain that had the potential to restrict the daily living activities. The patients were advised not to take paracetamol 48 h before visits.

Outcome measures The pain intensity levels sensed during rest, activity of the painful elbow, and nighttime were labeled on a 10-cm visual analog scale (VAS) (from no pain = 0 to unbearable pain = 10). Furthermore, the patients graded their pain levels on the lateral side of the elbow according to the same method during resisted wrist dorsiflexion and forearm supination.

The pain threshold on elbow was determined with Fischer's algometer. The investigator applied the algometer perpendicularly on the most painful point increasing the pressure by 1 kg/cm^2 every 3 s till the patient sensed the pain. The pressure value that caused the onset of pain sensation was determined as pain threshold. The lowest pressure value among three measurements within 20-s intervals was taken as the pain threshold.

Statistical analysis Statistical evaluation was performed by SPSS program (version 11.0). Differences among groups were analyzed by Kruskal–Wallis test and between-groups comparisons were performed by Mann–Whitney U test. Friedman test was used to investigate whether there was an impact of treatment within the groups. The between-visits comparisons were performed by nonparametric *t* test for dependant variables.

Results

Sixty patients with tennis elbow (45 women and 15 men) were allocated into three groups with equal number of patients (Table 1). All the participants completed the study. The mean age of the whole study attendants was 48.60± 7.05 and the mean duration of the disorder was 3.35± 2.26 months. There was no statistically significant difference among the groups for age, gender, and duration of disorder (p>0.05). Furthermore, no significant difference was found for VAS values of pain during rest, activity, nighttime, resisted wrist dorsiflexion, and forearm supination (Table 2). Moreover, pain threshold levels on lateral epicondyle determined by algometry were not different among the groups at the baseline evaluation (Table 2).

All the pain parameters improved after therapy (3 weeks after the first visit) and at the third month in Groups 1, 2, and 3 (Table 3).

There were statistically significant differences for VAS values of pain during rest and activity, pain levels during resisted wrist dorsiflexion and forearm supination, and algometric pain threshold values on the epicondyle among the groups at the post therapy evaluation. VAS values during activity and pain levels during resisted wrist dorsiflexion were found to be significantly lower in Group 3 than Group 1. When compared with sham PEMF (Group 2), patients treated with corticosteroid injection (Group 3) had lower pain levels during rest, resisted wrist dorsiflexion, and forearm supination and higher algometric pain threshold levels on the epicondyle at the third week. Only the pain levels during resisted wrist dorsiflexion and forearm supination were found to be lower in Group 1 when compared with sham PEMF (Group 2) (Table 4).

All parameters showed differences among the groups at the third-month evaluation. Patients treated with PEMF had lower pain during rest, activity, and nighttime when compared with Group 3 patients treated with corticosteroid injection. All the pain parameters except for VAS value during nighttime were found to be significantly improved in Group 1 patients at the third month when compared with sham PEMF group. Group 3 had more favorable improvements than Group 2 in pain levels during resisted wrist dorsiflexion and algometric pain threshold values on the epicondyle (Table 4).

Discussion

The statement of Cyriax, which he made in 1936 about the prognosis of tennis elbow that "lateral epicondylitis usually resolves spontaneously in 8 to 12 months" still preserves its value [6]. In most of the placebo-controlled studies, pain levels of the patients who had received placebo also improved and the patients who had received active therapy [29–31]. We similarly found improvements in pain levels in our patients who were treated with sham PEMF at post therapy and evaluations after 3 months. This positive effect in this group may possibly be related with placebo effect but may be due to splint applications, rest, and daily living activity modifications as well.

The usual spontaneous pain resolution period is too long for a patient and a physician to wait. Most of the patients usually expect to reach painless status and functional independence as quickly as possible. The earlier relief of pain seems to be usually provided successfully with corticosteroid injections according to the previous data on the point. In a previous study, local corticosteroid injections were found to be more beneficial for pain and functional incapacities of tennis elbow patients when compared with placebo and naproxen at the fourth week of evaluation. However, there was no difference between placebo and corticosteroid injection and similar relapse rates were observed in three groups at the 12th month [15]. In another study, Smidt et al. [8] compared the efficacy of a combination of physical therapy interventions including pulsed ultrasound, deep friction massage, and exercise with local corticosteroid injections and reported that the injection therapy was more successful to relive pain and restore grip strength upon evaluation 6 weeks after therapy. But recurrence rate in the injection group was high and long-term differences between injections and physiotherapy were significant in favor of physiotherapy. Perhaps greater initial reduction in pain in the injection group may have led them to greater early increases in activity and subsequent reaggravation of the condition. The findings of our study supported the early benefits of corticosteroid injections mentioned in these studies as pain parameters in our patients treated with injection resolved significantly when compared with sham and active PEMF group after 3 weeks. In our study, we also observed that patients treated with PEMF had lower pain levels during rest, activity, and nighttime when compared with patients treated with corticosteroid injections after 3 months, although pain during resisted wrist dorsiflexion and forearm supination maneuvers and algometric values were not different.

The only randomized controlled trial on the effect of PEMF in tennis elbow was conducted by Devereaux et al. [23] in 1985. No difference of pain reduction effect could be found after 6 weeks after active PEMF therapy when compared with sham PEMF. Although the improvements in the hand grip strength and thermographic parameters were greater in the PEMF group and continued until the eight week, the difference between the groups did not reach statistical significance. The study of Devereaux et al. was stopped in the eight week and the long-term benefits of PEMF therapy could not be evaluated [23]. The applied frequency and dosage were different from the parameters that we used in our study. The difference between the application methods, dosage, and the evaluation time may be the main reasons of the opposite findings that we found in our study.

A few weeks of time for human tissues may not be sufficient for the completion of biological effects and the total recovery that were observed in in vitro cellular and animal studies. Despite the lack of strict clinical evidence on the point, the various efficacy levels of different frequencies and dosages may lead to different findings on healing tissues. For instance, in an experimental study, 17-Hz-pulsed magnetic field was reported to suppress extravascular edema in all stages of an Achilles tendonitis, while a 46-Hz PEMF only suppressed accompanying edema to the inflammation in the late phases of the study [29]. If we could have performed histopathological investigations to explain the clinical improvements, we would have put forward stronger evidence. However, this was impossible because of ethical concerns and impossibility of getting consent from the patients. Short-term follow-up in this study can be criticized but the self-limiting character of lateral epicondylitis in about 8–12 months could have confused long-term results.

Another limitation of our study was that we did not evaluate the daily living activities and functional status of the tennis elbow patients. However, resisted wrist dorsiflexion and forearm supination are two of the key motions during daily living activities and pain investigation during these motions, we think, can give some idea about the functional status.

In conclusion, PEMF seems to reduce pain better than sham PEMF and may be a helpful modality in the treatment of lateral epicondylitis. Although the treatment time is quite long and necessitate compliance, it can be used in patients avoiding invasive approaches. Corticosteroid and anesthetic agent injections can be used in patients for rapid return to activities.

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Effects of 50 Hz electromagnetic fields on electroencephalographic alpha activity, dental pain threshold and cardiovascular parameters in humans

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Abstract

Recent studies indicate that exposure to extremely low frequency magnetic fields (ELF MFs) influences human electroencephalographic (EEG) alpha activity and pain perception. In the present study we analyse the effect on electrical EEG activity in the alpha band (8–13 Hz) and on nociception in 40 healthy male volunteers after 90-min exposure of the head to 50 Hz ELF MFs at a flux density of 40 or 80 μT in a double-blind randomized sham-controlled study. Since cardiovascular regulation is functionally related to pain modulation, we also measured blood pressure (BP) and heart rate (HR) during treatment. Alpha activity after 80 μT magnetic treatment almost doubled compared to sham treatment. Pain threshold after 40 μT magnetic treatment was significantly lower than after sham treatment. No effects were found for BP and HR. We suggest that these results may be explained by a modulation of sensory gating processes through the opioidergic system, that in turn is influenced by magnetic exposure.

Keywords: 50 Hz electromagnetic fields; EEG alpha activity; Pain; Attention; Cardiovascular; Human

In the last years a vast number of studies have been done on the effect of exposure to extremely low frequency magnetic fields (ELF MFs) in the 50–60 Hz frequency range (i.e. electrical power-line) on various physiological parameters in humans [34]. However, these studies have often provided controversial results. A recent publication reviewing the effects of exposure to weak ELF MFs on human electrophysiology suggests that magnetic exposure influences human EEG activity [7]. One of the most consistent results is a higher resting and evoked EEG alpha (8–13 Hz) activity subsequent to ELF MFs exposure [3,4,8,26,27].

In addition, several reports indicate that exposure to ELF MFs affects pain perception in various animal species [6,12], including humans [15]. Hyperalgesia (i.e. reduction of pain threshold) was observed in animal models for various MF frequencies [5,10,11,21,33]. In humans, we have performed a number of studies in which we observed a reduction of pain threshold after exposure to randomly varying sinusoidal ELF MFs at a frequency <1 Hz [31,35] and to regularly oscillating ELF MFs at 37 Hz [15]. To our knowledge, no studies have been reported on the effect of 50 Hz MFs on human pain sensitivity.

In this study we investigated in a group of normal subjects the effect of 50 Hz MFs on EEG alpha activity and pain perception. Since there are important relationships between cardiovascular and pain regulation [14] we also measured the effects on blood pressure and heart rate. Plasma levels of catecholamines and cortisol were also determined.

Forty healthy male students (26.8 ± 4.1 years, mean ± S.D.) participated to this study, which was approved by the local Ethical Committee ("Comitato per lo studio del farmaco sull'uomo"). Informed consent was obtained from all subjects. Assumption of psychoactive substances (including coffee, tea, tobacco and alcohol) was not allowed in the 2 h preceding the experiment, and neuro- or psychotropic drugs had been prohibited for three previous months.

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Magnetic exposure was delivered by two circular Helmholtz-like coils (44 cm in diameter, each composed of an aluminium ring on which 50 copper coils [0.8 mm diameter] were winded) connected to a voltage-controlled oscillator that produced sinusoidal alternate current at 50 Hz. Sham-controlled studies were done at 40 and 80 μT (peak) intensity, as measured at the centre of the coils by a Gaussmeter (Model EFA-2, 1000 μT setting measurement range, the measurement accuracy specified was ±8%, Wandel and Goltermann, Eningen, Germany).

Prior to the beginning of the study subjects were randomly assigned to one of the two exposure intensity groups (40 μT = 20 subjects and 80 μT = 20 subjects). In addition, in each group, the subjects were also randomly assigned to two subsets: one subset performed the sham first and then the magnetic exposure and the other subset followed the reverse order. Investigators' blindness was ensured by means of a switch with two positions, ON and OFF, that were not known by the investigators for the entire experimental part of the study. Each volunteer underwent two 90-min sessions at an interval of 1–2 weeks. All studies were performed from 3.00 p.m. to 6.00 p.m. Each subject was comfortably seated in a quiet room. Prior to magnetic/sham exposure, the pain sensitivity, EEG alpha activity, blood pressure and heart rate were recorded.

Pain sensitivity was assessed by determining dental pain threshold. This parameter was measured by means of a commercial non-invasive tooth pulp tester (American Analytic Technology, Missoula, MT, USA). The instrument gave automatic intermittent bursts of electrical stimuli of negative polarity at increasing voltage, through a probe applied to the tooth. The subject had to indicate, by lifting his hand, when the stimulation became painful enough to require interruption, and the corresponding value (ranging from 0 to 80, arbitrary units) on the instrument's display was taken as the pain threshold. Two healthy, unfilled teeth (two incisors, the right lateral inferior and the left lateral superior) were tested and the average value was used for the subsequent analysis.

A Modulab 800, SATEM (Rome, Italy) equipment was used for EEG alpha activity recording. The EEG alpha activity was monopolarly recorded by means of a surface Ag/AgCl cup electrode, 14 mm diameter, placed over the scalp at Oz position of the 10–20 International System. An equal electrode over the left earlobe was used as reference. Impedance was kept below 5 kΩ. The ground was located on the forehead. The EEG signal was band-pass filtered between 8 and 13 Hz (24 dB/octave roll-off), thus selecting only the alpha rhythm. Data were sampled at 128 Hz. The EEG was recorded for six consecutive minutes and divided into three periods lasting 2 min each, respectively, with the eyes closed, opened and then closed again. The output data of the instrument were the peak-to-peak amplitude (μV) averaged every 500 ms. The mean value of the two recordings with closed eyes was used as an estimate of the EEG signal intensity in the alpha band frequencies. Respectively, 10 and 5 subjects had to be discarded because of technical problems or bad signal quality of recordings in the 40 and 80 μT group.

Blood pressure and heart rate were measured every 3 min on the right arm by an automatic oscillometric device (Dinamap 845, Critikon, Tampa, Florida) for 15 min.

After these basal measurements the coils were positioned around the head of the subject with the head at the centre of the coils (Fig. 1), and the magnetic or sham exposure was started. The subject was invited to read a magazine for the following 105 min and blood pressure and heart rate continued to be measured every 3 min. All values collected were averaged over 15-min intervals.

Immediately after the end of magnetic or sham exposure, dental pain threshold and EEG alpha activity were measured again and 20 ml of blood were drawn from the antecubital vein. Catecholamines were determined by high pressure liquid chromatography (HPLC) and electrochemical detection and cortisol by a fluorescence polarization immunoassay (TDx/TDx/FLx Cortisol, Abbott, Rome, Italy).

Data were analysed with a repeated-measure analysis of variance (ANOVA) with two within-factor variables (exposure condition and sequence). Two levels were considered for exposure condition: sham and magnetic exposure. As to the sequence, two levels (i.e. basal and after exposure) were considered for pain threshold and EEG alpha activity; for what concerns systolic and diastolic blood pressure and heart rate, eight levels were considered (corresponding to the eight 15-min intervals of the total duration of study including basal measurement). Post hoc comparisons were done applying Bonferroni correction. For hematochemical parameters, ANOVA with one within-factor (i.e. exposure condition; two levels: sham and magnetic) was applied. All analyses were run with the statistical packages Statview 4 and SuperAnova (Abacus Concepts, Inc., Berkeley, CA, USA) implemented on an iMac computer (Apple, Inc.). A statistical significant effect was defined when the level of significance was $P < 0.05$.

Table 1 shows the comparison of the effects of sham and magnetic exposure on EEG alpha activity. In the 40 μT group the overall ANOVA did not show any significant main effect, whereas in the 80 μT group significant main effects of exposure condition ($F_{1,14} = 11.100$, $P = 0.0049$), of sequence ($F_{1,14} = 19.591$, $P = 0.0006$) and of their interaction ($F_{1,14} = 11.463$, $P = 0.0044$) were found. Post hoc comparison in this group revealed that alpha activity after magnetic treatment was significantly ($P < 0.05$) greater than after sham treatment.

Table 1
EEG alpha activity (μV) after sham and magnetic exposure

	Sham exposure		Magnetic exposure	
	Before	After	Before	After
40 μT group	23.6 ± 8.8	25.5 ± 9.8	24.1 ± 10.4	23.9 ± 8.4
80 μT group	23.2 ± 9.2	24.4 ± 9.0	22.6 ± 9.2	39.9 ± 20.2*

Mean values ± S.D. are reported.

* $P < 0.05$, post hoc comparison between values after sham and after magnetic exposure in the 80 μT group.

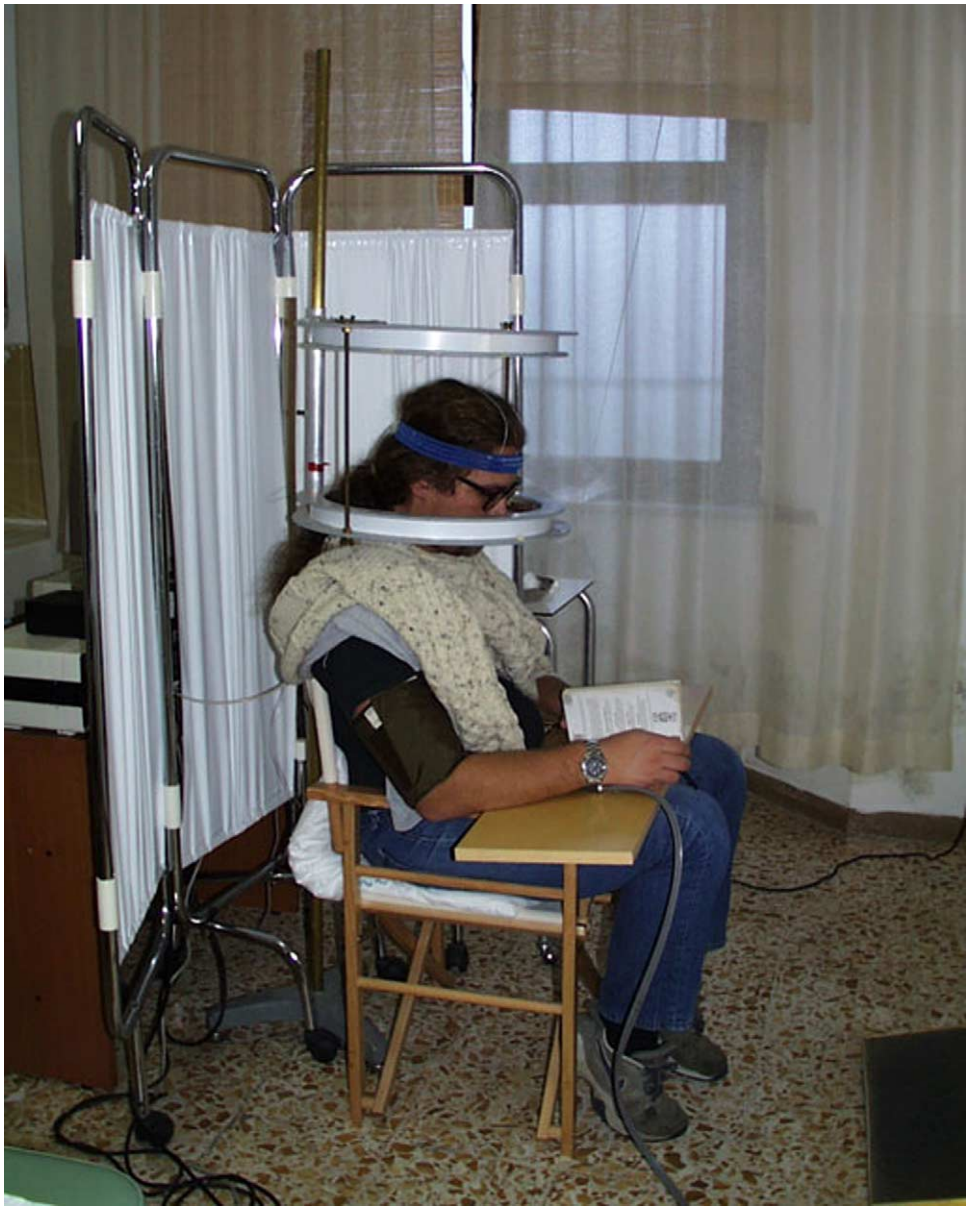

Fig. 1. A subject during a session of the study.

As to pain threshold (Table 2), the overall ANOVA did not show any significant main effect after 80 μT, whereas there was a significant main effect of exposure condition after 40 μT ($F_{1,18} = 5.347$, $P = 0.0328$). Post hoc comparison revealed that pain threshold after magnetic treatment was significantly ($P = 0.05$) lower than after sham treatment.

Table 2
Pain threshold (arbitrary units) after sham and magnetic exposure

	Sham exposure		Magnetic exposure	
	Before	After	Before	After
40 μT group	41.2 ± 12.7	42.3 ± 14.2	38.3 ± 12.0	37.0 ± 12.4*
80 μT group	37.0 ± 10.2	38.4 ± 10.3	37.8 ± 9.0	37.9 ± 8.9

Mean values ± S.D. are reported.
* $P < 0.05$, post hoc comparison between values after sham and after magnetic exposure in the 40 μT group.

The effects on blood pressure and heart rate are shown in Fig. 2. The overall ANOVA did not reveal significant effects of exposure condition either after 40 or 80 μT. A significant main effect of sequence (i.e. after exposure versus basal), presumably due to relaxation, was observed both for 40 and 80 μT for blood pressure, which initially decreased and then remained unchanged; the same was observed also for heart rate, which slowly decreased throughout the study.

Finally, Table 3 reports mean plasma levels of the cortisol and catecholamines. The overall ANOVA did not show any significant effect of exposure condition and sequence for all parameters both after 40 and 80 μT.

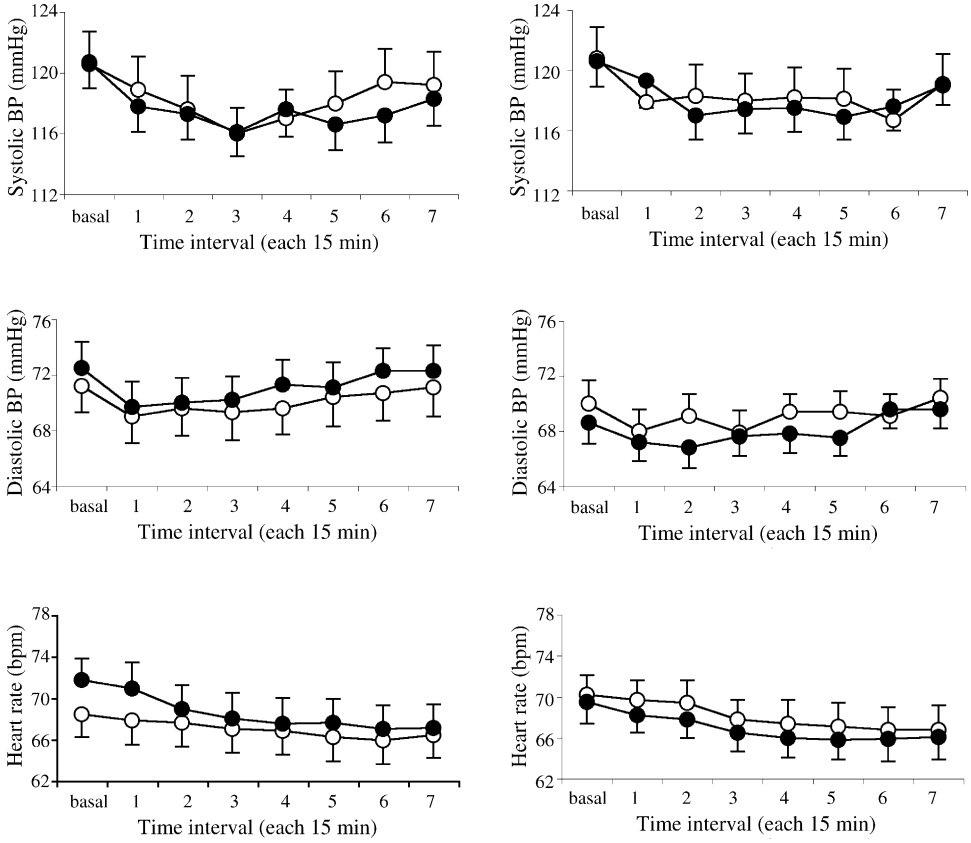

Fig. 2. Time course of mean values (±S.E.M.) of systolic, diastolic blood pressure (BP) and heart rate during exposure to electromagnetic field (full symbols) and under sham condition (empty symbols). Left panels refer to the 40 μT group and right panels to the 80 μT group.

The most prominent finding of this study is that ELF MFs exposure influenced EEG activity. This is in agreement with previous findings obtained both in animals [41] and humans [2,3,4,8,17,26–28,36,37,42].

Particularly, we have found an enhancement in the alpha band, recorded at medial occipital site under eye-closed resting wakefulness. Such a result has been found by other investigators under exposure conditions that were similar for frequencies (ranging from 10 to 60 Hz) but different for intensity and duration [3,8,26,27].

Increased alpha rhythm ("synchronization") is traditionally related to cortical inactivity (for review [32]) and its decrease ("desynchronization") to active information processing (for review [22]), although "paradoxical" alpha synchronization has been recently observed during cognitive performances over cortical areas not directly involved [9,23,24].

In our study, subjects had to remain under eye-closed resting wakefulness and cognitive tasks were not required. In this condition, however, the brain is not inactive because of internally driven mental operations that imply inward attention switching [9] and are characterized by a bilateral fronto-parietal alpha desynchronization (excitation) and a mid-cingulate and occipital alpha synchronization (inhibition) [16,25]. From this point of view, the enhanced occipital alpha activity observed in our study after electromagnetic field exposure could be explained by an enhanced inward attention. This possibility is also mentioned by Cook et al. [8].

The second result of this study is that magnetic field exposure increased pain sensitivity, as measured by graded electrical tooth pulp stimulation. To our knowledge, no studies were done to compare this method to other pain sensitivity assessment techniques, such as pressure and heat pain threshold measurement. However, a good agreement between intradental nerve activity and pain perception in response to graded stimulation applied to teeth has been reported [1]

Table 3
Mean levels (±S.D.) of hematochemical parameters after sham and magnetic exposure

	Cortisol (ng/ml)		Adrenaline (pg/ml)		Noradrenaline (pg/ml)	
	Sham exposure	Magnetic exposure	Sham exposure	Magnetic exposure	Sham exposure	Magnetic exposure
40 μT group	101.3 ± 38.7	111.0 ± 41.3	46.5 ± 28.5	52.0 ± 26.4	348.6 ± 93.5	347.8 ± 119.4
80 μT group	111.2 ± 69.8	99.6 ± 33.7	45.3 ± 26.6	42.8 ± 36.8	354.0 ± 98.9	323.3 ± 92.8

and tooth pulp test has been used to assess pain threshold in various clinical studies [13,14]. Hyperalgesia after magnetic exposure has been observed in various studies in humans [15,31,35] and animals (for review [6]). Recently, however, exposure to specific complex frequency-modulated magnetic fields has been shown to induce hypoalgesia both in animals [29,30,38,40] and humans [39].

It is uncertain whether these observations are linked, since enhanced alpha activity was observed at 80 μT but not 40 μT and hyperalgesia was observed at 40 μT but not 80 μT. If a link exists, a possible explanation is that both EEG alpha activity and nociception could be affected by sensory gating processes modulated by the opioidergic system, that in turn is influenced by magnetic exposure. In fact, some evidence indicates that MFs possess an anti-opioidergic naloxone-like action [11,18–20] and naloxone is reported to improve selective attention by enhancing sensory gating [2]. Following up this line, it is tempting to speculate that magnetic fields (1) facilitate, in a quiet environment, inward directed attention, resulting in increased occipital alpha activity and (2) facilitate, in the presence of an expected painful stimulus, outward directed attention on the nociceptive channel, resulting in hyperalgesia.

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Effects of Pulsed Electromagnetic Fields on Interleukin-1β and Postoperative Pain: A Double-Blind, Placebo-Controlled, Pilot Study in Breast Reduction Patients

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Background: Surgeons seek new methods of pain control to reduce side effects and speed postoperative recovery. Pulsed electromagnetic fields are effective for bone and wound repair and pain and edema reduction. This study examined whether the effect of pulsed electromagnetic fields on postoperative pain was associated with differences in levels of cytokines and angiogenic factors in the wound bed.

Methods: In this double-blind, placebo-controlled, randomized study, 24 patients, undergoing breast reduction for symptomatic macromastia received pulsed electromagnetic field therapy configured to modulate the calmodulin-dependent nitric oxide signaling pathway. Pain levels were measured by a visual analogue scale, and narcotic use was recorded. Wound exudates were analyzed for interleukin (IL)-1β, tumor necrosis factor-α, vascular endothelial growth factor, and fibroblast growth factor-2.

Results: Pulsed electromagnetic fields produced a 57 percent decrease in mean pain scores at 1 hour ($p < 0.01$) and a 300 percent decrease at 5 hours ($p < 0.001$), persisting to 48 hours postoperatively in the active versus the control group, along with a concomitant 2.2-fold reduction in narcotic use in active patients ($p = 0.002$). Mean IL-1β concentration in the wound exudates of treated patients was 275 percent lower ($p < 0.001$). There were no significant differences found for tumor necrosis factor-α, vascular endothelial growth factor, or fibroblast growth factor-2 concentrations.

Conclusions: Pulsed electromagnetic field therapy significantly reduced postoperative pain and narcotic use in the immediate postoperative period. The reduction of IL-1β in the wound exudate supports a mechanism that may involve manipulation of the dynamics of endogenous IL-1β in the wound bed by means of a pulsed electromagnetic field effect on nitric oxide signaling, which could impact the speed and quality of wound repair. (*Plast. Reconstr. Surg.* 125: 1620, 2010.)

Postsurgical pain increases patient morbidity and slows healing, particularly if narcotics, even by means of pain pumps, are used for pain management.[1–3] Therefore, surgeons are continually looking for other means of delivering postoperative analgesia. There is a growing body of clinical evidence that noninvasive, nonpharmacologic pulsed electromagnetic field therapy can have physiologically significant effects on inflammation and tissue repair.[4–6] Outpatient pulsed electromagnetic field therapy has been used extensively for the treatment of recalcitrant bone fractures for more than 30 years, and is reported to be as successful as an initial bone graft.[7] Recent advances in knowledge of the mechanism of the effects of pulsed electromagnetic field on tissue repair have led to the development of signals that can target the antiinflammatory cascade involving the calmodulin-dependent nitric oxide pathway. A pulsed electromagnetic field signal based on this model[8] has been shown to accelerate wound repair in a rat cutaneous wound model by 60 percent

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at 21 days[9] and Achilles tendon repair in a rat model by 70 percent at 21 days.[10] The same pulsed electromagnetic field signal has been reported to accelerate postsurgical pain relief in breast augmentation patients by 2.7-fold, with a concomitant decrease in the use of pain medication.[11]

Soft-tissue healing is a complex process involving the interactions of multiple cell types and a variety of molecules.[12] Cytokines, the humoral mediators of inflammation, are induced within minutes to hours after tissue damage, and serve as signals to engulf damaged tissue, destroy infectious agents, and clear the wound bed for healing.[13] Angiogenesis provides new vascular conduits for oxygen, nutrients, and hormones, and numerous studies indicate that pulsed electromagnetic fields can have a significant impact on angiogenesis both in vitro and in vivo.[14–18] Two of these studies showed, using specific antagonists, that modulation of fibroblast growth factor (FGF)-2 production was involved.[17,18] Indeed, many studies have demonstrated that pulsed electromagnetic fields modulate both cytokine and growth factor synthesis,[19–21] but to date there have been no examinations of the effects of pulsed electromagnetic fields on the molecular indices of pain, inflammation, and angiogenesis in postsurgical patients.

This pilot study was designed to determine whether pulsed electromagnetic field therapy, provided as a supplement to the current standard of care, could reduce postoperative pain, and to examine the content of wound exudates for levels of inflammatory cytokines and angiogenic factors as indices to better understand how pulsed electromagnetic field therapy may act on these processes.

Disclosures: *The pulsed electromagnetic field devices used in this study are cleared by the U.S. Food and Drug Administration for relief of postoperative pain and edema in superficial soft tissues and were donated by Ivivi Technologies, Inc. (Montvale, N.J.); Christine Rohde, M.D., Austin Chiang, and Omotinuwe Adipojou, M.D., have no financial interest or connections with Ivivi Technologies, Inc. They have no financial interests or sources of support to disclose. Diana Casper, Ph.D., receives research support from Ivivi Technologies, Inc., for unrelated cellular studies at Montefiore Medical Center and had no contact with patients in this study. Arthur A. Pilla, Ph.D., is a paid scientific consultant to Ivivi Technologies, Inc., and had no contact with patients in this study.*

MATERIALS AND METHODS

This study was approved by the Institutional Review Board at Columbia University Medical Center. Before the start of this study, a sample size analysis, assuming a clinically meaningful 50 ± 40 percent decrease in pain scores from pulsed electromagnetic field treatment,[22] suggested that 11 patients per group were needed. Thus, 24 healthy women, aged 27 to 59 years, who were candidates for breast reduction for symptomatic macromastia, were admitted to this double-blind, placebo-controlled, randomized study. All patients undergoing breast reduction surgery were asked to participate and all enrolled patients gave informed consent. Randomization was performed by the blinded assignment of devices from a list of their serial numbers. Breast reductions were performed by the same surgeon (C.R.) using standard breast reduction techniques with superomedial pedicles. Use of pulsed electromagnetic field coils was the only addition to the current standard of care. As is the routine practice for this surgeon, 10-mm Jackson-Pratt drains were placed into each breast and brought out through the incision. These drains were left in place until the first postoperative morning, when they were removed at the bedside before the patient was discharged from the hospital. This permitted the collection of wound exudates in the immediate postoperative stages of healing. Exudates were collected into 15-ml polypropylene tubes and stored at −80°C for subsequent analysis.

Patients were randomly assigned a disposable dual-coil pulsed electromagnetic field device (SofPulse Duo; Ivivi Technologies, Inc., Montvale, N.J.), placed within the postsurgical support bra normally used for all patients, as shown in Figure 1. Devices were activated on transfer to the recovery stretcher. The pulsed electromagnetic field signal, configured a priori to modulate Ca^{2+} binding to calmodulin, consisted of a 2-msec burst of 27.12-MHz sinusoidal waves repeating at two bursts per second. Peak magnetic field was 0.05 G, which induced an average electric field of 32 ± 6 mV/cm in each breast.[8,23,24] An active pulsed electromagnetic field device automatically provides a 20-minute treatment every 4 hours for the first 3 days of treatment, then once every 8 hours for the next 3 days, then twice daily thereafter. The availability of this automatic regimen ensures patient compliance and allows these devices to be used throughout the various stages of wound repair.[25] Sham devices were used in exactly the same manner as active devices but produced no electromagnetic field in tissue. These pulsed electromag-

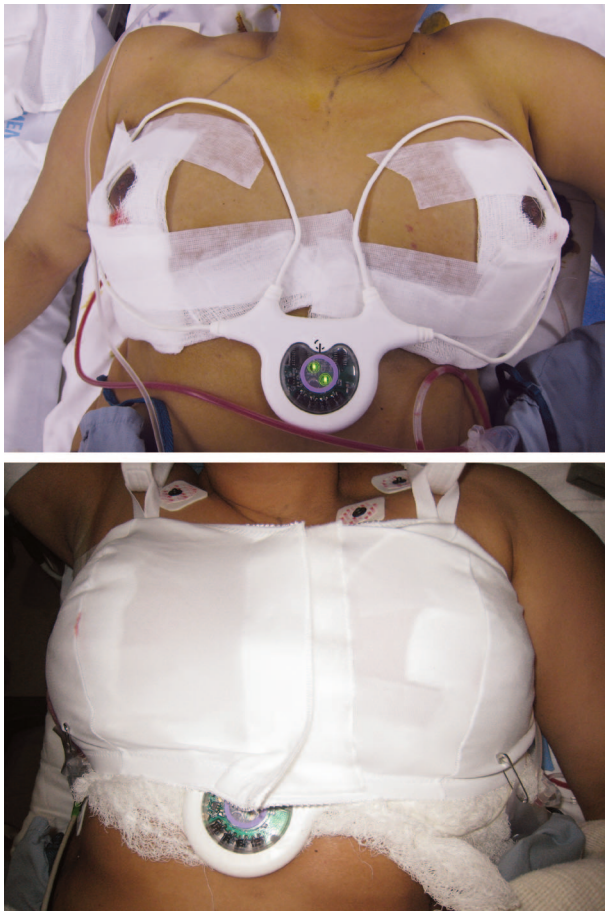

Fig. 1. The pulsed electromagnetic field device used in this study. (*Above*) Pulsed electromagnetic field device in place with each coil surrounding one breast. The battery-powered signal generator is at the bottom between the coils. Once activated, indicator lights flash approximately once per second for both active and sham devices. Note the surgical drains leading from each breast. (*Below*) Postsurgical support bra, normally used with this procedure, in place holding pulsed electromagnetic field device in position.

netic field devices do not produce heat or cause any sensation in tissue. The average in situ magnetic field induced by the pulsed electromagnetic field signal used in this study is at least 1000-fold below the earth's magnetic field and not detectable using standard gaussmeters. Therefore, only measurements with specialized laboratory equipment, not normally available in the recovery or hospital room or in the patient's home, could determine whether a device was active. Neither physicians nor patients knew whether a device was active throughout the study. General unblinding occurred after all data were collected.

Pulsed electromagnetic field signal amplitude and configuration was verified for each device by a third party, who had no contact with patients, at the beginning and end of pulsed electromagnetic field treatment with a calibrated field probe (model FCC-301-1-MR1; Fischer Custom Communications, Torrance, Calif.) connected to a calibrated 100-MHz oscilloscope (model 2358; Tektronix, Beaverton, Ore.). Measurement of the pulsed electromagnetic field signal distribution in a tissue phantom and in air provides an accurate map of the signal in tissue.[26] Such plots revealed that the amplitude dose of the electromagnetic field in the treated breast from active devices was uniform to within ±20 percent.

The two primary outcome measures in this study were (1) postsurgical pain and (2) cytokine and growth factor concentrations in wound exudates. Pain levels were assessed by self-evaluation with a visual analogue scale previously validated for postsurgical pain.[27,28] Visual analogue scale data were obtained at intervals starting at hour 1 postoperatively and at specified intervals thereafter for 48 hours. Use of narcotic pain medication (oxycodone/acetaminophen) over the first 48 hours was assessed by comparing pill counts for each group. All patients received oxycodone/acetaminophen (Percocet; Endo Pharmaceuticals, Newark, Del.) as soon as they were able to tolerate oral intake, usually within several hours after surgery, and discharged with a prescription. Because oxycodone/acetaminophen was the most common narcotic pain medication taken postoperatively, an equianalgesic table was used to convert other narcotics (e.g., morphine, hydromorphone, fentanyl, codeine, hydrocodone) given in the immediate postoperative period into Percocet equivalents.[29] This conversion enabled a comparison of pain medication use between the two groups.

Wound exudate was collected hourly starting at 1 hour postoperatively for the first 6 hours, and on the first postoperative morning before drain removal (at 15 to 24 hours postoperatively). All exudate fluid at each time point was removed completely, so that samples contained fluid drained only since the prior fluid collection. These samples were coded in a manner such that subsequent analyses were performed in a blinded fashion. For determination of cytokine and growth factor levels, exudates were thawed, cellular debris was pelleted by centrifugation, and resulting supernatants were divided into smaller aliquots and frozen at −80°C until analysis. Interleukin (IL)-1β, tumor necrosis factor (TNF)-α, vascular endothelial growth factor (VEGF), and FGF-2 were quantified using the appropriate enzyme-linked immunosorbent assay kit (R&D Systems, Minneapolis, Minn.). Pilot assays were performed initially with at least two samples to determine appropriate dilutions of exudates that fell within the linear range of quan-

tification for each assay. The Mann-Whitney rank sum test, analysis of variance, or repeated measures analysis of variance was used, as appropriate, to compare mean visual analogue scale scores and cytokine and growth factor levels. Significance was accepted at $p \leq 0.05$.

RESULTS

The portable and disposable pulsed electromagnetic field devices were well tolerated. No adverse events were reported. Data from 24 patients (12 active and 12 sham) were available for analysis. There was no significant difference between the two patient groups in terms of age, body mass index, or amount of tissue resected. Mean visual analogue scale scores over the 48-hour postsurgical period were compared. The results show a 57 percent decrease in mean pain at 1 hour ($p < 0.01$) and a 300 percent decrease at 5 hours, persisting to 48 hours postoperatively, in the active group compared with the untreated control group ($p < 0.001$). There was no significant change in mean visual analogue scale scores over the same postoperative time in the sham group. These results are summarized in Figure 2.

The mean pill count over the first 48 postoperative hours (using oxycodone/acetaminophen equivalents) in the active group was 5 ± 0.9 compared with 11 ± 1.2 in the sham group, demonstrating a significant 2.2-fold reduction in narcotic use in patients treated with pulsed electromagnetic field therapy ($p = 0.002$). Importantly, the magnitude of this effect correlates well with the decrease in mean visual analogue scale score over the same postoperative period in these patients. These results are summarized in Figure 3.

Wound exudates, collected from 22 patients at 1 to 24 hours postoperatively, were analyzed to quantify levels of IL-1β, a master cytokine induced at early times after tissue injury[30]; TGF-α, a cytotoxic factor[31]; VEGF, a central mediator of angio-

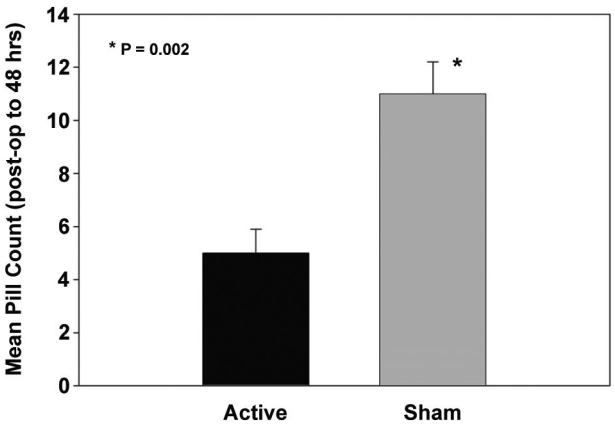

Fig. 3. Effect of pulsed electromagnetic field therapy on narcotic use (Percocet) following breast reduction surgery. The results show approximately 2.2-fold fewer pills were taken over the first 48 postoperative hours, correlating with the decrease in mean visual analogue scale score over the same period (Fig. 1).

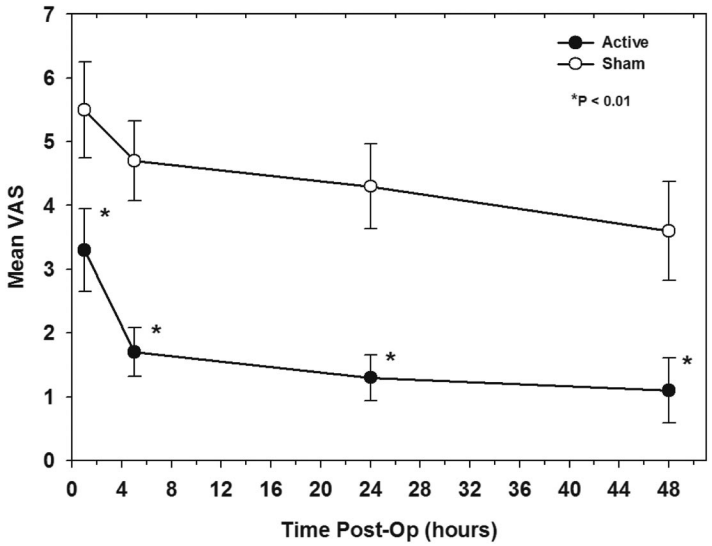

Fig. 2. Effect of pulsed electromagnetic field therapy on pain following breast reduction surgery. Mean visual analogue scale (*VAS*) score was significantly reduced starting at 1 hour postoperatively and approximately 3-fold lower at 5 hours postoperatively in the active group. There was no significant difference in mean visual analogue scale score in the sham group over the first 48 hours postoperatively.

genesis[32]; and FGF-2, an important factor in wound healing that can induce both fibroblast proliferation and angiogenesis.[33] The concentrations of these factors in the wound exudates collected in this study are consistent with levels reported in other studies.[34–37] No differences between active and sham groups were found for TNF-α, VEGF, or FGF-2 concentrations over the entire postoperative sampling period. In addition, the concentrations of these factors did not significantly increase over the same sampling period, as reported for the same early postoperative period in other studies.[38] In contrast, the overall mean IL-1β levels in the active cohort were approximately 275 ± 36 percent lower than in the sham cohort over the same postoperative sampling period ($p < 0.001$). A summary of the wound exudate data for the 6-hour sample is shown in Table 1.

Table 1. Mean Concentration, at the 6-Hour Postoperative Sample Time, of the Cytokines and Growth Factors in the Wound Exudates Evaluated in This Study*

Factor	Active (pg/ml)	Sham (pg/ml)	Active vs. Sham (%)	*p*
IL-1β	100 ± 11	208 ± 32	–200	0.001*
TNF-α	13 ± 3	15 ± 3	–14	0.572
FGF-2	577 ± 49	468 ± 59	+22	0.176
VEGF	970 ± 89	1026 ± 96	–5	0.671

*There is no significant difference for TNF-α, FGF-2, or VEGF. Only IL-1β was significantly lower in the active cohort ($p = 0.001$).

It is also of interest to compare the postoperative time course of the increase of IL-1β in the sham and active cohorts. This is shown in Figure 4, in which the concentration of IL-1β at each time point shown represents its accumulation in the wound exudate since the previous sample. IL-1β varied from 350 percent lower in exudates collected at 1 hour ($p < 0.001$), to 300 percent lower at 3 hours ($p < 0.001$), to 200 percent lower at 6 hours ($p < 0.001$), remaining at 200 percent lower at 15 to 24 hours (average, 18 hours) postoperatively ($p < 0.01$), than the sham group at the equivalent postoperatively time. These results correlate well with the temporal reduction in mean visual analogue scale pain scores and in the use of pain medication by patients in the active cohort. The mean volume of wound exudate collected between 6 and 15 to 24 hours was not significantly different from that collected between 5 and 6 hours, indicating that the rate of exudate accumulation in the wound bed had slowed significantly.

DISCUSSION

Results from this randomized, double-blind, placebo-controlled study demonstrate that pulsed

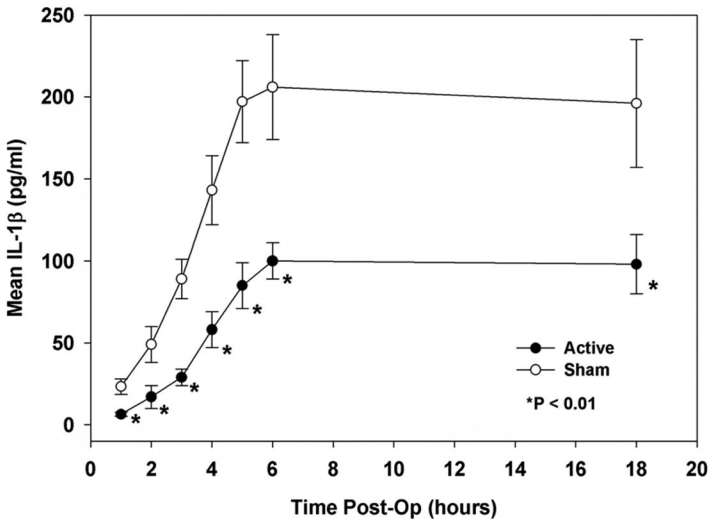

Fig. 4. Effect of pulsed electromagnetic field therapy on IL-1β concentrations in wound exudates following breast reduction surgery. Mean IL-1β concentration varied from approximately 350 percent lower at 1 hour postoperatively ($p < 0.001$), to approximately 300 percent lower at 3 hours, to approximately 200 percent lower at 6 hours ($p < 0.001$), and remaining approximately 200 percent lower at an average of 18 hours ($p < 0.01$) in the active group compared with the sham group. These results correlate well with the variation of mean visual analogue scale score over the same postoperative period (Fig. 2).

electromagnetic field therapy has a significant impact on postoperative pain. We believe that these findings could have major implications in developing new strategies for pain management. The effects of pulsed electromagnetic fields on pain reported here are of higher magnitude than those reported for pain pumps.[3] The effects on analgesia are also similar to those reported using the same pulsed electromagnetic field signal in an independent study on postoperative pain in breast augmentation patients performed in Sweden by different clinical investigators under different conditions,[11] supporting the consistency and validity of this type of therapeutic modality. The Swedish study did not include cytokine and/or growth factor analyses, but did include a third contralateral cohort in which one breast received pulsed electromagnetic field therapy and the contralateral breast received sham treatment. That study reported that the pulsed electromagnetic field effects on postoperative pain reduction for both active and sham breasts were not significantly different from each other or from that for the active cohort in which both breasts received pulsed electromagnetic field therapy. Analysis of the distribution of the pulsed electromagnetic field signal in both breasts in the contralateral cohort showed that the sham breast received approximately 60 percent of the signal amplitude present in the active breast. In other words, because of the spatial distribution of pulsed electromagnetic field signal propagation from the coil applicator, it was technically impossible to ensure zero signal amplitude in the sham breast. In view of the above, no attempt was made to include a contralateral cohort in the present study. It is also important to note that the effect of pulsed electromagnetic fields on pain was highly significant; mixed effect analyses[39] were not required.

No significant differences were found for TNF-α, VEGF, and FGF-2, which may be attributable to the slower kinetics of their appearance in the wound bed, as reported by other groups.[12,37] In contrast, quantitative data demonstrate that IL-1β levels in the wound exudates of patients treated with active pulsed electromagnetic field coils were concomitantly and significantly reduced. Interestingly, the postoperative time course of both pain and IL-1β reduction were similar, suggesting that a common mechanism produced both effects. The importance of this finding may be related to reports that the dynamics of IL-1β delivery in the wound bed affects the rate and quality of wound repair.[40] This is also supported by results from another study in which genetic disruption of IL-1 signaling reduced wound fibrosis and collagen deposition (scar), improved skin architecture, and increased tensile strength.[41] Although inflammation is essential for healing, it is the most painful stage of wound repair and, if not resolved quickly, can delay healing and lead to complications such as fibrosis, scarring, and keloid formation. Indeed, manipulation of the dynamics of IL-1β using pharmacologic antagonists to minimize or even eliminate scar formation is currently a highly discussed topic in wound repair research.[42,43] Our results suggest that pulsed electromagnetic field signals can produce endogenous changes in the dynamics of IL-1β availability, which should impact the many known subsequent inflammatory events that are mediated by this cytokine.[29] Importantly, pulsed electromagnetic field therapy is not systemic and not governed by pharmacokinetics. Indeed, the pulsed electromagnetic field signal appears instantaneously in all compartments of the target tissue where endogenous antiinflammatory and subsequent tissue repair processes can be modulated.

The mechanism of action of pulsed electromagnetic field signals in this study is not completely elucidated. However, it is intriguing to consider that the known effects of pulsed electromagnetic fields on the modulation of Ca^{2+} binding to calmodulin, with subsequent enzyme activation,[6,23,24] may be applicable here. Calmodulin-dependent activation of nitric oxide synthase to produce nitric oxide and its subsequent stimulation of cyclic guanosine monophosphate formation, which plays an orchestrating role in tissue repair,[44] has recently been reported to be sensitive to pulsed electromagnetic fields. DNA synthesis in cultured articular chondrocytes can be stimulated by pulsed electromagnetic fields through the calmodulin/nitric oxide/cyclic guanosine monophosphate pathway, where inhibition of calmodulin, nitric oxide synthase, and guanylate cyclase, individually, eliminated the effect.[45] Pulsed electromagnetic field effects on osteoblast proliferation and differentiation were also shown to be mediated by nitric oxide.[46] Direct evidence of the effect of a pulsed electromagnetic field signal configured for the Ca^{2+}/calmodulin pathway on real-time nitric oxide production in a neuronal cell line, which could be eliminated by calmodulin and nitric oxide synthase inhibitors, has also recently been reported.[47,48] Other effects of pulsed electromagnetic fields involving nitric oxide include the following: increased vasodilatation,[49] inhibition of the vasoconstrictor endothelin-1,[50] increased neuronal regeneration,[51] and increased nitric oxide in nasal and sinus mucosa.[52]

Neutrophils, the first cellular responder in the inflammatory phase of wound repair, produce IL-1β which, in turn, can up-regulate inducible nitric ox-

ide synthase activity, resulting in proinflammatory amounts of nitric oxide to be released into the wound bed.[53] Protracted exposure to nitric oxide leads to the induction of cyclooxygenase-2, increasing levels of prostaglandins and unnecessarily extending the inflammatory phase of healing, which can lead to pain, fibrosis, and other complications.[12] Pulsed electromagnetic fields have been reported to down-regulate inducible nitric oxide synthase at the mRNA and protein levels in monocytes,[54] supporting the notion that the actions of pulsed electromagnetic fields on tissue repair include an early antiinflammatory component. Furthermore, it has been reported that the calmodulin/endothelial nitric oxide synthase/nitric oxide signaling pathway down-regulates both IL-1β and inducible nitric oxide synthase.[55,56] It follows that the pulsed electromagnetic field signal used in this study, configured to target this calmodulin-dependent nitric oxide signaling pathway, could down-regulate both IL-1β and inducible nitric oxide synthase by means of its effect on nitric oxide signaling. In addition, inducible nitric oxide synthase activity would be indirectly attenuated because of its known dependence on IL-1β.[53] Together with the known effects of pulsed electromagnetic fields on vasodilatation,[16] these events could account for the accelerated pain relief experienced by patients in this study who received pulsed electromagnetic field therapy. This leads to the proposed mechanism depicted in Figure 5 for pulsed electromagnetic field attenuation of the inflammatory phase of wound repair, based on a general pulsed electromagnetic field mechanism for tissue repair reported elsewhere.[57] Additional basic and clinical studies will be necessary to further test the validity of this proposed mechanism.

It is also of importance that increased nitric oxide production by means of pulsed electromagnetic

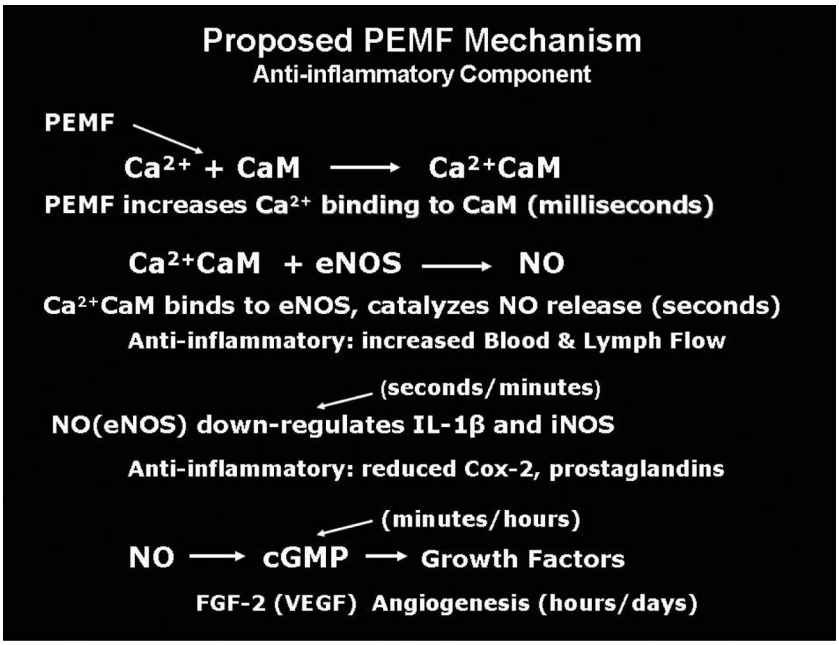

Fig. 5. Summary of proposed mechanism for the pulsed electromagnetic field (*PEMF*) effects on postoperative IL-1β and pain. The pulsed electromagnetic field signal was configured to modulate the kinetics of Ca^{2+} binding to calmodulin. This, in turn, modulates endothelial nitric oxide synthase activation and manipulates the concentration of IL-1β and inducible nitric oxide synthase activity in the wound bed. The result is accelerated postoperative pain relief. Also shown is the proposed pulsed electromagnetic field pathway for increased angiogenesis that has been reported in other studies (Tepper OM, Callaghan MJ, Chang EI, et al. Electromagnetic fields increase in vitro and in vivo angiogenesis through endothelial release of FGF-2. *FASEB J.* 2004;18:1231–1233; and Callaghan MJ, Chang EI, Seiser N, et al. Pulsed electromagnetic fields accelerate normal and diabetic wound healing by increasing endogenous FGF-2 release. *Plast Reconstr Surg.* 2008;121:130–141). *CaM*, calmodulin; *eNOS*, endothelial nitric oxide synthase; *NO*, nitric oxide; *iNOS*, inducible nitric oxide synthase; *cGMP*, cyclic guanosine monophosphate.

fields also leads to increased cyclic guanosine monophosphate production that may act to stimulate the synthesis and/or release of specific growth factors appropriate for each particular stage of healing if used during later postoperative periods as the inflammatory stage dissipates. Although no significant differences in levels of FGF-2 were detected in wound exudates within 24 hours postoperatively, it is still possible that a pulsed electromagnetic field effect on the production of this factor could be observed at a later time. Indeed, other studies have demonstrated increases in FGF-2 levels in response to pulsed electromagnetic field signals in vitro and in vivo,[17,18] and it is known that this factor, which has pleiotropic effects on fibroblasts and endothelial cells, can be induced by nitric oxide/cyclic guanosine monophosphate signaling.[58–60]

The clinical implications of our findings are significant. The pulsed electromagnetic field devices do not increase the normal effort or time required to place a postoperative dressing. The device weighs only 2.4 ounces, fits easily in a surgical bra and, once positioned and activated, requires no further intervention. Patients are instructed to remove the device only for bathing and to replace the device in its original position under the bra. The cost of the pulsed electromagnetic field device used in this study is approximately $100. As a comparison, implantable local anesthetic pain pump catheters cost from $200 to $280 per patient, and require more, and invasive, intervention.[61] It is also important to note that there are no known side effects associated with the use of pulsed electromagnetic field devices, whereas narcotic pain medications can cause side effects of nausea, vomiting, or constipation, and have addictive potential. With this in mind, the cost of the pulsed electromagnetic field device is a fraction of the potential cost of treating side effects from narcotics. The benefits of reducing the severity and duration of the inflammatory phase of wound repair with noninvasive, nonpharmacologic pulsed electromagnetic field therapy, which can manipulate the body's endogenous orchestration of wound repair with no known side effects, could thus have a major impact on the reduction of patient morbidity. This, in turn, may lead to a reduction in length of hospital stay with consequent reductions in the cost of health care. We are embarking on additional prospective studies that will compare pain levels, cytokine and growth factor analyses in exudate, and length of stay as outcome measures of pulsed electromagnetic field therapy in more complex reconstructive operations.

It is intriguing to speculate that the use of pulsed electromagnetic fields to manage postsurgical pain through its effect on inflammatory cytokines, followed by its anticipated modulation of endogenous growth factors,[6,19] may also lead to an overall acceleration of wound healing in humans.[25] Pulsed electromagnetic field therapy may also be accompanied by a concomitant reduction in scar formation, thus enhancing the quality of healing.

CONCLUSIONS

This study provides further evidence that pulsed electromagnetic field therapy can reduce pain levels and pain medication requirements in the immediate postoperative period. The concomitant reduction of IL-1β in the wound bed, possibly by means of nitric oxide/cyclic guanosine monophosphate signaling, suggests that pulsed electromagnetic field therapy could have a profound effect on wound repair outcomes. Larger clinical studies that include more extensive cytokine and growth factor analysis are clearly warranted. If these results are confirmed, the current availability of both economical and disposable pulsed electromagnetic field devices could easily translate to many, if not most, postsurgical situations, leading to lower morbidity, shorter hospital stays, increased productivity, and a reduction in the cost of health care.

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Exposure to a specific pulsed low-frequency magnetic field: A double-blind placebo-controlled study of effects on pain ratings in rheumatoid arthritis and fibromyalgia patients

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BACKGROUND: Specific pulsed electromagnetic fields (PEMFs) have been shown to induce analgesia (antinociception) in snails, rodents and healthy human volunteers.
OBJECTIVE: The effect of specific PEMF exposure on pain and anxiety ratings was investigated in two patient populations.
DESIGN: A double-blind, randomized, placebo-controlled parallel design was used.
METHOD: The present study investigated the effects of an acute 30 min magnetic field exposure (less than or equal to 400 μT_{pk}; less than 3 kHz) on pain (McGill Pain Questionnaire [MPQ], visual analogue scale [VAS]) and anxiety (VAS) ratings in female rheumatoid arthritis (RA) (n=13; mean age 52 years) and fibromyalgia (FM) patients (n=18; mean age 51 years) who received either the PEMF or sham exposure treatment.
RESULTS: A repeated measures analysis revealed a significant pre-post-testing by condition interaction for the MPQ Pain Rating Index total for the RA patients, $F(1,11)=5.09$, $P<0.05$, estimate of effect size = 0.32, power = 0.54. A significant pre-post-effect for the same variable was present for the FM patients, $F(1,15)=16.2$, $P<0.01$, estimate of effect size = 0.52, power =0.96. Similar findings were found for MPQ subcomponents and the VAS (pain). There was no significant reduction in VAS anxiety ratings pre- to post-exposure for either the RA or FM patients.
CONCLUSION: These findings provide some initial support for the use of PEMF exposure in reducing pain in chronic pain populations and warrants continued investigation into the use of PEMF exposure for short-term pain relief.

Key Words: *Analgesia; Fibromyalgia; Pain therapy; Pulsed electromagnetic fields; Rheumatoid arthritis*

Exposition à un champ magnétique pulsé de basse fréquence : résultats d'une étude à double insu, contre placebo, visant à évaluer les effets du traitement sur la cotation de la douleur chez des patientes souffrant de polyarthrite rhumatoïde ou de fibromyalgie

CONTEXTE : Certains champs électromagnétiques pulsés (CEP) produisent un effet analgésique (antinociceptif) chez les escargots, les rongeurs et les sujets volontaires humains.
BUT : L'étude avait pour but d'évaluer l'effet de l'exposition à un CEP d'une fréquence donnée sur la cotation de la douleur et de l'anxiété dans deux populations de patientes.
TYPE D'ÉTUDE : Il s'agissait d'une étude comparative contre placebo, menée à double insu, avec hasardisation, en mode parallèle.
MÉTHODE : L'étude portait sur les effets d'une exposition à un champ magn-étique, durant 30 min (densité égale ou inférieure à 400 μTmax; fréquence inférieure à 3 kHz), sur la douleur (questionnaire sur la douleur de McGill [QDM], échelle visuelle analogue [EVA] et sur l'anxiété (EVA) chez des femmes souffrant de polyarthrite rhumatoïde (PR) (n=13; âge moyen : 52 ans) ou de fibromyalgie (n=18; âge moyen : 51 ans), qui ont été soumises soit à un CEP réel, soit à un CEP fictif.
RÉSULTATS : Une analyse des mesures répétées a révélé une interaction significative entre les évaluations avant et après l'essai, selon l'affection, en ce qui concerne l'indice général de cotation de la douleur au QDM chez les patientes atteintes de PR ($F[1,11]=5,09$; $P<0,05$; estimation de l'importance de l'effet : 0,32; puissance : 0,54). Un effet important a aussi été noté avant et après l'essai pour la même variable chez les patientes atteintes de fibromyalgie ($F[1,15]=16,2$; $P<0,01$; estimation de l'importance de l'effet : 0,52; puissance : 0,96). Des effets similaires ont été observés aux autres volets du QDM et sur l'EVA de la douleur. Par contre, on n'a pas relevé de diminution importante de l'anxiété sur l'EVA, avant et après l'exposition au champ magnétique, ni chez les femmes souffrant de PR ni chez celles souffrant de fibromyalgie.
CONCLUSION : Les résultats obtenus justifient, dans un premier temps, le recours aux CEP pour le soulagement de la douleur chronique et, dans un deuxième temps, la poursuite des recherches sur le recours aux CEP pour le soulagement de la douleur aiguë.

Static, sinusoidal and low-frequency pulsed magnetic fields (PEMFs) have been shown to alter pain perception (nociception) and cognitive processing in both animals and humans (1-5). Our laboratory, and those of others, have demonstrated in snails (6), rodents (7), and humans (3) that single exposures to a sinusoidal, relatively weak PEMF tends to increase nociception. However, a single exposure to a specific low-frequency PEMF (8) can induce antinociception (ie, analgesia). To date, this has been observed in snails (5), rodents (9) and healthy volunteers (4,10). A single application of this PEMF has been shown to affect human electroencephalogram (2,11) and standing balance in both healthy humans (12) and

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patients with rheumatoid arthritis (RA) and fibromyalgia (FM) (13). The recent report (14) that a similar PEMF can reduce depression in patients with bipolar depression suggests that a PEMF can also influence affective state.

Taken together, these findings suggest that such weak PEMFs may alter pain perception in patients with chronic pain. We report here the effects of a 30 min exposure to a PEMF on pain levels in FM and RA patients using a double-blind, randomized, placebo-controlled parallel design.

PATIENTS AND METHODS

Participants

This study was approved by the Research Ethics Board for the Review of Health Sciences Research Involving Human Subjects at the University of Western Ontario, London, Ontario.

Thirteen female RA patients in study 1 (mean age 52.23 years, range 29 to 79 years) and 18 female FM patients in study 2 (mean age 51.28 years, range 35 to 67 years) were recruited from day treatment programs at St Joseph's Health Care (London, Ontario). Participation in the program required a physician referral following a positive diagnosis for RA or FM by a rheumatologist (15,16). There were standardized criteria for chronic pain patients to be included in the program, which included pain history, diagnostic criteria and chronic pain level. The authors did not have access to the patients' medical history, the population was not preselected in any way and selection bias was not applied. It was thought that this enrollment method provided the most robust and critical method for testing the treatment. Patients were narcotic free during the present study and were screened for depressive symptoms (concomitant depression was an exclusion criterion for the program). Subjects were numerically and randomly assigned on a computer-generated list and all blinding (data, equipment and exposure condition) was maintained by staff outside of the study.

Of the RA patients, seven were randomly assigned to the PEMF group (mean age 54 years, SD=15.87) and six were randomly assigned to the sham exposure group (mean age 50.71 years, SD=12.0). No patients withdrew from the study before completion of the study requirements. Nine of the FM patients were randomly assigned to each of the PEMF (mean age 51.5 years, SD=9.07) and sham (mean age 51 years, SD=9.90) exposure groups. One FM patient withdrew (sham group) before the exposure period due to feelings of anxiety unrelated to the research conditions.

Materials

All subjects were seated in a comfortable chair in a quiet room. A headset fitted with coils beneath the plastic ear coverings and connected by a wire to the portable PEMF generating unit was placed with the earpieces covering the patient's temples. The headset covered the area that extended from above the temple to just above and behind the ear, on both sides of the head. Consequently, the treatment area was the area of the central nervous system that went from immediately above the temple to just above and behind the ear, extending from the outer periphery of the cingulate cortex to the brain midline. The PEMF unit was designed to have two pulse sequence patterns: one pattern was set to deliver a zero-amplitude magnetic field (MF) exposure (sham), while the other pattern produced a PEMF of a maximum of 200 μT (2 Gauss) to the deep brain and a maximum of 400 μT (4 Gauss) at the headset. The frequency content of the MF as determined by Fourier analysis was less than 1 kHz. The pulse design used in the current study is described in the United States patent #6,234,953 (8).

The MF was not physically detectable by either the experimenter or the participant. No sound or vibration was emitted and there were no visual indicators on the unit other than a blinded 'a' or 'b' switch setting for conditions. The experimenter was provided with a randomized and blinded schedule of the 'a' or 'b' switch settings before each run of sessions for a day.

The McGill Pain Questionnaire (MPQ) (17) was used to assess subjective measures of clinical pain both before and after the delivery of the PEMF or sham exposure. This questionnaire consisted of four major classes of word descriptors: sensory, affective, evaluative and miscellaneous. Patients were asked to select the most fitting word in each of the 20 categories that pertained to their current pain level. A category was omitted if none of the words were relevant to the patient's pain. Words within each category were ranked in order of appearance; a sum of the selected words according to their ranking provided the clinician with a Pain Rating Index (PRI). In addition to the PRI, an overall Present Pain Intensity (PPI) measure was provided on the questionnaire. This question asked patients to indicate their level of current pain intensity on a six-point Likert scale, ranging from no pain (0) to excruciating pain (5). The MPQ has been successfully tested for reliability and validity (17).

Visual analogue scales (VAS) (18) were used to assess levels of pain and anxiety, both before (pre) and after (post) MF or sham exposure. The pain scales ranged from no pain to worst possible pain. The anxiety scale ranged from no anxiety to worst possible anxiety.

The Beck Depression Inventory-II (BDI-II; The Psychological Corporation, USA), the most widely used instrument for detecting depression, is consistent with diagnostic criteria listed in the *Diagnostic and Statistical Manual of Mental Health Disorders-IV* (19). This questionnaire was quick and easy to complete; it contained four to six sentences from which individuals were expected to select the one that best described their experiences over the previous two weeks. The BDI-II has been shown to provide reliable, internally consistent and valid scores in medical settings (20). This questionnaire was administered at the beginning of the study to verify the patients' depression level.

Procedure

Patients were randomly assigned to either the sham (no PEMF exposure) or the PEMF exposure conditions. The purpose of the study was explained and informed consent was obtained from the patients before the beginning of the experiment.

Once seated comfortably in the chair, patients completed the MPQ, the VAS for both pain and anxiety and the BDI-II. Patients were also asked to report their handedness and when their last menstrual cycle ended. The headset was then secured on the patients' temples. After 15 min of recording physiological data (heart rate and respiration), the PEMF device was set to deliver the random but blind condition. Following 30 min of PEMF or sham exposure, an additional 10 min of rest (with no exposure) was recorded after which the MPQ and the pain and anxiety VAS scales were completed a second time. Patients were left alone in the experiment room but were provided with a paging device to have access to the experimenter at any time. The specific settings for the sham and PEMF exposure were kept blind to both the patient and the experimenter, and the code was broken following all data collection. Participants were queried as to which condition they thought they had received and asked if they had anything else such as adverse events to report. Analysis indicated that the participants guessed their condition at a random rate (their guess was not significantly correlated to the actual condition).

TABLE 1
Summary of pain and anxiety ratings, pre- and post-magnetic field or sham exposure conditions for rheumatoid arthritis patients

	Magnetic field		Sham	
Measure	**Pre**	**Post**	**Pre**	**Post**
Pain rating index				
Total	25.86±6.67	12.14±8.78 (P=0.001, eta^2=0.872)	18.50±13.82	12.16±18.14 (P=0.052, eta^2=0.562)
Sensory	18.4±4.69	8.14±5.98 (P=0.004, eta^2=0.778)	12.30±5.50	7.67±10.0 (P=0.105, eta^2=0.438)
Affective	1.29±1.12	0.57±0.785 (P=0.094, eta^2=0.397)	1.84±2.40	1.00±2.45 (P=0.185, eta^2=0.321)
Evaluative	1.86±1.22	1.29±1.38 (P=0.508, eta^2=0.076)	1.50±1.76	0.667±1.21 (P=0.042, eta^2=0.595)*
Miscellaneous	4.28±2.87	2.14±2.27 (P=0.023, eta^2=0.606)	2.83±5.00	2.83±4.68 (P=1.00, eta^2=0.000)
Present pain intensity	1.57±0.535	1.43±0.535 (P=0.604, eta^2=0.048)	2.00±0.633	1.17±1.17 (P=0.042, eta^2=0.595)*
Visual analogue scale				
Pain	5.04±2.21	3.01±2.46 (P=0.031, eta^2=0.566)	4.35±1.22	4.17±3.21 (P=0.839, eta^2=0.009)
Anxiety	3.74±1.64	2.13±2.14 (P=0.071, eta^2=0.445)	3.17±2.36	3.12±2.92 (P=0.966, eta^2=0.000)

Data presented as mean ± SD. **Significant values (P<0.05). eta^2 Estimate of effect size*

All of the analyses were performed using SPSS version 11.0, (USA). Analyses were performed separately on each of these independent studies (study 1: RA patients; study 2: FM patients). Pre- versus post-exposure results (repeated measures) were tested a priori to account for possible confounding placebo effects in the sham exposure groups. Where interactions were not significant, particular attention was paid to alpha and estimate of effect size (eta^2) values. Pain and anxiety data were analyzed using repeated measures ANOVA. Covariates (eg, age, handedness, menstrual cycle phase and depression rating) were analyzed and not found to change any of the significance levels reported below. All hypothesis tests used α=0.05.

RESULTS

Study 1: RA patients

Demographic information: There was no significant difference in age between patients randomly assigned to the two groups, $t(11)$=–0.43, P>0.1.

Pain ratings: A significant interaction was found between the pre-post pain rating and type of exposure, ie, the effect of pre-test versus post-test condition on pain ratings differed across the exposure conditions, with a large reduction of pain noted in the PEMF-exposed group and a lesser reduction in the sham exposed group. Table 1 displays the specific numbers and significance values for the overall and subcomponent parts (including the PPI) of the MPQ. Specifically, a repeated measures ANOVA revealed the significant pre-post × condition interaction for the MPQ PRI (Total), F(1,11)=5.09, P<0.05, partial eta^2=0.32, power = 0.54. This was confirmed by *t* test due to the disparity in pre-exposure pain levels between the two groups (pre-score minus post-score tested between the sham and MF conditions [t=2.26, P<0.05]). There was also a significant main effect of pre-post testing, F(1,11)=37.51, P<0.01, partial eta^2=0.77, power = 1.0.

Similar findings were found for the miscellaneous subscale of the MPQ. Results from the sensory, affective and evaluative subscales, as well as the PPI of the MPQ, revealed significant main effects of pre-post testing; however, prepost testing × condition interactions were nonsignificant.

VAS – Pain: The only significant change using the VAS pain rating was found within the PEMF group: these patients had reduced pain ratings after the PEMF exposure. Conversely, PEMF versus sham exposure on the VAS pain rating did not differ between the pre-test and post-test times, and the effect of test time on its own did not lead to any changes in pain rating (Table 1).

Specifically, patients randomly assigned to the PEMF groups had significantly reduced pain ratings following their exposure period, F(1,6)=7.84, P<0.05, partial eta^2=0.57, power = 0.65; patients in the sham exposure group did not report significantly reduced VAS pain ratings, F(1,5)=0.05, P>0.10, partial eta^2=0.01, power = 0.05. The pre-post-testing × condition (PEMF versus sham exposure) interaction for VAS pain ratings was nonsignificant, F(1,11)=3.95, P>0.10, partial eta^2=0.26, power = 0.44. The main effect of pre-post-testing was also nonsignificant, F(1,11)=3.95, P>0.10, partial eta^2=0.26, power = 0.44.

VAS – Anxiety: Table 1 displays the mean anxiety ratings reported by RA patients randomly assigned to the PEMF and sham exposure groups both pre- and post-exposure. Analysis of these results revealed a nonsignificant reduction in anxiety ratings, F(1,11)=1.64, P>0.10, partial eta^2=0.13, power = 0.22. Furthermore, there was no significant condition by pre-post testing interaction for anxiety ratings, F(1,11)=1.45, P>0.10, partial eta^2=0.12, power = 0.20.

Study 2: FM patients

Demographic information: There was no significant difference in age between patients randomly assigned to the two groups, $t(15)$=0.11, P>0.10.

Pain ratings: Using the MPQ, the only decreases in pain ratings were made by the subjects that were assigned to the PEMF group. Repeated measures ANOVA revealed a significant overall pre-post-effect for the MPQ PRI (Total), F(1,15)=16.16, P<0.01, partial eta^2=0.52, power = 0.96. The PEMF group, F(1,8)=17.60, P<0.01, partial eta^2=0.69, power = 0.96, but not the sham group, F(1,7)=3.98, P=0.09, partial eta^2=0.36, power = 0.41 showed a significant decrease in the overall pain rating following the exposure period. There was no significant interaction between pre-post-testing and condition (sham versus PEMF exposure) on this pain rating measure, F(1,15)=0.32, P=0.58, partial eta^2=0.02, power = 0.08.

TABLE 2
Summary of pain and anxiety ratings, pre- and post-magnetic field or sham exposure conditions for fibromyalgia patients

	Magnetic field		Sham	
Measure	**Pre**	**Post**	**Pre**	**Post**
Pain rating index				
Total	24.89±14.94	14.44±11.88 (P=0.003, eta^2=0.688)*	26.50±9.93	18.63±12.28 (P=0.086, eta^2=0.362)
Sensory	15.11±9.01	9.22±6.70 (P=0.01, eta^2=0.558)*	16.88±5.62	11.88±7.30 (P=0.098, eta^2=0.342)
Affective	3.00±2.74	1.67±2.24 (P=0.035, eta^2=0.444)*	2.63±1.92	0.88±1.73 (P=0.105, eta^2=0.331)
Evaluative	2.67±1.32	1.11±1.17 (P=0.008, eta^2=0.605)*	2.25±1.70	1.63±1.06 (P=0.351, eta^2=0.125)
Miscellaneous	4.11±3.95	2.44±2.70 (P=0.105, eta^2=0.294)	4.75±2.43	4.25±3.65 (P=0.681, eta^2=0.026)
Present pain intensity	2.33±0.866	1.33±0.707 (P=0.003, eta^2=0.692)*	2.63±0.916	1.50±0.535 (P=0.002, eta^2=0.779)*
Visual analogue scale				
Pain	5.69±2.78	3.78±2.44 (P=0.001, eta^2=0.793)*	7.64±1.74	5.93±2.79 (P=0.045, eta^2=0.516)*
Anxiety	2.78±3.26	1.68±1.54 (P=0.136, eta^2=0.288)	4.99±4.41	2.90±2.54 (P=0.159, eta^2=0.301)

Data presented as mean ± SD. **Significant values (P<0.05). eta^2 Estimate of effect size*

Similar findings were found for the sensory, affective and evaluative subscales of the MPQ. Table 2 displays the specific numbers and significance values for the overall and component parts (including the PPI) of the questionnaire.

The miscellaneous subscale of the questionnaire did not yield the same results; there was no significant effect of pre-post-testing across groups (F[1,15]=2.19, P=0.16, partial eta^2=0.13, power = 0.28), of pre-post-testing for the PEMF (F[1,8]=3.33, P=0.11, partial eta^2=0.29, power = 0.36) or sham groups (F[1,7]=0.18, P=0.68, partial eta^2=0.03, power = 0.07), or of pre-post-testing by condition interaction (F[1,15]=0.64, P=0.44, partial eta^2=0.04, power = 0.12).

In contrast, there was a significant pre-post effect across groups (F[1,28]= 35.05, P=0.001, partial eta^2=0.56, power = 1.00) for the PPI scores. These scores were significantly decreased pre- to post-exposure for both the PEMF-exposed group of patients (F[1,9]=18.00, P=0.003, partial eta^2=0.69, power = 0.96) and the sham-exposed patients (F[1,7]=24.65, P=0.002, partial eta^2=0.78, power = 0.99).

VAS – Pain: Using the pain ratings from the VAS, a significant decrease in pain ratings was found after both sham and PEMF exposure (Table 2). A significant pre-post exposure effect was noted for VAS pain ratings, F(1,13)=23.70, P<0.001, partial eta^2=0.65, power = 1.00, with decreased pain scores present following the exposure period. Patients randomly assigned to both the PEMF and sham groups had significantly reduced pain ratings following their exposure period, F(1,7)=26.85, P<0.01, partial eta^2=0.79, power = 0.99 and F(1,6)=6.39, P<0.05, partial eta^2=0.52, power = 0.56 for the PEMF and sham groups, respectively. No pre-post-testing by condition interaction existed.

VAS – Anxiety: Table 2 displays the average anxiety ratings reported by patients randomly assigned to the PEMF and sham exposure groups both pre- and post-exposure. Analysis of these results revealed a significant overall reduction in anxiety ratings across the entire patient pool, F(1,13)=5.21, P<0.05, partial eta^2=0.29, power = 0.56; however, anxiety ratings did not significantly change across pre-post-testing for patients when analyzed separately by group, F(1,7)=2.83, P=0.14, partial eta^2=0.29, power = 0.31 and F(1,6)=2.59, P=0.16, partial eta^2=0.30, power = 0.27 for the PEMF and sham groups, respectively. Furthermore, there was no significant condition by pre-post-testing interaction for anxiety ratings, F(1,13)=0.50, P=0.49, partial eta^2=0.04, power = 0.10.

DISCUSSION

The results indicate that exposure to a specific low-frequency PEMF appears to have some beneficial analgesic properties, particularly in patients with RA. The results for the FM patient sample were mixed.

Pain ratings (MPQ and VAS)

Both the RA and FM patients randomly assigned to the sham and PEMF exposure groups reported decreased pain ratings following the 30 min trial period. Specifically, the RA patients exposed to the PEMF experienced a larger reduction in pain ratings than patients in the sham exposure group according to the pain rating on the MPQ (total) and VAS. For the FM patients, those in the PEMF group also had post-exposure pain ratings on the MPQ (total) that were more reduced by the exposure period (a priori hypothesis) compared with the control subjects; however, on the VAS, the FM patients who received both the PEMF and sham exposure showed a decrease in pain, with a greater decrease in the PEMF-exposed group. For RA patients, these findings were supported by the presence of a significant condition by time of testing interaction. Patients randomly assigned to the PEMF group had a significantly greater reduction in MPQ PRI scores than those in the sham exposure group.

All patients in the present study reported decreased pain ratings across time, an occurrence that can be attributed to the placebo effect. As defined by Kleinman et al (21), the placebo effect is the observation of a psychological or physiological change associated with inert treatments, sham procedures or therapeutic encounters. In the present study, patients were exposed to a therapeutic encounter: administration of either PEMF or sham exposure. For some of these patients, no treatment modality administered before participation in the current study, either pharmacological or non-pharmacological, was providing pain relief. The presence of a potentially effective and beneficial therapeutic treatment was likely encouraging to these patients; the potential benefit may have driven these patients to voluntarily participate in the study and expect a benefit.

The partial eta^2 values obtained for patients in the two exposure groups (PEMF and sham) are consistent with the view that PEMF exposure confers a benefit greater than that obtained by expectancy or the placebo effect. For RA patients, the average partial eta^2 value obtained for the pain ratings was 0.87 for patients in the PEMF group and 0.56 for the sham-exposed patients. Values for the FM patients were 0.69 and 0.36 for the PEMF and sham exposure groups, respectively.

Aside from the placebo effect, decreases in pain ratings for patients randomly assigned to the sham group can be attributed to relaxation. Staud et al (22) have reported that patients with FM report improvements in chronic pain following periods of rest. In the present study, the 55 min experimental period in which patients were seated in a comfortable chair could be considered a setting of relaxation; this time period of relaxation may have been the catalyst for reduced pain ratings post-exposure. Alternatively, activities in which the patients partook before enrollment and/or participation in the present study (eg, exercise training, household work), which were not controlled by the study administrators, may have exacerbated the patients' pain symptoms (22), resulting in elevated pre-exposure pain ratings for patients in both the PEMF and sham exposure groups. Even if relaxation or prior activity participation were the cause of altered pain ratings, patients in the PEMF group benefited from significantly reduced pain ratings on a number of the tested scales (eg, PRI) post-exposure while patients in the sham exposure group did not.

Pain ratings assessed via the PPI and the VAS provided mixed results for both patient populations. Patients in the PEMF group for both patient populations reported significantly reduced VAS scores; however, of the sham-exposed patients, only the patients in the FM sample reported significantly reduced scores. For the PPI, significantly reduced scores were reported for RA patients in the sham exposure group and FM patients in the PEMF and sham exposure groups. PPI scores were not significantly reduced for RA patients in the PEMF group. One possible explanation for these results is that both the VAS and PPI refer to the intensity of the experienced pain in contrast to the quality of pain that is measured through the PRI. By memory alone, patients can improve their pain rating on the PPI. While both of the PPI and VAS measures have been shown to be valid and reliable indicators of subjective pain ratings, the finding that the MPQ had significantly reduced pain ratings only for the PEMF-exposed patients (and that a significant condition by time of testing interaction was present for RA patients for the MPQ) provides evidence, albeit mixed, of the analgesic conferring properties of exposure to the PEMF. Furthermore, it is a possibility that PEMF exposure influences the quality of experienced pain but not pain intensity itself.

Results of the present study are consistent with past research such that exposure to a specific PEMF has been shown to increase latencies on a hot plate in snail (5,23,24) and rodent models (9), as well as increase thermal (4) and electric (10) thresholds in humans. The effect of such extremely low-frequency PEMFs on pain and other behavioural responses is likely due to a direct sensing mechanism within tissues and cells (6). Finally, exposure to PEMFs has been studied for a large variety of clinical indications and has been shown to have encouraging results for most of the conditions studied (25).

CONCLUSION

Results of the current study confirm past findings in snails, rodents and humans exposed to acute pain that exposure to a specific PEMF has a modest pain-reducing effect in patients with RA. For these patients, exposure to a low-frequency PEMF produced decreases in pain beyond those found for a sham treatment control group. Interestingly, this effect was not found when FM patients were compared using an identical protocol. Future research using possibly more optimal PEMF parameters should be conducted to better understand how and when PEMFs produce reductions in clinical pain.

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ORIGINAL ARTICLE

Healing of Chronic Arterial and Venous Leg Ulcers With Systemic Electromagnetic Fields

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Background. Mitogen-activated autologous peripheral blood mononuclear cells applied locally on the ulcer surface promote healing of chronic arterial and venous leg ulcers. *In vitro*, extremely low frequency electromagnetic fields (ELF) interact with peripheral blood mononuclear cells (PBMC) via Ca^{++} channels, activating signal transduction cascades, promoting cytokine synthesis, and changing cell proliferation patterns.

Methods. ELF frequencies were configured to interact *in vitro* with the proliferation patterns of PBMC obtained from normal human volunteers. These ELF were then applied peripherally as the sole treatment to 26 patients with 42 chronic leg ulcers of predominantly arterial or venous etiology unresponsive to previous medical and/or surgical treatments in a phase I before-after design.

Results. At admission, age of ulcers had a skewed distribution with a median of 639 days. Wound healing or deleterious effects began in all patients during the first 2 weeks after ELF exposure, permitting their previously unresponsive ulcers to function as internal controls. After ELF exposure, 69% of all lesions were cured or healed >50% in a period <4 months. Defective wound healing was observed in lesions associated with important arterial occlusion, uncontrolled arterial hypertension, severe lipodermatosclerosis, non-pitting edema, and obesity (body mass index >30). Lesions worsened in patients with autoimmune diseases.

Conclusions. Systemic effects are hypothetically explained by ELF activation of PBMC and their subsequent transportation to the ulcer site via humoral route. This therapy is effective in selected patients with chronic arterial and venous leg ulcers.

Key Words: Leg ulcers, Skin ulcers, Electromagnetic fields, Cell activation.

Introduction

Chronic arterial and venous leg ulcers can be thought of as dysregulated inflammatory processes produced by inadequate blood supply, tissue anoxia, edema, cell death, and infection, among other factors (1). These changes alter interaction among structural components of affected tissues and between these and immune cells in a manner that impedes wound healing. Existing hypotheses on the pathophysiology of chronic arterial and venous leg ulceration concentrate on local effects induced by hemodynamic alterations (2–8). Treatments at present focused on alleviating these local changes include hemodynamic preventive

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measures, ulcer dressings, topical treatments, and surgical or endovascular repair of the macrovasculature (8,9). Pharmacologic agents and locally applied growth factors have in general shown poor results (8,9). Successful experimental treatments include intramuscular gene transfer (10), allogeneic skin grafts (11), and directly applied electromagnetic fields (ELF) (12,13). Central to the present study is the successful treatment of non-healing skin ulcers with autologous-activated mononuclear cells (14), and *in vitro* experiments that have shown that ELF elicit changes in cells of the immune system through Ca^{++} signaling (15–19), including up-regulated cytokine synthesis (20,21) and increased cell proliferation (16,21). Hypothetically, activation of peripheral blood mononuclear cells (PBMC) could be induced in the body of patients with chronic leg ulcers by using ELF frequencies that interact with PBMC. To test this hypothesis, ELF frequencies were specifically configured to interact with PBMC obtained from normal human volunteers *in vitro*. Subsequently, these ELF were applied to patients with chronic leg ulcers at a site far from the lesion site. The prompt effects of this treatment on chronic leg ulcers were monitored. Results are summarized in this preliminary report.

Materials and Methods

In vitro *Studies*

PBMC proliferation. Heparinized venous blood from healthy human volunteers was fractionated on a Ficoll gradient (Amersham Co., Arlington Heights, IL, USA). The fraction containing PBMC was washed in phosphate-buffered saline and resuspended in sterile culture medium (RPMI 1640, Sigma Chemical Co., St. Louis, MO, USA) to a final concentration of 5×10^6 cells/mL. Eppendorf tubes were filled with 0.2-mL aliquots of PBMC suspension plus 1 mg/mL of phytohemagglutinin (Gibco BRL Life Technologies, Inc., Gaithersburg, MD, USA). Four subsets in triplicate were studied. The first set had no ELF or static magnetic fields (SMF); the second was exposed to a combination of ELF and SMF; the third was exposed to ELF alone, and the fourth to SMF only. Four similar subsets without phytohemagglutinin were used as controls. All tubes were incubated 58 h at 37°C in a CO_2 humidifier incubator (Forma Scientific, Inc., Marietta, OH, USA); then, 1 μCi of [Methyl-^{3}H] thymidine (specific activity 185 GBq/mmol, Amersham) was added to all tubes and incubated for an additional 14 h. Tubes were harvested at 72 h and washed through glass fiber filter paper with a Skraton cell harvester (Molecular Device Corp., Göteborg, Sweden). [Methyl-^{3}H] thymidine incorporation was determined using a liquid scintillator counter (Beckman model LS 6000 SE, Beckman, San Diego, CA, USA). Mean number of counts per minute was obtained in triplicate for each sample. Cell viability was always >90% determined on the basis of trypan blue exclusion.

PBMC magnetic field exposure conditions. Homogeneous SMF of 503 ± 45.9 SD Gauss were generated inside the exposure chamber with permanent magnets around the coil (Figure 1 A,a). ELF were produced inside a 4-cm length coil composed of four layers of 22 AWG wire (272 total turns). Measured inductance was 5.61 mH. (Inductimeter, Beckman model LM22A). An alternating current power source (120 V/60 Hz) was connected to a transformer and a rectifier bridge, which supplied 100 mA rms to the coil (Figure 1 A,b). ELF magnetic field strength was 8.02 Gauss. Cells were exposed inside Eppendorf tubes placed in the outer edge of an acrylic rack inside a cylindrical exposure chamber 7 cm in diameter and 8 cm in length (Figure 1A,c). Exposure chambers were shielded from the magnetic fields of the cell culture incubator using μ-metal. Magnetic fields ambient background levels were <0.44 Gauss.

Patients. A phase I before-after trial to document ELF's systemic effects on chronic arterial and venous leg ulcers was approved by the Hospital Juárez Institutional Review Board/Ethics Committee (September 25, 1995). Pregnant women and patients with cancer were excluded. All recruited patients had chronic leg ulcers resistant to medical and surgical treatment and were under medical care prior to admission. After signing a voluntary consent form, 30 patients with 49 chronic leg ulcers and two paraplegic patients with decubitus ulcers below the spinal lesion were accepted into the protocol. During the study, three patients with chronic leg ulcers (five wounds) were excluded, one with factitious ulcers, one who employed unauthorized treatments, and one with secondary effects of amlodipine. The paraplegic patients and one patient with two ulcers diagnosed as pyoderma gangrenosum associated with chronic venous disease were analyzed separately. A total of 26 patients with 42 chronic leg ulcers of predominantly arterial or venous etiology unresponsive to medical and surgical treatments were studied. Standards recommended by the Society for Vascular Surgery/North American Chapter and the International Society for Cardiovascular Surgery were used as criteria to define the contribution of arterial and venous disease in chronic leg ulcer etiology (23,24). Patients were exposed to ELF alone (five patients) or to a combination of ELF and SMF (21 patients). Eight patients had 17 chronic leg ulcers of predominantly arterial etiology and 18 patients had 25 chronic leg ulcers of predominantly venous origin. Their characteristics are summarized in Table 1. Patients were allowed to continue systemic treatments for pain, rheumatoid arthritis, arterial hypertension, and diabetes. Other systemic medications and preventive measures were discontinued. Local treat-

ments were limited to cleaning the ulcer with soap and water and subsequently covering it.

Patient exposure conditions. Patients were exposed to magnetic fields by placing either arm inside a cylindrical exposure chamber 10 cm in diameter by 25 cm in length. Its internal structure was similar to the *in vitro* exposure system (Figure 1A). Average exposure time was 2–3 h/day three times weekly (Figure 1B). ELFs were generated inside a coil 25 cm in length with three layers of 22 AWG wire (1,059 total turns). Measured inductance was 43 mH (Inductimeter Beckman model LM22A). An alternating current power source (120 V/60 Hz) was connected to a transformer and to a rectifier bridge, which supplied 680 mA rms to the coil. Spectral frequencies distribution of ELF were measured as an induced voltage through a 4.7-kΩ shunt resistor, bridging the leads of a 1-cm probe made of 190 turns of number 42-gauge magnet copper wire placed perpendicularly to the ELF direction. The probe was connected to a digitizing oscilloscope (TDS 420 Tektronik) and to a spectral analyzer (model SR 7609, Stanford Research Systems) (Figure 1C). ELF magnetic field strength was 36.36 Gauss. Maxwell equations defined induced variable electric fields perpendicular to magnetic field flux lines with amplitude proportional to strength and time variation of magnetic fields (dB/dt). Homogeneous static magnetic field of 522 ± 93.6 SD. Gauss were generated inside the exposure chamber with permanent magnets around the coil (Figure 1D). SMF strength was measured with a calibrated Hall effect probe (RFL model 912) placed perpendicularly to uniform SMF. A map of homogeneous magnetic field generated by combined ELF and SMF inside the exposure chamber is depicted in Figure 1E. Magnetic fields ambient background levels were 1.0 Gauss (patent pending). Magnetic field ambient background levels were 1.0 Gauss (Figure 1B).

Statistics. Follow-up ulcer size and appearance were consecutively documented with photographs that in turn were digitized and processed with Internet Scion Image software (25). Areas were calculated by delineating ulcer contours, counting the number of pixels contained inside the area and comparing it with the number of pixels contained in 1 cm^2 measured above a ruler placed near the ulcer (Figures 3–5). JMP 3.2.1 statistical software from the SAS Institute (Cary, NC, USA) was used for descriptive statistics (mean ± SD, medians and ranges), χ^2, ANOVA F, *t* tests, and linear regression.

Results

In vitro *Studies.* PBMC exposure to ELF alone decreased the stimulation produced by phytohemagglutinin by 62.2% with statistical significance $p < 0.05$ (Tukey test). This effect was inhibited when PBMC were exposed to a combination of ELF and SMF. Data were analyzed by two-way ANOVA, with patients as blocks and experimental conditions. Patients: $p < 0.04$; conditions: $p < 0.0001$ (F test), and Tukey test was done for conditions (Figure 2).

Patients. According to their response to treatment, patients were divided into two groups: responders when all wounds healed or with a >50% size reduction during the first 4 months and non-responders when at least one ulcer had a <50% size reduction or increased in size within the same time period (Figures 3 and 4) (Table 1). Age and initial size of chronic leg ulcers at admission had a skewed distribution. Age range was as follows: 30–4,745 days, median: 639 days; quartile 25%: 171 days, and quartile 75%: 2,281 days. Initial size range: 0.43–350.7 cm^2, median: 6.45 cm^2; quartile 25%: 1.97 cm^2, and quartile 75%: 24.93 cm^2. Healing or deleterious effects began in all patients during the first 2 weeks after initiation of treatment (Figures 3–5). Negative secondary effects were absent during treatment and follow-up periods. ELF alone or associated with SMF produced similar healing or deleterious effects (Figures 3–5).

Responders. Twenty-nine ulcers of different size and age previously unresponsive to conventional treatments began to heal after ELF arm exposure. Healing velocity as percentage of area reduction/day was calculated for each chronic arterial and venous leg ulcer by linear regression with values of R^2 around 0.9 and $p < 0.01$ for all cases. In 93% of chronic arterial and venous leg ulcers, this value ranged from 0.3 to 3.0%; the remaining 7% varied from 3.0 to 12.0%. No statistical differences in healing speed were observed between chronic arterial and venous leg ulcers (ANOVA for regression coefficients, $p > 0.05$). Healed ulcers remained healed for at least 6 months and up to 2 years after the conclusion of treatment. In the affected legs of patients with chronic arterial leg ulcers, the superficial vascular network became visible and skin temperature increased after 4–8 weeks of treatment (Figure 3 a–c). In chronic leg ulcers of predominantly venous etiology, pain, edema, and weeping were reduced significantly or eliminated 3–6 weeks after the initiation of treatment (Figure 3 e–f).

Non-responders. Thirteen ulcers healed poorly or increased in size. Most were of predominantly venous etiology, ulcers were older and larger in size, and patients of this group had higher body mass index (BMI) (Table 1). In ulcers of predominantly arterial origin, deficient wound healing was associated with severe arterial occlusion (Figures 4 a–d) and uncontrolled arterial hypertension. In ulcers of predominantly venous etiology, non-healing was associated with obesity and/or non-pitting edema. Figures 4 e–g depict different responses to treatment in separate ulcers of the same patient. Two ulcers located in areas with non-pitting edema increased in size (only one is shown, top), while the third ul-

Table 1. Chronic leg ulcers of predominantly arterial etiology (CALU) and chronic leg ulcers of predominantly venous etiology (CVLU)

		Responders	Non-responders	All patients	Test	*p* value
# patients	CALU	6	2	8	Fisher exact	
	CVLU	10	8	18		<0.42
	Total	16	10	26		
Patient (age/years)	Range	33−85	46−80	33−85	Student *t*	<0.661
	Mean	59.4	62.1	60.5		
	SD	17.0	10.43	14.6		
Sex	Male	2	4	6	Fisher exact	<0.163
	Female	14	6	20		
Body mass index	Range	15.8−32.5	23−45.32	15.8−45.32		
	Mean	25.85	31.4	27.98	Student *t*	<0.026
	SD	4.94	6.97	6.31		
# ulcers	CALU	15	2	17		
	CVLU	14	11	25	Chi square	<0.027
	Total	29	13	42		
Ulcer age at admission	Range/days	30−2,550	91−4,745	30−4,745		
	Median	274	2,190	639	Wilcoxon	<0.0018
Ulcer size at admission	Range/cm^2	0.43−116.05	2.19−350.7	0.43−350.7		
	Median	2.92	32.3	6.45	Wilcoxon	<0.0002

cer (bottom) located in an area without non-pitting edema healed by 87.5%. Poor healing response was observed in severe lipodermatosclerosis (Figures 4 h–i). Pain was only partially reduced 4–6 weeks after initiation of treatment. In one patient with pyoderma gangrenosum associated with chronic venous disease, ulcer size increased after magnetic field exposure (Figures 5 a–c).

To ascertain whether systemic effects were caused by magnetic field interaction with action potentials, we measured differences in amplitude or latency of radial nerve somatosensory-evoked potentials before, during, and after magnetic field exposure. No changes in nerve conduction parameters associated with magnetic fields exposure were found (unpublished results). The healing response of two paraplegic women with chronic skin wounds resistant to medical and surgical treatments below the spinal lesion was studied in the patients' homes. Activation of the wound repair process began the second week after ELF exposure; healing speed was similar to that of patients with chronic wounds, intact nervous systems, and under the same treatment conditions (Figures 5 d–g).

Discussion

It has been reported that ELF (15–19) and SMF (26) elicit changes in cells of the immune system through Ca^{++} signaling. The *in vitro* studies were intended to configure optimal ELF frequencies that could interact with PBMC proliferation and to investigate SMF and ELF/SMF effects on PBMC proliferation. PBMC were obtained from healthy human volunteers to exclude interference of a diseased status. These experiments showed that ELF exposure decreases PBMC proliferation and ELF combined with SMF inhibited the *in vitro* ELF effect (Figure 2). In both cases, cell viability was maintained during the incubation period, indicating that the magnetic fields used interacted with phytohemagglutinin-induced PBMC proliferation. Hypothetically, it was possible for *in vitro* interactions between

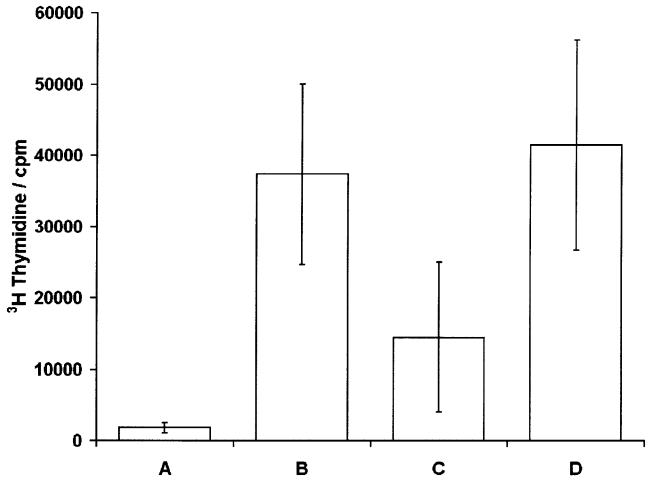

Figure 2. ELF modifies PBMC proliferation patterns without reducing cell viability. Each column represents the results of three independent experiments done in triplicate. A) PBMC without phytohemagglutinin. Mean 1,775 ± 675 SD. B) PBMC proliferation increased 21 times with phytohemagglutinin. Mean 37,394 ± 12,674 SD. C) PBMC with phytohemagglutinin exposed to ELF. Mean 14,514 ± 10,528 SD. Proliferation was reduced by 62.2% with statistical difference $p < 0.05$ (Tukey test) with (B) and (D), and D) PBMC with phytohemagglutinin exposed to ELF combined with SMF. Mean 41,514 ± 14,717 SD. No statistical difference was found between (D) and (B); $p > 0.05$ (Tukey test). Error bar = mean ± SD.

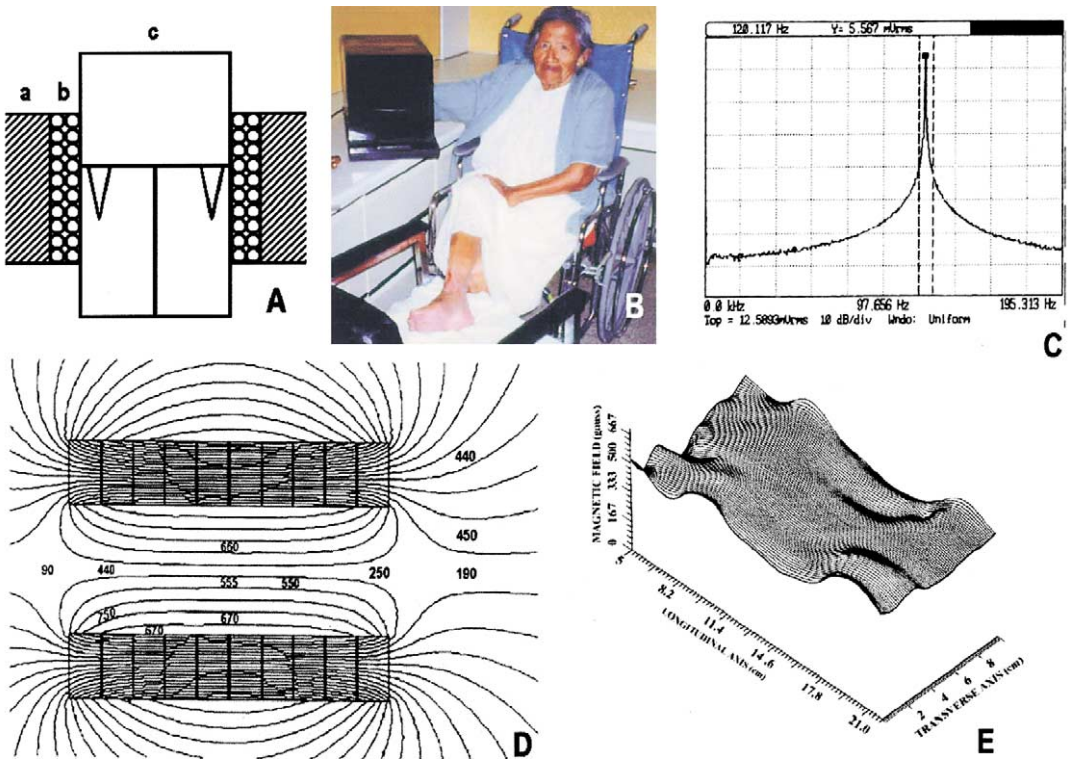

Figure 1. Exposure conditions and magnetic field parameters (See Materials and Methods).

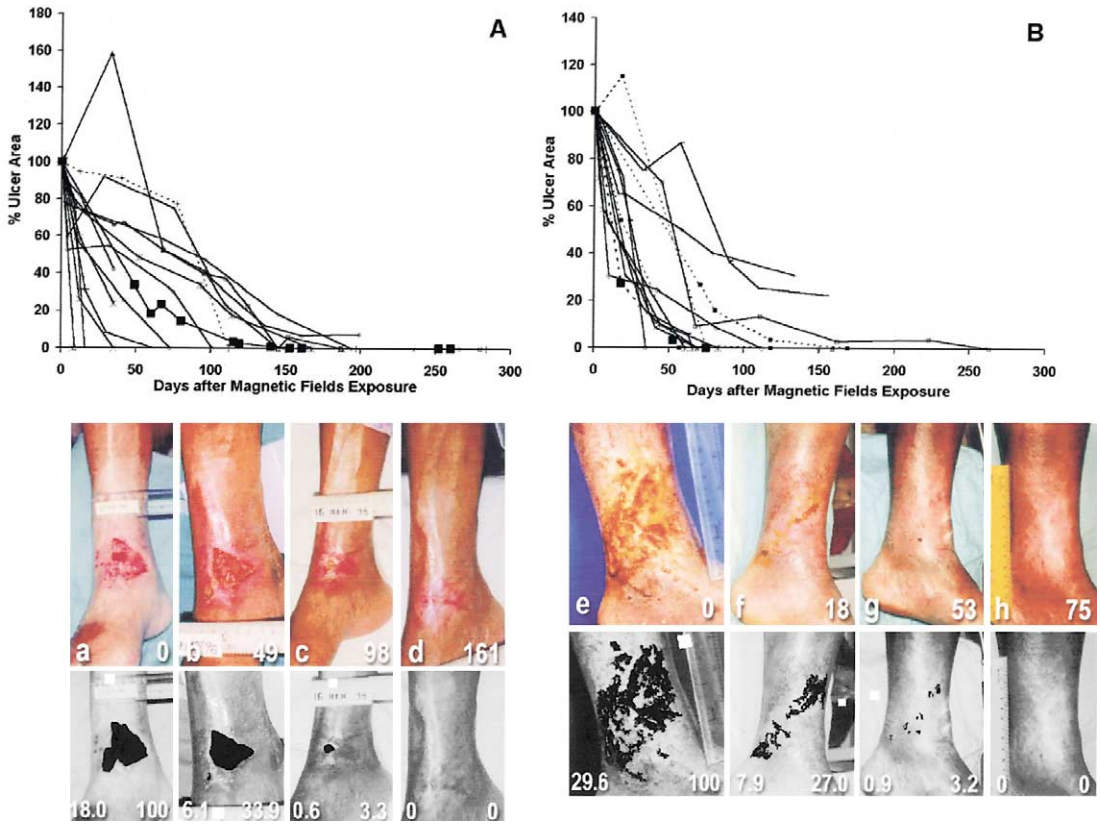

Figure 3. Chronic leg ulcer evolution in responder patients. (A) Predominantly arterial etiology. (B) Predominantly venous etiology. Graphs in Figures 3–5 depict ulcer-size evolution as change in percentage over time of magnetic field exposure. Dotted lines (ELF alone). Solid lines (ELF + SMF). Solid squares (photographic examples). Color picture numbers: days of exposure; black and white pictures numbers: left (area in cm^2), right (area as percentage). See Results.

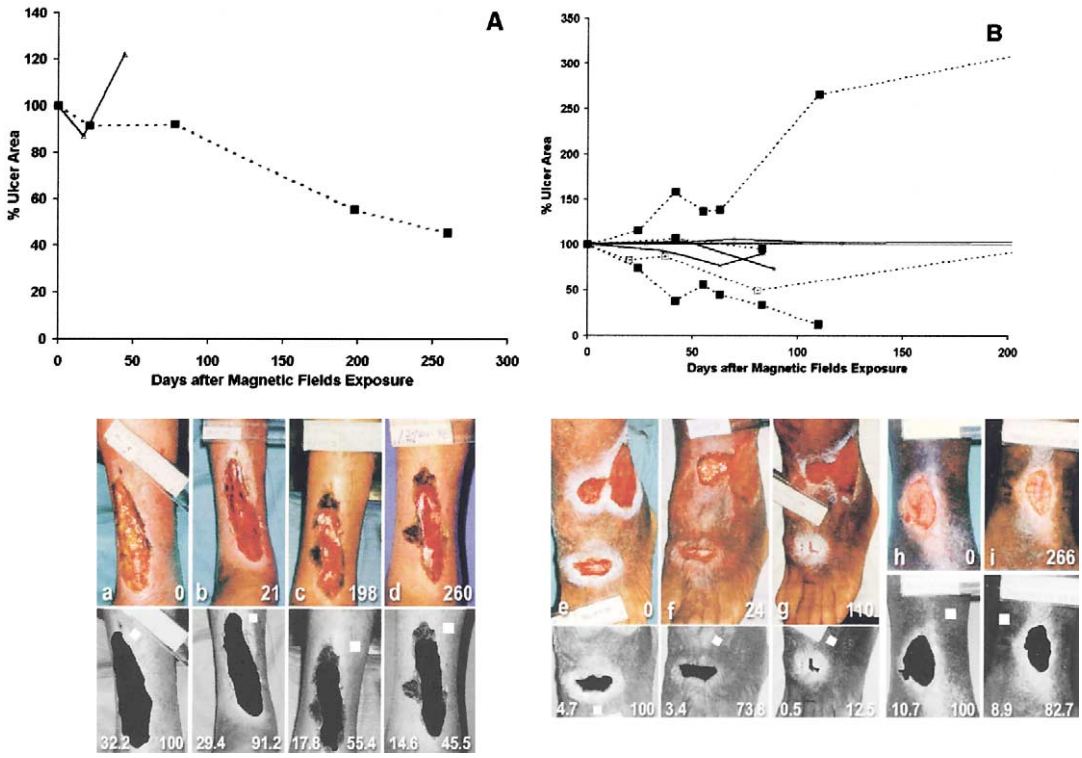

Figure 4. Evolution of chronic leg ulcers in non-responder patients. (A) Predominantly arterial etiology. (B) Predominantly venous etiology. See Results.

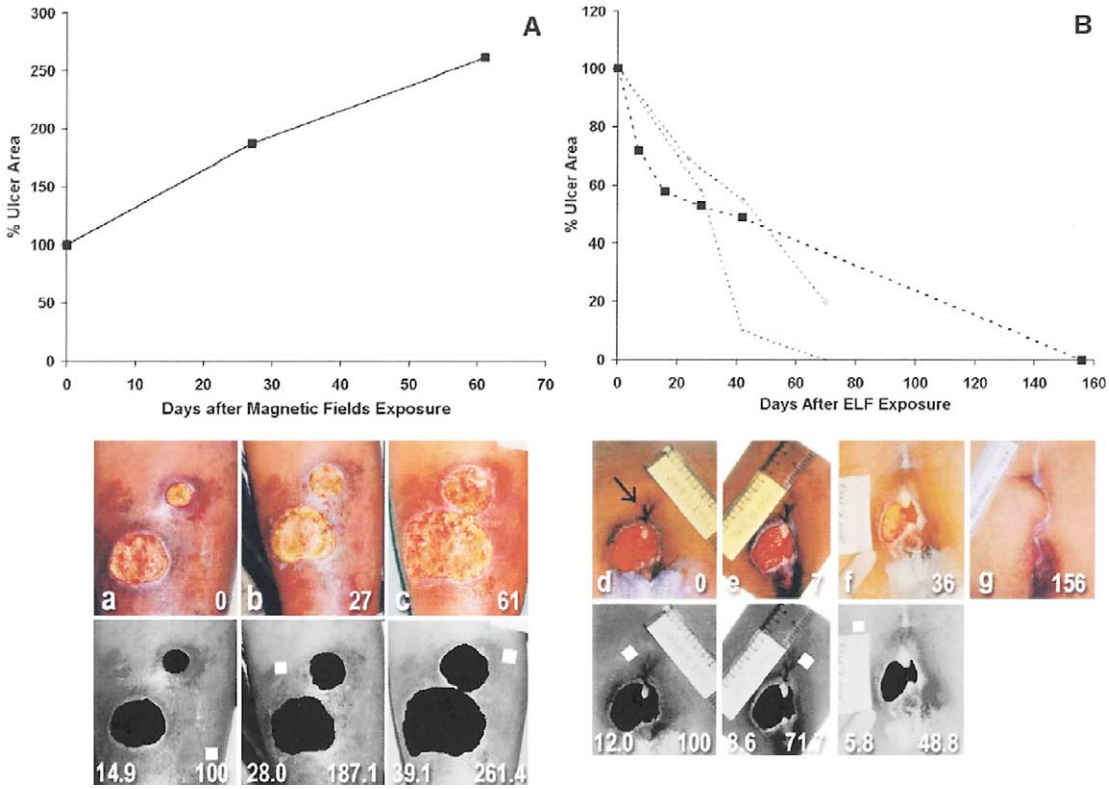

Figure 5. (A) Pyoderma gangrenosum associated to chronic venous disease. (B) Decubitus ulcer in sacral region of paraplegic patient. Arrow indicates suture from previous unsuccessful surgery. See Results.

ELF and ELF/SMF with PBMC to occur *in vivo* because the human body is transparent to magnetic fields. Under this assumption, ELF alone or combined with SMF was applied to patients with chronic leg ulcers in a clinical phase 1 study. In the responder group, ulcers had been under medical treatment prior to admission and their median age was 639 days. Wound healing or deleterious effects began in all patients during the first 2 weeks after ELF or ELF/SMF exposure. Healing time was comparable to other present treatments (1,8–14,27) and healing was independent of lesion etiology or environmental location of patients (Figures 3 and 5 d–g). These responses were partially explained in chronic arterial leg ulcer patients by the increased vascular network associated with ulcer healing (Figures 3 a–c), and in chronic venous leg ulcers by edema reduction during the first weeks after magnetic fields exposure (Figures 3 e–h). These changes indicated that interaction between PBMC and other wound-repair active molecules with local tissues (28) had been restored. In the non-responder group, poor healing was partially explained in chronic leg ulcers of predominantly arterial etiology by severely reduced blood supply, as in critical leg ischemia (8) (Figures 4 a–d), increased sympathetic activity in uncontrolled arterial hypertension (29), and endocrine alterations in obesity (30). In chronic leg ulcers of predominantly venous etiology, ulcers located in different skin areas of the same patient responded differently to treatment. In areas with normal skin structure, edema reduction activated ulcer healing; in areas with non-pitting edema, reduction of extracellular fluid caused altered skin structures to retract, increasing ulcerated areas (Figures 4 e–g). In lipodermatosclerosis (2), destruction of skin structures impeded the normal wound healing process from taking place (Figure 4 h and i), and in pyoderma gangrenosum reactivation of the disease process could be attributed to activated immune cells (31) (Figures 5 a–c). Healing of chronic leg ulcers and some deleterious effects appear associated with magnetic fields exposure and argue against a placebo effect of this treatment.

SMF interaction *in vitro* with human leukocytes (26) has been reported. How could magnetic fields systemic effects be explained? Exposure time and magnitude of the SMF used in these experiments do not produce measurable effects in humans (32). Transmission of nerve impulses was not altered by SMF exposure and a hydrodynamic effect is excluded by the magnitude of SMF (32). Therefore, a mechanism based on changes induced by SMF in exposed arm tissues appears unlikely.

Propagation of electric potential differences produced by ELF through the skin and other tissues is theoretically implausible because according to the Faraday law of induction, electric potential differences are confined within the exposure chamber. Alteration of blood-transported molecules was eliminated due to the low energy of ELF. A mechanism based on molecules secreted by exposed arm tissues and acting at a distance appeared improbable because most molecules that activate wound healing act in an autocrine or paracrine manner. Absence of magnetic field effects on somatosensory-evoked potentials and the wound healing response observed in paraplegic patients suggested a humoral route. Although it is not possible to extrapolate to patients with leg ulcers *in vitro* results obtained with cells harvested from healthy human donors, clinical results suggested as a plausible framework for consideration the peripheral activation of PBMC followed by their transportation to the ulcer site via humoral route.

This phase I before-after study defined the conditions under which to conduct a randomized, placebo-controlled study. In patients, similar healing effects were observed with ELF alone or combined with static magnetic fields (Figure 3). These data, preliminary in nature, suggested that PBMC activation was necessary for wound repair and not a specific pattern of cell proliferation. To define a biological correlation between ELF and PBMC activation, the clinically controlled study should be compared with *in vitro* ELF or ELF/SMF activation of PBMC obtained from the same patients. Cellular activation should be documented with quantitative experiments of gene activation such as cytokine expression using RNA messenger identification by reverse transcriptase/polymerase chain reaction. In addition, PBMC harvested from patients with chronic leg ulcer should be exposed to ELF or ELF/SMF *in vitro* and reintroduced into the blood or applied on the surface of the ulcer (14) of donor patients to observe whether healing is stimulated. It has been reported that activated PBMC, in particular memory/effector T cells, concentrate in sites of chronic inflammation (33) where they participate as a source of cytokines (34,35). CLA lymphocytes (33) can be collected from ulcer fluid before and after ELF- or ELF/SMF-induced healing effects are observed, their status of cellular activation determined and compared with similar data from CLA lymphocytes before and after *in vitro* ELF or ELF/SMF exposure.

Therapeutic applications of magnetic fields have grown over the last three decades, gaining acceptance in some medical specialties. However, the majority of the medical community remains unconvinced. This could be attributed to a) difficulty in reproducing clinical results under the same experimental conditions, b) fear of undesirable side effects, and c) the broad spectrum of interaction mechanisms between magnetic fields and living tissues (36). The following solutions to these problems have been offered: Standards for reporting work with magnetic fields are available (37,38); the controversy over its undesirable health effects is under control (39,40), and some cellular effects have been unveiled (16–22). These advances will progressively encourage physician interest in understanding the therapeutic applications of magnetic fields.

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ORIGINAL ARTICLE

A 1-μT extremely low-frequency electromagnetic field *vs.* sham control for mild-to-moderate hypertension: a double-blind, randomized study

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The effects of extremely low-frequency electromagnetic fields (ELF-EMFs) on blood pressure (BP) are controversial. In this double-blind, randomized, sham-controlled study, we examined the effects of repeated exposure to a 1-μT ELF-EMF on BP in 20 humans with mild-to-moderate hypertension. Subjects were randomly assigned to either the ELF-EMF group or the sham group. Subjects in the ELF-EMF group were exposed to an ELF-EMF (6- and 8 Hz, respectively, peak magnetic field 1 μT, peak electric field 10 V m^{-1}) for at least two 10- to 15-min sessions per week, over a period of 4 weeks. In the sham group, the EMF-generating apparatus was not active. We obtained systolic and diastolic BP (SBP and DBP, respectively) measurements at registration and before and after each ELF-EMF exposure session. Subjects in the ELF-EMF and sham groups had mean ages of 52.8 and 55.1 years, and were exposed to a mean of 9.9 and 9.0 sessions, respectively. There was a significant difference between the ELF-EMF and sham groups with respect to change in SBP value between baseline and the end of the exposure regimen (P=0.02), but not with respect to change in DBP (P=0.21). There were no adverse events other than mild paresthesia of the hands of two subjects in the ELF-EMF group. Our results suggest that repeated exposure to an ELF-EMF has a BP-lowering effect on humans with mild-to-moderate hypertension.
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Keywords: blood pressure; ELF; magnetic field; randomized trial

INTRODUCTION

Hypertension is an important public health concern worldwide.[1] There are an estimated 30 million hypertensive patients in Japan and an estimated 43 million in the USA.[2,3] It is well established that alleviating hypertension can reduce the incidence of cardiovascular events;[4] however, 30–46% of patients undergoing medical treatment for high blood pressure (BP) are not compliant with drug therapy for various reasons, including treatment cost and adverse effects.[5] Perhaps because of this, there has been growing interest in other treatment modalities for lowering BP, including complementary and alternative medicine approaches such as acupuncture and qigong, and new methods such as the use of electromagnetic fields (EMFs).[6–10]

In a recent review, Okano discussed various studies showing that static magnetic fields have a hypotensive effect on BP in animals (including rats, mice and rabbits).[8] In several studies, extremely low-frequency (ELF)-EMFs have been shown to have no effect on systolic BP (SBP) or diastolic BP (DBP) in humans.[11–15] However, in a self-controlled study of 60 hypertensive subjects, each of whom was exposed to ten 12- to 15-min sessions of a 50-Hz 30-mT EMF, Chiuich and Orekhova found that the ELF-EMF induced a significant decrease in BP.[16] In that study, posttreatment peripheral vascular resistance was decreased compared with pretreatment,[16] which may have acted to ameliorate hypertension.[16]

In the 1950s, Schumann hypothesized that EMF signals could resonate in the cavity between the Earth's surface and the ionosphere.[17] The Schumann resonances are simply the electromagnetic resonances of the global Earth–ionosphere (quasi) spherical-shell cavity.[18] It consists of a spectrum of ELF resonant peaks with a fundamental frequency of about 7.8 Hz and broad resonant peaks typically at 14-, 20-, 26-, 33-, 39-, 45- and 51 Hz.[19] The Schumann resonance modes happen to be within the frequency range of electroencephalogram bands (that is, alpha 8–13 Hz and beta 14–30 Hz).[19,20]

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Interestingly, Mitsutake *et al.* researched the relationship between human BP and Schumann resonance, and found that SBP and DBP were lower on enhanced Schumann resonance days than on other days.[20] Such ELF-EMF has been shown to affect BP. Studies of rats exposed to ELF-EMFs in the frequency band of 0.01–100 Hz (with magnitudes of 5, 50 and 5000 nT) have revealed that ELF-EMFs at frequencies of 0.02, 0.5–0.6, 5–6 and 8–11 Hz had the greatest impact on the circulatory system.[21] We previously conducted a self-controlled study of 30 subjects, each of whom was exposed to at least fifteen 10-min sessions of a 6- and 8-Hz 1-μT ELF-EMF, and we found that the ELF-EMF induced a significant decrease in the BP of subjects with hypertension.[22] Based on the results of these previous studies, we considered it possible that ELF-EMFs could represent an alternative approach for controlling hypertension. To test this hypothesis, we conducted the present double-blind, randomized, sham-controlled study on the effects of a 1-μT 6- and 8-Hz ELF-EMF on BP in hypertensive human subjects.

METHODS

At enrollment, for each subject, we obtained demographic information, physical measurements (height and weight) and information on medical history (history of hypertension, use of antihypertensive drugs and inclusion/exclusion criteria as listed below). We also measured BP and performed electrocardiograms and chest X-rays. At enrollment, at the end of the exposure period and 6 months after the exposure, all subjects underwent urine analysis and blood tests, including complete blood counts and blood biochemistry tests (albumin, creatinine, aspartate aminotransferase, alanine aminotransferase and lactate dehydrogenase levels).

All subjects enrolled in this study were aged between 20 and 74 years, were employees of Ichikawa Construction and had mild-to-moderate hypertension according to the World Health Organization/International Society of Hypertension criteria (SBP of 140–179 mm Hg and/or DBP of 90–109 mm Hg).[23] Interday differences in SBP and DBP were no more than 30 and 15 mm Hg, respectively. Both men and women were included in the study.

Exclusion criteria were as follows: severe essential hypertension, secondary hypertension, or malignant hypertension; history or symptoms of cerebrovascular accident; history of myocardial infarction; history or symptoms of angina pectoris, atrial fibrillation, arrhythmia, or cardiac failure; renal dysfunction (serum creatinine >2.1 mg per 100 ml); severe hepatic dysfunction; uncontrolled diabetes; allergy, drug hypersensitivity, or chronic skin disorder; peptic ulcer; pregnancy, suspected pregnancy, or breastfeeding; depression requiring treatment; hypertension controlled using an antihypertensive drug; and other causes for exclusion as determined by the principal investigator or coinvestigators. Hypertensive subjects whose condition was not successfully controlled by using an antihypertensive drug were included in this study. Subjects continued to use antihypertensive drugs during the study.

The study was done in accordance with the International Conference on Harmonisation and the Declaration of Helsinki, and subsequent revisions. The study protocol and other relevant documents were reviewed and approved by the Ethics Committee of the Kyoto University Graduate School and Faculty of Medicine, and the Ethics Committee of Ichikawa Construction. Written informed consent was obtained from all participants. The study was monitored by an independent safety monitoring board. There was no external funding source.

Electromagnetic devices

The ELF-EMF was generated by an electromagnetic device (Ichikawa Construction, Gifu, Japan) comprising a pair of square-shaped coils, each of which was mounted within a housing frame (height (H), 300 cm; length (L), 170 cm; and diameter 2.5 cm). The device was set up in a room 121.6 m^3 in size. The axis of the coil frames was placed perpendicular to the geomagnetic field. The distance between the coil frames was 300 cm. During exposure sessions, subjects sat on a chair placed between the coils. The paired coils produced a sinusoidal 6- and 8-Hz EMF with peak magnetic field of 1 μT and a peak electric field of 10 $V\,m^{-1}$ at the point where subjects sat. The EMF was controlled by two functional generators (DF1905; NF, Kanagawa, Japan), and the peak values were measured using an EMF meter (ME3830B; Gigahertz Solutions GmbH, Langenzenn, Germany and MGM-1DS; Aichi Micro Intelligent, Aichi, Japan). The background value of the geomagnetic field in our laboratory was ~46 to 47 μT (data from the World Data Center for Geomagnetism, Kyoto, Japan). At the point where the subjects sat, there was low-level urban EMF noise (a few nT).

The sham exposure apparatus involved an identical apparatus installed in another room of the same size. Both the two rooms were located in the offices of Ichikawa Construction, and were very similar in all respects. In the EMF room, the exposure system was switched on between 0800 hours and 1200, so to ensure that subjects underwent treatment or sham treatment during the same time period, we used two different rooms. The only difference between the two rooms was whether or not there was an electrical cable connecting the generator and the coils, but in any case the subjects could not see this. Both rooms were kept at $22.0 \pm 1.0\,^{\circ}C$. Only one employee of the Ichikawa Construction knew which subjects were in the exposure group and which in the sham group, but he kept this information strictly confidential, was not involved in administering the study and was not a subject. None of the subjects had been involved in any way with the manufacturing or setup of this system.

Study design and procedures

We performed a randomized, double-blind, sham-controlled trial. Subjects were randomly assigned to either the ELF-EMF group or the sham group. Neither the subjects nor the medical staff overseeing the exposure session and taking BP measurements knew which group each subject was in.

Subjects in the ELF-EMF group were exposed to the 1-μT ELF-EMF for at least two 10- to 15-min sessions per week for 4 weeks. Only one session was permitted per day. The sham group was treated in the same way as the ELF-EMF group, except that the EMF-generating apparatus was not turned on. During the exposure or sham exposure period, medical personnel observed the subjects. After exposure/sham treatment, the subjects were asked questions relating to any physical and mental changes that they had experienced during the exposure. Medical doctors asked subjects about their condition at the end of the exposure period and 6 months after the exposure. Adverse events were recorded when they occurred over the course of the study. During the 6-month follow-up period, subjects were free to receive any medical treatment.

BP measurements

Registration values were recorded 1 month or less before the start of the exposure regimen, and these measurements were used to assess whether subjects were hypertensive. At each exposure session, subjects' BP and pulse rate were measured three times just before the exposure and three times just after the exposure. The mean of the three readings was used for analysis. Measurements were made by a trained nurse using an automated sphygmomanometer (TM-2655P; A&D, Tokyo, Japan) with an appropriate cuff size, with the arm at heart level and with the subject in the sitting position. All BP measurements were performed between 0800 hours and 1200 at a fixed time for each individual.

Study outcomes

The primary outcome of this study was the difference between the ELF-EMF and sham groups with respect to the absolute change in SBP value between baseline (the average of the registration and preexposure values for the first session for each subject) and the end of the exposure regimen (the average of the preexposure values for the last two sessions and the values obtained 1 week after the treatment ended for each subject). The secondary outcomes were the difference between the ELF-EMF and sham groups with respect to the absolute change in DBP value between baseline (the average of the registration and preexposure values for the first session for each subject) and the end of the exposure regimen (the average of the preexposure values for the last two sessions and the values obtained 1 week after the treatment ended), the change in both SBP and DBP values between preexposure and postexposure for each session (averaged over the regimen) and the incidence of adverse events.

Statistical analysis

Data management and statistical analysis were conducted at the Department of Clinical Trial Design and Management, Translational Research Center, Kyoto University Hospital. Based on the results of a previous self-controlled study,[22] the sample size (n=10 in each arm) was calculated to detect a 12 mm Hg reduction in SBP, assuming a s.d. of 9 mm Hg, a two-sided significance level of 0.05 and a power of 80%. Differences between the two groups with respect to the changes in SBP and DBP values between baseline and the end of the exposure regimen and each pre- and postexposure session were tested using the t-test. A value of 0.05 indicated statistical significance. Statistical analyses were performed using SAS ver. 9.1 (SAS Institute, Cary, NC, USA). This study is registered with the ClinicalTrials.gov (no. NCT00709930).

RESULTS

Subject characteristics

The first subjects were enrolled on 28 January 2008. All subjects had their first exposure or sham exposure session on 18 February 2008. Subject characteristics, hematologic data and blood biochemistry data at registration, at the end of the exposure regimen and 6 months after the end of the exposure regimen are summarized in Table 1. One subject was excluded from analysis because after enrollment her BP turned out not to meet the eligibility criteria (as described in the Methods section). The mean ages of subjects in the ELF-EMF and sham groups were 52.8 years (range 38–69 years) and 55.1 years (range 47–74 years), respectively. Subjects in the ELF-EMF and sham groups were exposed to a mean of 9.9 sessions (range 8–15 sessions) and 9.0 sessions (range 8–15 sessions), respectively. Four subjects in the sham group took antihypertensive drugs during the study period. The subjects were taking (1) amlodipine besylate 5 mg and telmisartan 40 mg, (2) amlodipine besylate 2.5 mg, (3) valsartan (dose unknown) and (4) amlodipine besylate (dose unknown). Two subjects in the ELF-EMF group took drugs during the study period. These subjects took (1) losartan potassium 50 mg and (2) unknown.

BP and adverse events outcomes

There were no adverse events other than mild paresthesia of the hands in two subjects in the ELF-EMF group, who described the feeling as a lack of sensation that resolved quickly and spontaneously. Thus, no statistical analysis was performed on adverse event data. Data on baseline and pre- and postexposure BP measurements are given in Table 2. There was a statistically significant difference between the ELF-EMF and sham groups with respect to the absolute change in SBP value between baseline and the end of the exposure regimen (-11.7 ± 6.0 mm Hg in the ELF-EMF group *vs.* -3.2 ± 8.3 mm Hg in the sham group, P=0.02; t-test; Table 2). However, there was no statistically significant difference between the ELF-EMF and sham groups with respect to the absolute change in DBP value between baseline and the end of the exposure regimen (-5.6 ± 3.7 mm Hg in the ELF-EMF group *vs.* -3.1 ± 4.5 mm Hg in the sham group, P=0.21; t-test; Table 2). There was no statistically significant difference between the ELF-EMF and sham groups with respect to the change in either SBP or DBP values between pre- and postexposure for each session (P=0.23 and P=0.49, respectively; t-test). There was, however, a statistically significant difference between the ELF-EMF and sham groups with respect to the change in SBP values between pre- and postexposure in the first week (considering all exposure sessions in the first week; P=0.02, t-test; Table 2 and Figure 1).

Additional analysis

As shown in Table 1, hematologic and blood biochemistry findings were almost identical in the two groups. In the ELF-EMF group, pre- and postexposure SBP values were below 140 mm Hg, except for preexposure SBP in the first week (Table 2). In contrast, in the sham group, pre-and postexposure SBP values were above 140 mm Hg, except for postexposure SBP in the fourth week (Table 2).

Two-way repeated-measures analysis of variance was used to compare the response patterns in terms of SBP values in the ELF-EMF and sham groups. There were significant differences between the ELF-EMF and sham groups with respect to SBP (including baseline values and values 1 week after the treatment ended) (P=0.04) and measurement date (baseline, first to fourth weeks, 1 week after treatments ended) (P=0.0018), but there was no significant difference between the preexposure and postexposure

Table 1 Demographic and hematological characteristics of subjects in the ELF-EMF and sham groups at registration (data are ± s.d.)

	ELF-EMF group			*Sham group*		
	Registration	*End of the exposure regimen*	*6 months after the end of the exposure regimen*	*Registration*	*End of the exposure regimen*	*6 months after the end of the exposure regimen*
Sex (male/female)	10/0			9/0		
Age (years)	52.8 ± 10.2			55.1 ± 7.9		
Height (cm)	172.2 ± 5.0			170.3 ± 6.5		
Bodyweight (kg)	78.5 ± 11.1			73.9 ± 9.1		
Albumin (g per 100 ml)	4.6 ± 0.3	4.4 ± 0.2	4.4 ± 0.3	4.8 ± 0.1	4.6 ± 0.2	4.7 ± 0.2
AST (IU l^{-1})	31.1 ± 21.1	27.3 ± 18.3	34.2 ± 39.8	32.3 ± 12.6	25.4 ± 6.1	24.1 ± 4.9
ALT (IU l^{-1})	45.2 ± 52.9	38.2 ± 48.1	39.7 ± 48.3	36.2 ± 19.9	29.8 ± 19.2	24.6 ± 14.1
LDH (IU l^{-1})	190.9 ± 23.1	181.7 ± 23.4	206.9 ± 33.7	187.4 ± 22.7	167.0 ± 24.1	179.9 ± 25.8
Creatinine (mg per 100 ml)	0.9 ± 0.1	0.8 ± 0.1	0.9 ± 0.1	1.0 ± 0.2	1.0 ± 0.2	0.96 ± 0.2
Leukocyte count (per μl)	6630 ± 2838	5990 ± 1530	6730.0 ± 2382	6688.9 ± 1465	6455.6 ± 1415	6288.9 ± 1622
Platelet count ($\times 10^4$ per μl)	25.1 ± 9.7	22.6 ± 3.2	22.4 ± 4.2	24.5 ± 3.6	23.8 ± 4.0	24.7 ± 4.4
Neutrophil (%)	58.8 ± 7.2	56.5 ± 5.2	56.1 ± 5.3	57.5 ± 6.3	58.2 ± 6.1	56.7 ± 6.7
Eosinophil (%)	2.9 ± 1.9	2.9 ± 1.4	3.1 ± 1.5	4.6 ± 2.7	5.0 ± 2.7	5.1 ± 2.9
Basophil (%)	0.9 ± 0.5	0.8 ± 0.4	0.7 ± 0.7	0.7 ± 0.3	0.6 ± 0.5	0.2 ± 0.4
Lymphocyte (%)	31.9 ± 6.3	33.0 ± 5.1	35.0 ± 4.4	30.8 ± 4.6	29.9 ± 7.2	33.0 ± 6.3
Monocyte (%)	5.5 ± 1.0	6.8 ± 1.5	5.1 ± 1.0	6.4 ± 1.9	6.3 ± 1.6	5.0 ± 1.3

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; ELF-EMF, extremely low-frequency electromagnetic field; LDH, lactate dehydrogenase.

Table 2 Mean baseline and pre- and postexposure SBP and DBP (mm Hg) values for the ELF-EMF and sham groups (data are shown as mean ± s.d.)

	Baseline	*1st week*	*Post—pre (1st week)*	*2nd week*	*Post—pre (2nd week)*	*3rd week*	*Post—pre (3rd week)*	*4th week*	*Post—pre (4th week)*	*Post-pre (Overall, 1st-4th weeks)*	*1 week after the treatment ended*	*End of the exposure regimen*
ELF-EMF group												
Pre-SBP	145.8 ± 11.7[a]	142.1 ± 12.1		139.2 ± 10.8		137.4 ± 11.0		133.1 ± 9.0			135.2 ± 13.6	134.1 ± 8.7[a]
Post-SBP		135.0 ± 9.8	−7.1 ± 6.0	132.4 ± 11.1	−6.8 ± 8.6	134.8 ± 11.8	−2.6 ± 10.6	131.6 ± 9.5	−1.5 ± 8.4	−4.5 ± 8.8[b]		
Pre-DBP	93.1 ± 9.1[c]	91.3 ± 9.2		90.5 ± 8.0		88.8 ± 8.8		86.0 ± 8.7			86.0 ± 8.1	87.5 ± 7.4[c]
Post-DBP		88.8 ± 8.1	−2.5 ± 2.7	88.8 ± 8.0	−1.6 ± 4.0	89.1 ± 8.3	0.2 ± 6.1	85.5 ± 8.0	−0.4 ± 5.1	−1.1 ± 4.7[b]		
Pre-PR		75.8 ± 12.0		75.3 ± 13.1		76.8 ± 11.6		73.4 ± 14.3				
Post-PR		74.3 ± 11.7	−1.6 ± 2.4	73.7 ± 11.3	−1.5 ± 3.4	76.3 ± 10.9	−0.5 ± 3.2	72.2 ± 13.4	−1.2 ± 3.3			
Sham group												
Pre-SBP	146.1 ± 13.5[a]	149.6 ± 13.1		146.7 ± 10.1		142.6 ± 14.2		143.4 ± 12.7			143.8 ± 20.8	142.9 ± 14.0[a]
Post-SBP		146.9 ± 12.3	−2.7 ± 5.2	143.0 ± 11.0	−3.7 ± 8.7	141.1 ± 11.6	−1.4 ± 8.1	139.1 ± 9.8	−4.3 ± 6.2	−3.0 ± 7.2[b]		
Pre-DBP	97.9 ± 8.9[c]	100.5 ± 10.0		100.0 ± 7.9		96.0 ± 11.3		95.6 ± 9.0			94.6 ± 11.7	94.8 ± 9.2[c]
Post-DBP		99.5 ± 10.5	−1.0 ± 4.6	98.5 ± 9.4	−1.5 ± 6.7	95.0 ± 8.6	−1.0 ± 6.5	92.6 ± 9.1	−3.0 ± 4.4	−1.6 ± 5.6[b]		
Pre-PR		73.9 ± 7.8		74.2 ± 8.1		73.9 ± 7.2		75.9 ± 9.2				
Post-PR		72.0 ± 6.3	−1.9 ± 2.9	72.6 ± 7.4	−1.6 ± 2.7	72.5 ± 7.0	−1.4 ± 2.2	73.6 ± 7.5	−2.2 ± 3.8			

Abbreviations: DBP, diastolic blood pressure; ELF-EMF, extremely low-frequency electromagnetic field; Post, postexposure; PR, pulse rate; Pre, preexposure; SBP, systolic blood pressure.
Data for each week are averages for all sessions in that week. Post—pre indicates the postexposure value minus the preexposure value.
[a]Primary outcome: there was a statistically significant difference between the ELF-EMF and sham groups with respect to the absolute change in SBP value between baseline and the end of the exposure regimen (−11.7 ± 6.0 mm Hg in the ELF-EMF group *vs.* −3.2 ± 8.3 mm Hg in the sham group, $P=0.02$; *t*-test).
[b]Secondary outcome: there was no statistically significant difference between the ELF-EMF and sham groups with respect to the change in either SBP or DBP values between pre- and postexposure for each session ($P=0.23$ and $P=0.49$, respectively; *t*-test).
[c]Secondary outcome: there was no statistically significant difference between the ELF-EMF and sham groups with respect to the absolute change in DBP value between baseline and the end of the exposure regimen (−5.6 ± 3.7 mm Hg in the ELF-EMF group *vs.* −3.1 ± 4.5 mm Hg in the sham group, $P=0.21$; *t*-test).

Figure 1 Differences between the ELF-EMF and sham groups with respect to mean absolute changes between preexposure and postexposure SBP.

values ($P=0.35$). The interaction between group (ELF-EMF group *vs.* sham group) and measurement date was statistically significant ($P=0.0003$).

DISCUSSION

Given that the effects of ELF-EMFs on BP are controversial, we conducted a carefully designed, randomized, controlled study to examine the effects of a 1-μT ELF-EMF on BP in human subjects. In our study, there was a significant difference between the ELF-EMF and sham groups with respect to absolute change in SBP value between baseline and the end of the exposure regimen ($P=0.02$; *t*-test). However, there were no significant differences between the ELF-EMF and sham groups with respect to absolute change in DBP value between baseline and the end of the exposure regimen ($P=0.21$; *t*-test), nor with respect to change in SBP and DBP values pre- and postexposure session ($P=0.23$ and $P=0.49$; *t*-test). Two-way repeated-measures analysis of variance was used to compare the response patterns in terms of SBP values in the ELF-EMF and sham groups. The interaction between group (ELF-EMF group *vs.* sham group) and measurement date was statistically significant ($P=0.0003$), indicating that the response patterns differed significantly between the ELF-EMF and sham groups.

A potential limitation of this study was the small sample size. However, we calculated an appropriate sample size based on data obtained in a previous study,[22] and the power of this study approximately corresponded with the planned value (see statistical analysis); hence we conclude that the small sample size is not a major problem. To calculate the actual power of this study, we took the difference in SBP value between the two groups (see Table 2: −11.7+3.2=−8.5 mm Hg) as delta and arrived at a value of 0.60 (s.d.=8.2 in the control and ELF-EMF groups). We enrolled subjects who were taking antihypertensive drugs, but whose BP had not normalized. These subjects kept taking their antihypertensive drugs during the study. Although the differing antihypertensive medications and doses being taken by subjects represent a potential confounding factor, four subjects in the sham group and two in the EMF group were taking antihypertensive drugs (that is, fewer in the EMF group). Therefore, we think that the factor is not likely to have affected our conclusion that EMF has some effect on BP. Another limitation of our study was that smoking and alcohol consumption were not controlled, both of which have an effect on BP. Both smoking and alcohol consumption were permitted during the study period, but subjects were asked to refrain from smoking before treatment.

No serious adverse events were reported by subjects during the ELF-EMF exposure regimen period or during the follow-up period. Of all hematological factors, only the change in monocyte count over the exposure regimen (value at the end of the exposure regimen minus value at registration) differed significantly between the ELF-EMF and sham groups (P=0.04, t-test). At present, the significance of the increase in monocyte count following ELF-EMF exposure is unknown.

The results of the present study correspond to some extent with those of a study by Chiuich and Orekhova.[16] In that study, hypertensive subjects were treated with 10 sessions of a 50-Hz 30-mT ELF-EMF for 12–15 min, applied to either the forehead or neck, resulting in a significant lowering in SBP.[16] The differences between the results of Chiuich and Orekhova and those of studies in which it was found that ELF-EMFs had no effect on BP[11–15] may have been caused by differences in the EMF frequency, magnetic flux density, number of exposure sessions, exposure sites (whole body, head or neck) or subject characteristics. In Chiuich and Orekhova's study, the subjects were exposed to 10 sessions of ELF-EMF,[16] whereas in other studies, subjects were exposed to only 1 or 2 sessions.[11–13,15] In addition, in Chiuich and Orekhova's study, subjects were hypertensive, whereas the subjects were normotensive in other studies.[11–15] In our study, subjects with clinically well-defined hypertension were involved; thus, the status of the subjects seems to be important in eliciting an effect of ELF-EMFs on BP. This argument may be supported by our observation that ELF-EMF exposure seemed to have a greater effect on subjects in the ELF-EMF group at the start of the study (for example, during the first week, there were significant differences between the ELF-EMF and sham groups with respect to change in SBP values pre- and postexposure session), but had a lesser effect as subjects became normotensive (Table 2 and Figure 1). This may indicate that ELF-EMF acts to normalize BP. Static magnetic fields have been found to have a normalizing effect on BP; that is, an antihypertensive effect on hypertensive animals and an antihypotensive effect on hypotensive animals.[8] Therefore it is possible that ELF-EMF does not decrease BP beyond a normotensive level. In fact, in this study, there was a statistically significant difference between the ELF-EMF and sham groups with respect to the change in SBP values between pre- and postexposure in the first week (considering all exposure sessions in the first week; P=0.02, t-test; Table 2 and Figure 1). In the second week, this tendency was also evident, but not in the third and fourth weeks when SBP values in the ELF-EMF group reached normotensive levels (Figure 1).

The exposure level used by Chiuich and Orekhova was 30 mT, 30 000 times stronger than the 1 μT field used in our study. Moreover, these authors used a 50 Hz field, as opposed to the 6–8 Hz used in the present study. Therefore, the underlying mechanism of the effects observed may differ from those observed in our study, but frequency may explain why moderate-intensity (mT range) static magnetic fields or ELF-EMFs and weak-intensity (μT range) ELF-EMFs may have the same effect on BP. Animals seem to be most sensitive to ELF-EMFs below 10 Hz . For example, studies of rats exposed to ELF-EMFs have revealed that frequencies of 0.02, 0.5–0.6, 5–6 and 8–11 Hz had the greatest impact on the circulatory system (as described in the Introduction).[21] One hypothesis explaining the results of these previous studies is that humans may be especially physiologically sensitive to ELF-EMFs below 10 Hz, even when low magnetic flux densities are used. If true, this may be beneficial for the future clinical use of ELF-EMFs because the weak field used in our study (6- and 8 Hz, 1 μT, 10 V m^{-1}) meets the guidelines of the International Commission on Non-Ionizing Radiation Protection.[24]

The potent effects of static magnetic fields on BP have been linked to the nitric oxide pathway, the Ca^{2+}-dependent pathway, the sympathetic nervous system (for example, baroreflex sensitivity and the actions of sympathetic agonists or antagonists) and the neurohumoral regulatory system (for example, production and secretion of angiotensin II and aldosterone), as reviewed by McKay *et al*.[25] The precise mechanism by which ELF-EMFs might ameliorate hypertension is unknown; however, there are two hypotheses that may explain the effect. One potential hypothesis is that the effect of EMFs may be mediated by melatonin release. There is good evidence showing that EMF affects melatonin release,[26] and Reiter *et al*. suggest that the night time rise in endogenous circulating melatonin levels may be inversely related to the reduction in night time BP.[27] In this study, we asked each subject to undergo their BP measurements at the same time of day (as far as possible) over the entire duration of the study period. Therefore, the effect of circadian rhythm on BP would be minimal. The other hypothesis involves an effect of ELF-EMF on blood vessel diameter. Trakov *et al*. investigated changes in blood vessel diameter during and after application of three different frequencies (10-, 16- and 50 Hz) of ELF-EMF for 10 min.[28] In the 16-Hz exposure group, significant vasodilatation was observed in the postexposure period compared with the preexposure and exposure periods, but no significant effects were shown for the 10- and 50-Hz exposure groups.[28] This result suggests that there may be a 'window effect' at 16 Hz for mean blood vessel diameter.[28] One of the authors of the present study, K. Mohri, is a magnetic sensor specialist and inventor of a magnetoimpedance sensor. The magnetoimpedance sensor can detect 50 pT magnetic fields without shield room.[29] In a pilot study, using an magnetoimpedance sensor, we found that exposure of humans to our ELF-EMF for 10 min altered their blood flow. Subjects were a 68-year-old male and female who were exposed to 10 min ELF-EMF.[30] The magnetoimpedance sensor head was set to measure at the right side of cervical spine.[30] Using this apparatus, they clearly detected the increased magnetic signal resulting from blood flow after ELF-EMF exposure.[30] Thus, blood vessel resistance is decreased and blood flow may be increased.

There was a statistically significant difference between the ELF-EMF and sham groups with respect to the absolute change in SBP value between baseline and the end of the exposure regimen. Our findings suggest that repeated ELF-EMF exposure has an effect on SBP. This finding warrants a larger controlled clinical trial to determine whether long-term repeated exposure to 1-μT ELF-EMFs has a beneficial effect on hypertensive humans, such that it could reduce dependence on or obviate the need for pharmacotherapy.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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Involvement of Mitochondrial Activity in Mediating ELF-EMF Stimulatory Effect on Human Sperm Motility

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It has recently been reported that the exposure of human spermatozoa to an extremely low frequency (ELF) electromagnetic field (EMF) with a square waveform of 5 mT amplitude and frequency of 50 Hz improves sperm motility. The functional relationship between the energy metabolism and the enhancement of human sperm motility induced by ELF-EMF was investigated. Sperm exposure to ELF-EMF resulted in a progressive and significant increase of mitochondrial membrane potential and levels of ATP, ADP and NAD^+ that was associated with a progressive and significant increase in the sperm kinematic parameters. No significant effects were detected on other parameters such as ATP/ADP ratio and energy charge. When carbamoyl cyanide *m*-chlorophenylhydrazone (CICCP) was applied to inhibit the oxidative phosphorylation in the mitochondria, the values of energy parameters and motility in the sperm incubated in the presence of glucose and exposed to ELF-EMF did not change, thus indicating that the glycolysis was not involved in mediating ELF-EMF stimulatory effect on motility. By contrast, when pyruvate and lactate were provided instead of glucose, the energy status and motility increased significantly in ELF-EMF-treated sperm. Under these culture conditions, the inhibition of glycolitic metabolism by 2-deoxy-D-glucose (DOG) again resulted in increased values of energy and kinematic parameters, indicating that gluconeogenesis was not involved in producing glucose for use in glycolysis. We concluded that the key role in mediating the stimulatory effects exerted by ELF-EMF on human sperm motility is played by mitochondrial oxidative phosphorylation rather than glycolysis. Bioelectromagnetics

Key words: human sperm; ELF-EMF; energy metabolism; mitochondrial activity

INTRODUCTION

Much research activity has focused on the influence of extremely low frequency (ELF) electromagnetic fields (EMFs) on biological systems. The ELF-EMFs have been found to produce a variety of biological effects, from simple enzyme reactions to the far more complex gene induction and protein [Goodman and Blank, 1998; De Mattei et al., 2005; Delle Monache et al., 2008; Piacentini et al., 2008; Goodman et al., 2009]. Recently, studies carried out on mammalian sperm suggest that ELF-EMFs can negatively [Bernabò et al., 2007] or positively [Iorio et al., 2007; Roychoudhury et al., 2009] influence sperm motility according to the specific characteristics of the EMF applied and/or to the type of sperm used. In humans, the exposure of spermatozoa to an ELF-EMF with a square waveform of 5 mT amplitude and frequency of 50 Hz resulted in an increase in motility and kinematic parameters [Iorio et al., 2007].

Energy metabolism plays a central role in sperm motility, and mitochondrial activity could support flagellar movement because of its high efficiency in ATP production. Actually, the involvement of oxidative phosphorylation (OXPHOS) in the supply of energy for sperm motility is demonstrated by studies showing that

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mitochondrial activity correlates with sperm motility [Gopalkrishnan et al., 1995] and that oxygen consumption is associated with the flagellar movement in human and bull sperm [Ford and Harrison, 1981; Halangk et al., 1990]. Moreover, in human sperm, the functional status of mitochondria is related to sperm motility since high values of mitochondrial membrane potential ($\Delta\Psi_m^{high}$) are correlated with high values of kinematic parameters [Marchetti et al., 2004; Gallon et al., 2006]. The relevance of OXPHOS to sperm motility has also been suggested by studies regarding the effect of specific inhibitors of the respiratory complexes on sperm motility. In particular, rotenone, an inhibitor of respiratory complex I, and antimycina A, an inhibitor of respiratory complex III, depleted ATP and negatively affected sperm motility [Ford and Harrison, 1981; Halangk et al., 1985; de Lamirande and Gagnon, 1992; Ruiz-Pesini et al., 2000].

Recent observations indicate that the energy processes involved in energy transduction could be affected by ELF-EMF. Biological studies with in vitro cell-free systems have focused on the possibility that ELF-EMF could increase the activity of cytochrome oxidase, a key enzyme of the mitochondrial redox chain, apparently accelerating electron movements associated with the reaction [Blank and Soo, 1998a,b, 2001].

On the whole, these findings could suggest that the stimulatory effect induced by ELF-EMF on human sperm motility [Iorio et al., 2007] could be due to a stimulation of mitochondrial metabolism. However, the possibility of an involvement of glycolysis in supplying energy for flagellar movements in ELF-EMF-treated sperm could also exist, in view of the potential role played by this pathway in supporting sperm motility. In fact, some studies have suggested that the energy necessary for sperm motility is generated by glycolysis rather than oxidative phosphorylation [Miki et al., 2004; Mukai and Okuno, 2004]. Mukai and Okuno [2004] found that sperm motility and the amount of ATP were maintained in mouse sperm incubated in the presence of pyruvate or lactate but dramatically decreased when 2-deoxy-D-glucose (DOG), an inhibitor of the glycolysis, was applied. The authors proposed that sperm motility could not be maintained in the presence of respiratory substrates unless glycolysis is functional. They suggested that when pyruvate and lactate are present as the only energy substrates, sperm could activate gluconeogenesis in the midpiece, producing glucose which is metabolized by glycolysis to provide energy for flagellar movement. In addition, it has been reported that mice with a gene knockout for the sperm-specific isoform of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were infertile, and showed sperm with very minimal movement and extremely low levels of ATP [Miki et al., 2004].

Because energy metabolism plays a central role in supporting flagellar movement, the aim of this study was to investigate the relationship between the motility and the energy metabolism in human sperm exposed to ELF-EMF. Moreover, we assessed the energy contribution of mitochondrial respiration and glycolysis to the enhancement of sperm motility induced by ELF-EMF. Therefore, we examined the effect of ELF-EMF treatment on mitochondrial membrane potential ($\Delta\Psi_m$), nucleotide (ATP, ADP, AMP) and coenzyme ($NAD^+ + NADH$) content, ATP/ADP ratio and energy charge in swim-up selected spermatozoa. In addition, the same evaluations were carried out when glicolitic or mitochondrial metabolism were inhibited.

Our study revealed a key role of mitochondria in mediating the ELF-EMF stimulatory effect on motility of human sperm.

MATERIALS AND METHODS

Reagents

5,5′,6,6′-tetrachloro-1,1′3,3′-tetraethylbenzimidazolecarbocyanine iodide (JC-1) and carbamoyl cyanide *m*-chlorophenylhydrazone (ClCCP) were obtained from Molecular Probes (Eugene, OR). All other reagents were obtained from Sigma Chemical (St Louis, MO).

ELF-EMF Exposure System

The ELF-EMF exposure system consisted of two identical apparatuses, each containing a waveform generator, a current amplifier and a solenoid (Fig. 1) [Iorio et al., 2007]. The waveform generator was composed of a PCI DAQ (Digital Acquisition NI PCI 6040E; National Instruments, Milan, Italy), placed in a personal computer with the waveform editor software. In our experiments, for the magnetic field generation we employed a cylindrical coil 200 mm long with a 160 mm diameter. The coil was wrapped with a single strand and the number of turns was 511, giving a resulting resistance of 5.5 Ω and a total inductance of 25 mH, as experimentally determined. The coil used for the Sham experiments (200 mm long; 160 mm diameter; total number of turns 511) was in a bifilar, counter-wound configuration so that the current could flow in the opposite direction. In this case, the magnetic field produced by the counter-wound coil cancels each other, allowing a ''true'' Sham system where the current and the power dissipation are the same as in the coil used for the magnetic field generation, but the magnetic flux density is theoretically zero [Kirschvink, 1992]. A

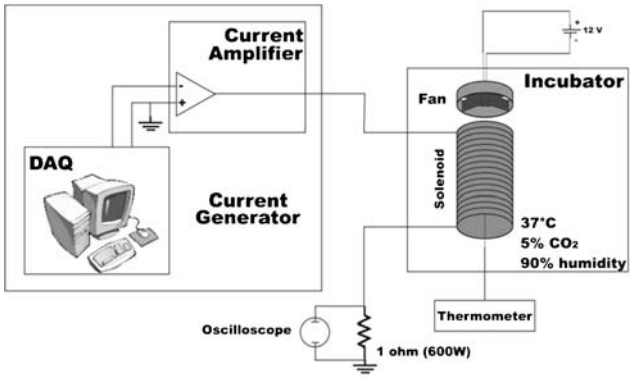

Fig. 1. Experimental apparatus employed for oscillating magnetic field generation.

current generator (BOP 72-6M; Kepco, Flushing, NY) was employed to compensate the effects due to the induced current, and the input current was continuously monitored by means of an oscilloscope (3054 digital oscilloscope, Tektronix, Beaverton, OR) which checked the voltage difference between the 1 Ω (600 W) resistor endings. In order to verify the uniformity of the magnetic field in the solenoid core, some measurements were performed by using a Gaussmeter (Model 912; RFL Industries, Boonton, NJ) connected to a magnetic probe (1 mm^2). The magnetic field was constant inside a 10 mm long cylindrical region (coaxial to the solenoid) with a 10 mm diameter, and the variation in the magnetic field amplitude was about 2.4% at a distance of 20 mm from the centre (moving along the solenoid axis). The system also has the possibility to have different B values by changing the current in the solenoid. For this purpose, we found experimentally the B versus I (Fig. 2), monitoring the pulse current by the ammeter (AC mode) connected in series to the solenoid. We used two exposure systems, one for Sham and the other for field exposure. Each solenoid was placed within two separate

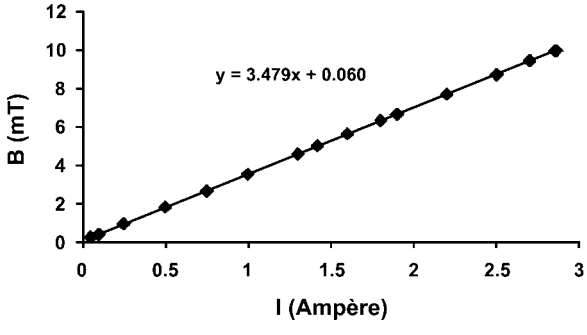

Fig. 2. B versus I at the centre of the solenoid. The best fitting straight line of the measured values was obtained using the method of least squares.

incubators (Steri-Cult 200; Forma Scientific, Marietta, OH) at a temperature of 37 °C and an atmosphere of 95% air/5% CO_2 and 100% relative humidity. The temperature inside the solenoid coils was controlled by a temperature sensor and was maintained at 37 °C ± 0.1 by means of a fan (120 mm, 12V, 100 m^3/h) which was continuously operating during the experiments. The generated magnetic field used in this work was a square waveform with a 50% duty cycle and an amplitude of 5 mT ($I_{max} = 1.4$ A) and frequency of 50 Hz.

Collection, Preparation and Treatment of Semen Samples

The basic culture medium used in all experiments was Biggers, Whitten and Wittingham (BWW) [Biggers et al., 1971], supplemented with 1% human serum albumin (HSA), fraction V. All incubations were conducted at 37 °C, in an atmosphere of 5% CO_2/95% air and 100% relative humidity. Semen samples were collected according to the World Health Organization (WHO) recommended procedure [WHO, 1999] by masturbation from healthy normozoospermic donors. All samples were allowed to liquefy for 30 min at room temperature and then analysed in terms of volume, pH, sperm concentration, motility, viability and morphology. From the collected semen samples, we chose those with a concentration greater than 20×10^6 sperm/ml and a progressive motility greater than 50%. Motile sperm suspensions were obtained by swim-up procedure. Briefly, spermatozoa were washed twice (700g for 7 min) in BWW–HSA. After the second centrifugation, supernatants were removed by aspiration leaving 0.5 ml on the pellet, which was incubated for 30 min at 37 °C in an atmosphere of 5% CO_2/95% air. Supernatants containing highly concentrated motile sperm were carefully aspirated and used to examine the effect of ELF-EMF on energy and motility parameters. In the experiments designed to evaluate which biochemical pathway was involved in mediating ELF-EMF stimulatory effect on motility, the swim-up selected spermatozoa were washed (700g for 7 min) in glucose-, lactate- and pyruvate-free BWW–HSA medium (BWW-GLP) and resuspended in BWW–HSA deprived of glucose (BWW-G), or in BWW–HSA deprived of lactate and pyruvate (BWW-LP). For the inhibition experiments of metabolic pathways, two experimental protocols were used. In the first, to inhibit mitochondrial activity, the swim-up selected spermatozoa were incubated for 30 min in BWW–HSA containing 0.5 µmol/L ClCCP, washed in BWW-GLP and resuspended in BWW-LP. In the second, to inhibit glicolytic metabolism, the swim-up selected spermatozoa were washed in BWW-GLP and resuspended in BWW-G containing 5.56 mmol/L DOG. Each sample,

resuspended in the appropriate medium, was incubated for 3 h at 37 °C in an atmosphere of 5% CO_2/95% air and 100% relative humidity in the presence (ELF-EMF-exposed group) or absence (Sham-exposed and control groups) of ELF-EMF. The ELF-EMF-exposed group was placed in the core of the solenoid where a homogeneous square magnetic field was generated, while Sham-exposed and control groups were placed in a separate incubator, inside and outside the Sham system, respectively.

Cytofluorometric Assessment of Mitochondrial Membrane Potential

Changes in the mitochondrial membrane potential ($\Delta\Psi_m$) were analysed using the lipophilic cation JC-1 as described by Marchetti et al. [2004]. After the different treatments, sperm samples (5×10^5 spermatozoa) were incubated in the presence of 2 μmol/L JC-1 for 30 min at 37 °C in a humidified incubator, immediately washed, resuspended in 0.5 ml of phosphate-buffered saline (PBS), and analysed by flow cytometry. According to the manufacturer, JC-1 changes reversibly its fluorescence from green (monomeric status) to orange (multimeric status) when $\Delta\Psi_m$ is high. All flow cytometry experiments were performed on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Data were acquired using CELLQuest software (Becton Dickinson). Forward and side-scatter channels were gated on the major population of normal-sized cells and a minimum of 10000 cells was analysed. The fluorescence signals of JC-1 monomers and aggregates were detected through the FL-1 (525 ± 5 nm band pass filter) and FL-2 channels (575 ± 5 nm band pass filter), which detect green and red fluorescence, respectively. ClCCP, previously described by Marchetti et al. [2004], was used to provide a positive control for the abolishment of the $\Delta\Psi_m$ of spermatozoa. ClCCP is a protonophore that uncouples oxidation from phosphorylation by dissipating the chemiosmotic gradient and induces dissipation of $\Delta\Psi_m$. Spermatozoa (5×10^5) were incubated in the presence of 0.5 μmol/L ClCCP for 30 min at 37 °C in a humidified incubator, and then stained with the potentiometric dyes as described above.

Assay of Adenine Nucleotides and NAD^+ Concentrations

The adenine nucleotides (ATP, ADP, AMP) and NAD^+ concentrations were determined by high-pressure liquid chromatography (HPLC) according to Stocchi et al. [1987]. Briefly, at fixed intervals, sperm samples (50×10^6 spermatozoa) kept under different experimental conditions were withdrawn and extracted with 0.55 mol/L perchloric acid. After 30 min on ice, the samples were processed by centrifuge at 20000*g* for 5 min, and the supernatants were neutralized with an equimolar addition of K_2CO_3. Adenine nucleotides and NAD^+ levels were measured by absorbance at 254 nm by reverse-phase HPLC on a Supelcosil LC-18-T (Sigma–Aldrich, St. Louis, MO) under gradient conditions. Buffer solutions consisted of 0.1 M KH_2PO_4, 4 mM tetrabutylammonium hydrogen sulphate, pH 6.0 (Buffer A) and 70% Buffer A, 30% methanol, pH 7.2 (Buffer B). Concentrations of ATP, ADP, AMP and NAD^+ were determined by automatic integration based on the appropriate nucleotide standards.

Motility Evaluation With Computer-Assisted Semen Analysis

The motility exhibited by ELF-EMF-exposed, Sham-exposed and control groups was evaluated at intervals of 60 min. The semen samples were loaded into the Makler chamber and computer-assisted semen analysis (CASA) was accomplished by using an ATS 20 system (JC Diffusion International, Gauville, France). For each evaluation, at least 200 motile spermatozoa were selected.

The assessed motility parameters consisted of: (1) the progressive motility; (2) the straight-line velocity (VSL; the straight-line distance from the beginning to the end of a spermatozoon track divided by the elapsed time) given in μm/s and (3) the average path velocity (VAP) given in μm/s.

Statistical Analysis

All experiments were replicated at least five times and the statistical significance of each difference observed among the mean values was determined by standard error analysis. The Sigma Stat 2.03 (SPSS, Chicago, IL) was used to test the statistical significance of differences between group means (one-way ANOVA followed by Tukey's test); $P < 0.05$ was considered to be statistically significant. The data presented in the graphs were expressed as a percentage of control cells.

RESULTS

Effect of ELF-EMF on Mitochondrial Membrane Potential

Mitochondrial membrane potential ($\Delta\Psi_m$) is a major parameter which reflects mitochondrial functionality and is correlated with sperm motility [Troiano et al., 1998; Marchetti et al., 2002]. In particular, high values of $\Delta\Psi_m$ ($\Delta\Psi_m^{high}$) are related to good sperm motility and high-velocity values [Marchetti et al., 2004; Gallon et al., 2006]. In order to evaluate whether the stimulatory effect induced by ELF-EMF (square wave; 5 mT; 50 Hz) on sperm motility [Iorio et al.,

2007] was associated with changes in $\Delta\Psi_m$, we assessed the activity of mitochondria by flow cytometric analysis using JC-1 staining. For our study, we evaluated both the percentage of cells with $\Delta\Psi_m^{high}$ (high fluorescence of JC-1, red-orange in the upper quadrant) and the mean fluorescence intensity of the sperm cells with polarized mitochondria. As shown in Figure 3A, at 2 and 3 h of incubation the mean fluorescence intensity was significantly higher in spermatozoa exposed to ELF-EMF when compared with the controls. The percentage of increase was 6 ± 2 ($P = 0.024$) and 11 ± 4 ($P = 0.016$) at 2 and 3 h, respectively, indicating a progressive hyperpolarization of mitochondrial membrane in the treated spermatozoa compared to the controls. No remarkable differences were found when comparing Sham-exposed groups with control groups. Moreover, at 2 and 3 h of incubation, the percentage of cells with $\Delta\Psi_m^{high}$ increased (~4%) in ELF-EMF-exposed groups with respect to control groups, although the values were not significant (data not shown). Figure 3B,C show that ELF-EMF substantially increased the mean fluorescence intensity of the sperm cells with polarized mitochondria (Sham-exposed group not shown). Figure 3D represents ClCCP-treated samples used as positive control for the abolishment of the $\Delta\Psi_m$.

Effect of ELF-EMF on Sperm Energy Metabolism

Because of the key role of mitochondrial functionality in sperm energy metabolism, we next examined whether the variations in $\Delta\Psi_m$ observed in sperm exposed to ELF-EMF were reflected by proportional differences in: (1) sperm nucleotide concentrations (ATP, ADP, AMP); (2) ATP/ADP ratio; (3) adenylate energy charge [(ATP) + 0.5 (ADP)]/[(ATP) + (ADP) + (AMP)] and (4) total contents of NAD (NADH + NAD^+). However, in our experimental conditions

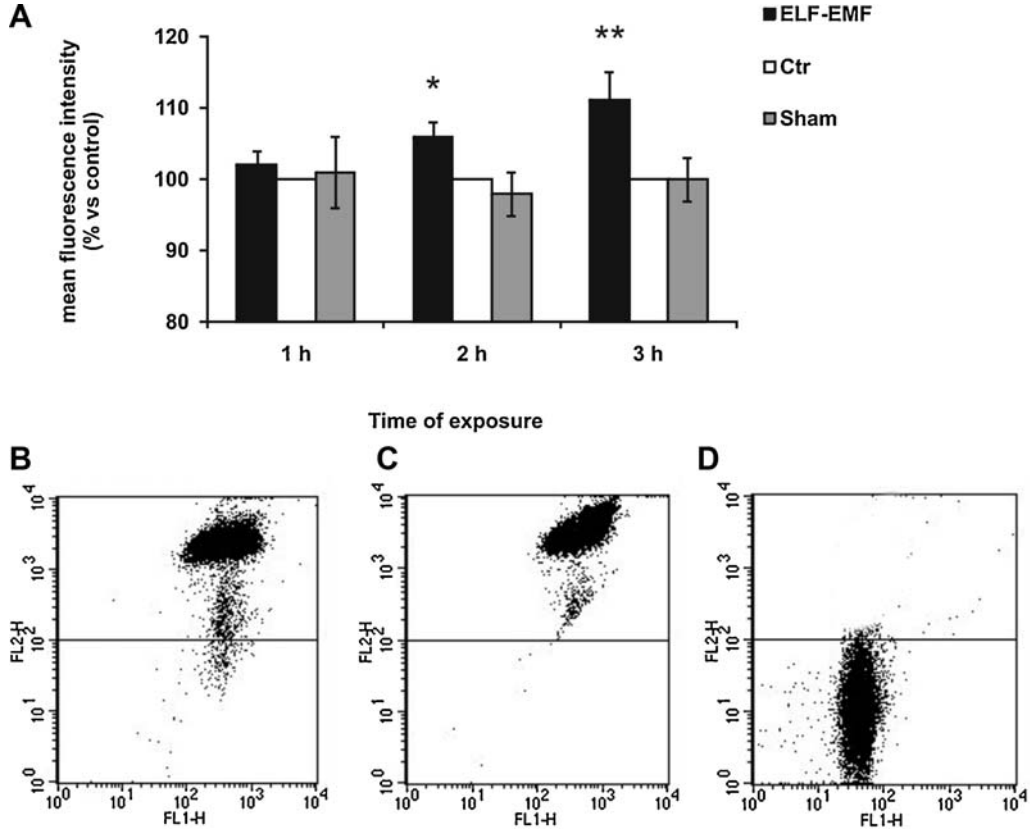

Fig. 3. Effect of ELF-EMF (square wave; 5 mT; 50 Hz) on mitochondrial membrane potential in swim-up selected spermatozoa. Mitochondrial membrane potentials ($\Delta\Psi_m$) were measured by means of JC-1 (2 μmol/L) staining. **A**: Time-dependent effect of ELF-EMF on $\Delta\Psi_m$ in swim-up selected spermatozoa. ELF-EMF = ELF-EMF-exposed groups; Ctr = Control groups; Sham = Sham-exposed groups. Data from 8 different experiments were expressed as a percentage of control cells. $^*P = 0.024$, $^{**}P = 0.016$. **B**: Representative results at 3 h of incubation for control group. **C**: ELF-EMF-exposed group and (**D**) spermatozoa incubated with 0.5 μmol/L ClCCP as a positive control for the abolishment of the $\Delta\Psi_m$.

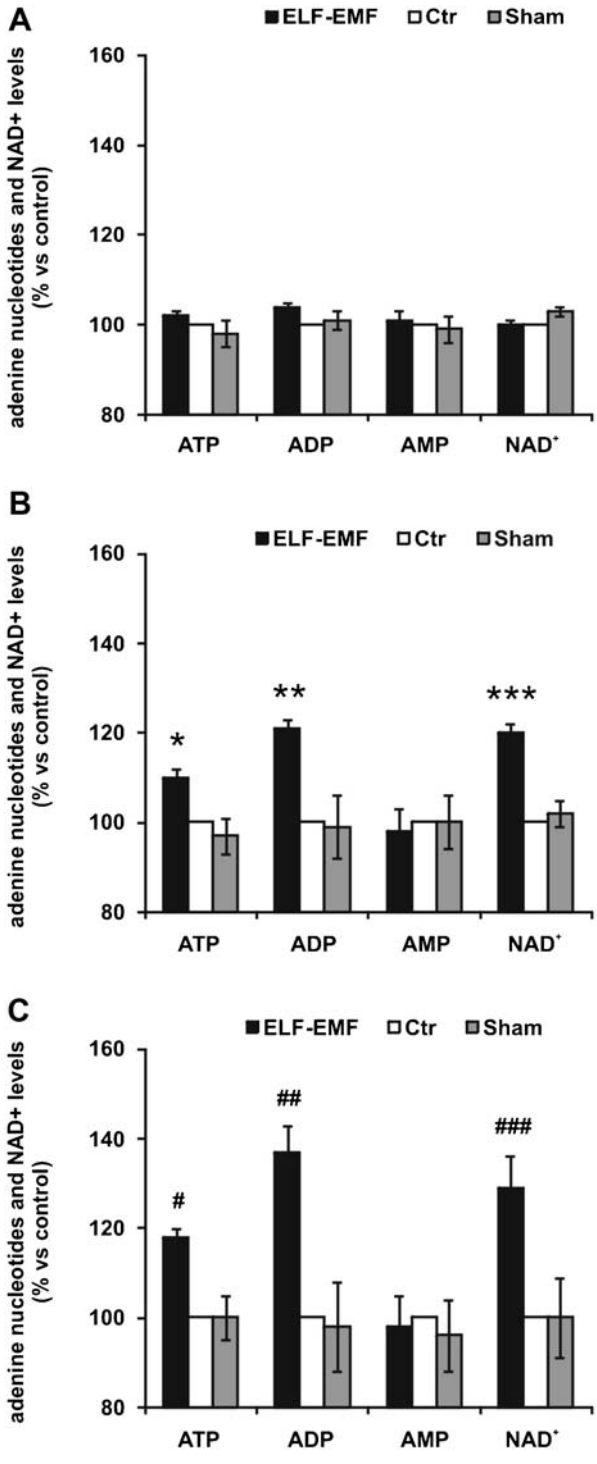

Fig. 4. Effect of ELF-EMF (square wave; 5 mT; 50 Hz) on adenine nucleotides and NAD^+ levels in swim-up selected spermatozoa. At 1 h (**A**), 2 h (**B**) and 3 h (**C**) of incubation, the samples were removed and fixed for nucleotides assay. ELF-EMF = ELF-EMF-exposed groups; Ctr = Control groups; Sham = Sham-exposed groups. Data from 16 different experiments were expressed as a percentage of control cells. $^{*}P = 0.007$, $^{**}P = 0.001$, $^{***}P = 0.001$; $^{\#}P = 0.001$, $^{\#\#}P = 0.002$, $^{\#\#\#}P = 0.001$.

we were unable to measure NADH directly in the same sample used to determine the NAD^+ levels because the extraction conditions used for separating and quantifying coenzyme (acid extraction procedures with trichloroacetic acid) were specific for the stabilization of oxidized compounds. Consequently, we could not quantify the total content of NAD and we reported only the concentration of NAD^+. As shown in Figure 4, the exposure to ELF-EMF significantly enhanced the energy metabolism of the human spermatozoa and the effect was time-dependent. In particular, at 2 h of incubation, the ATP, ADP and NAD^+ levels were significantly higher in sperm exposed to ELF-EMF when compared to the control (Fig. 4B; percentage of increase: ATP = 10 ± 2; ADP = 21 ± 2; NAD^+ = 20 ± 2) reaching maximum levels at 3 h of ELF-EMF exposure (Fig. 4C; percentage of increase: ATP = 18 ± 2; ADP = 37 ± 6; $NAD^+ = 29 \pm 7$). By contrast, no remarkable differences were found when comparing Sham-exposed cells with control cells (Fig. 4B,C), and no significant effects were detected on other parameters such as the ATP/ADP ratio and energy charge when ELF-EMF-exposed groups were compared to control groups (Table 1; Sham-exposed groups not shown). Figure 5 shows the HPLC elution pattern of a sperm extract at 3 h of incubation (Fig. 5A, control group; Fig. 5B, ELF-EMF-exposed group). The Sham-exposed group was not shown.

Effect of ELF-EMF on Sperm Kinematics

According to Iorio et al. [2007], incubation of human sperm in the presence of ELF-EMF resulted in a significant stimulation of the VAP at 2 h and 3 h of exposure (Fig. 6A), and of the VSL at 3 h of exposure (Fig. 6B). No remarkable differences were found when comparing Sham-exposed groups with control groups. However, under our experimental condition, the positive effect of ELF-EMF on the percentage of sperm with progressive motility, previously described by Iorio et al. [2007], was not demonstrable (data not shown) because more than 90% of swim-up selected spermatozoa already exhibited progressive motility.

Involvement of Mitochondrial Metabolism in Mediating ELF-EMF Stimulatory Effect on Motility

Although, under normal conditions, it has been suggested that most of the energy required for sperm movement is generated by glycolysis rather than by OXPHOS [Mukai and Okuno, 2004], our results showed that the stimulatory effect exerted by ELF-EMF on sperm motility characteristics was associated with increases in $\Delta\Psi_m$ and levels of ATP, ADP and

TABLE 1. Energy Charge and ATP/ADP Ratio in Control and ELF-EMF-Exposed Groups After 1, 2 and 3 h of Incubation

Time	1 h		2 h		3 h	
Parameters	ATP/ADP	Energy charge	ATP/ADP	Energy charge	ATP/ADP	Energy charge
Control groups	3.69 ± 0.18	0.62 ± 0.01	3.75 ± 0.09	0.62 ± 0.03	3.22 ± 0.23	0.61 ± 0.02
ELF-EMF-exposed groups	3.55 ± 0.31	0.61 ± 0.02	4.37 ± 0.29	0.65 ± 0.03	3.61 ± 0.25	0.63 ± 0.03
Comparison	$P = 0.71$	$P = 0.53$	$P = 0.11$	$P = 0.22$	$P = 0.27$	$P = 0.41$

Data represent means ± SEM from 16 different experiments.

NAD^+, suggesting an important role of mitochondrial metabolism in the energy production required for flagellar movement. In order to investigate which biochemical pathway plays a major role in mediating the ELF-EMF stimulatory effect on sperm motility, we examined, at 2 and 3 h of incubation, the effect of ELF-EMF on energy production ($\Delta\Psi_m$, ATP, ADP, AMP, NAD^+) and sperm motility (VAP, VSL) in the presence of different energy substrates such as glucose or pyruvate and lactate. Under these experimental conditions, the same evaluations were carried out when mitochondrial activity or glycolysis metabolism were inhibited. As shown in Figure 7, when glucose was present as the only source of energy (BWW-LP) and mitochondrial activity was inhibited by ClCCP, no remarkable differences were found in the energy status (Fig. 7A,B) and motility parameters (Fig. 8A) when comparing sperm exposed to ELF-EMF with control cells. These results indicate that glycolysis was not involved in mediating the ELF-EMF stimulatory effect on sperm motility and point out the critical role of mitochondrial metabolism in the enhancement of the energy status and sperm motility caused by ELF-EMF. Accordingly, when the samples were incubated in the absence of glucose but in the presence of pyruvate and lactate (BWW-G), the ELF-EMF exerted a significant stimulatory effect on the energy metabolism (Fig. 7C,D, Fig. 9A) and the sperm motility characteristics (Fig. 8B), confirming that the enhancement in the sperm functionality induced by ELF-EMF resulted from mitochondrial activity. However, in the latter experimental condition, we cannot exclude the possibility that gluconeogenesis might be activated to produce glucose for use in glycolysis using the energy obtained from respiration. Therefore, to investigate this possibility, we used DOG to block glycolysis since it can be phosphorylated by hexokinase but not further metabolized. As shown in Figures 7E,F, 9B and 8C, when human sperm was incubated in BWW-G containing DOG and exposed to ELF-EMF, the sperm kinematics and the energy parameters remained significantly higher than those observed in the control cells.

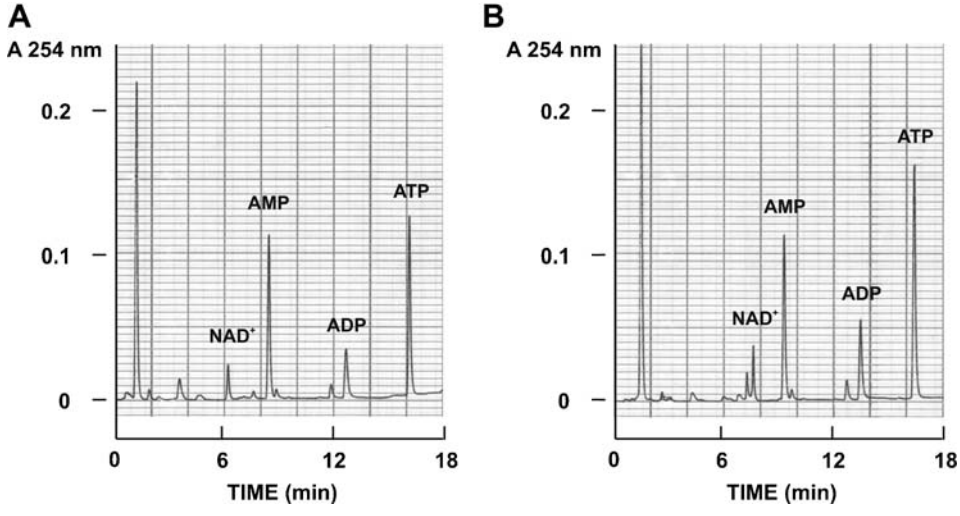

Fig. 5. High-pressure liquid chromatography eluition of a sperm extract. Following 3 h of ELF-EMF exposure (square wave; 5 mT; 50 Hz), the sperm cells were subjected to nucleotide determinations. Nucleotides (ATP, ADP, AMP) and NAD^+ were measured by absorbance at 254 nm by reverse-phase HPLC on a Supelcosil LC-18 column. (**A**) Control group; (**B**) ELF-EMF-exposed group.

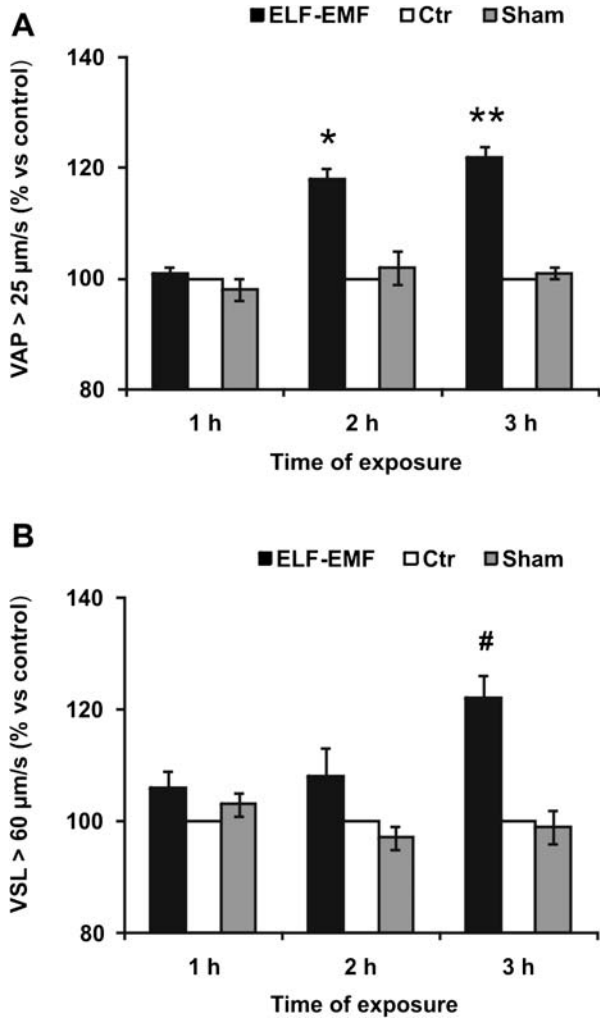

Fig. 6. Effect of ELF-EMF (square wave; 5 mT; 50 Hz) on the VAP (**A**) and VSL (**B**) in swim-up selected spermatozoa. At 1, 2 and 3 h of incubation in BWW–HSA, ELF-EMF-exposed groups, control groups and Sham-exposed groups were analysed for VAP and VSL. ELF-EMF = ELF-EMF-exposed groups; Ctr = Control groups; Sham = Sham-exposed groups. Data from 6 different experiments were expressed as a percentage of control cells. $^{*}P = 0.008$, $^{**}P = 0.006$; $^{\#}P = 0.01$.

DISCUSSION

We previously described that ELF-EMF with a square waveform of 5 mT intensity and 50 Hz frequency is able to promote motility in human sperm [Iorio et al., 2007]. In this study, we examined the functional relationship between energy metabolism and the enhancement of flagellar movement induced by ELF-EMF in human sperm in order to determine the metabolic pathway(s) involved in this stimulatory process. The central findings obtained in the present work indicate a direct activity of mitochondrial metabolism in supplying the extra energy required to enhance sperm motility, and exclude the involvement of glycolysis in mediating ELF-EMF stimulatory effects on sperm motility. These conclusions are based on the following observations: sperm exposure to ELF-EMF resulted in a progressive and significant increase in mitochondrial membrane potential ($\Delta\Psi_m$) as well as the total content of intracellular nucleotides (ATP, ADP) and NAD^+ levels; this progressive increase in the cell energy status was associated with a progressive and significant increase in the sperm kinematic parameters; and the stimulatory effects exerted by ELF-EMF on energy status and motility of human sperm were prevented by inhibition of mitochondrial OXPHOS when the glycolytic process was active.

Previous studies showed an association of $\Delta\Psi_m$ with sperm motility [Troiano et al., 1998; Marchetti et al., 2002] and in particular, it was demonstrated that higher sperm motility is associated with higher $\Delta\Psi_m$ [Marchetti et al., 2004; Gallon et al., 2006]. According to these findings, the exposure of human sperm to ELF-EMF leads to a progressive mitochondrial membrane hyperpolarization that occurs in parallel with a progressive increase in sperm motility, confirming the strong link between the functional status of mitochondria and sperm motion.

The $\Delta\Psi_m$ is a good indicator for the energetic and functional state of the mitochondria and reflects the activity of the respiratory chain and the electrogenic transport systems [Ly et al., 2003]. Since the OXPHOS in the living cell is not functioning to its fullest because the maximal respiratory rate is usually higher than the spontaneous respiratory rate, a first and simple interpretation of our results on mitochondrial activity is that there is a stimulatory effect of the magnetic field on the OXPHOS rate. If this is the case, ELF-EMF should be able to enhance the rate of mitochondrial respiration in two possible ways: one, by modulating the specific activity of the enzyme complexes; and the other by regulating the electron flux at the level of electrogenic transport systems. In this regard, a role for EMFs in the reactions involving moving charges has previously been suggested. Studies on Na, K-ATPase [Blank, 1992; Blank and Soo, 1992, 1996] and cytochrome oxidase [Blank and Soo, 1998a,b] have demonstrated that low frequency magnetic fields increase the activity of two membrane enzymes, determining effects on their biochemical reactions. Since the two enzyme reactions studied are always accelerated in magnetic fields, conversely, with the basal reaction rate, it has been proposed that magnetic fields accelerate charge movements associated with the reaction (mobile charge interaction model, MCI). The affected charge movements in the Na, K-ATPase have not been identified, but those

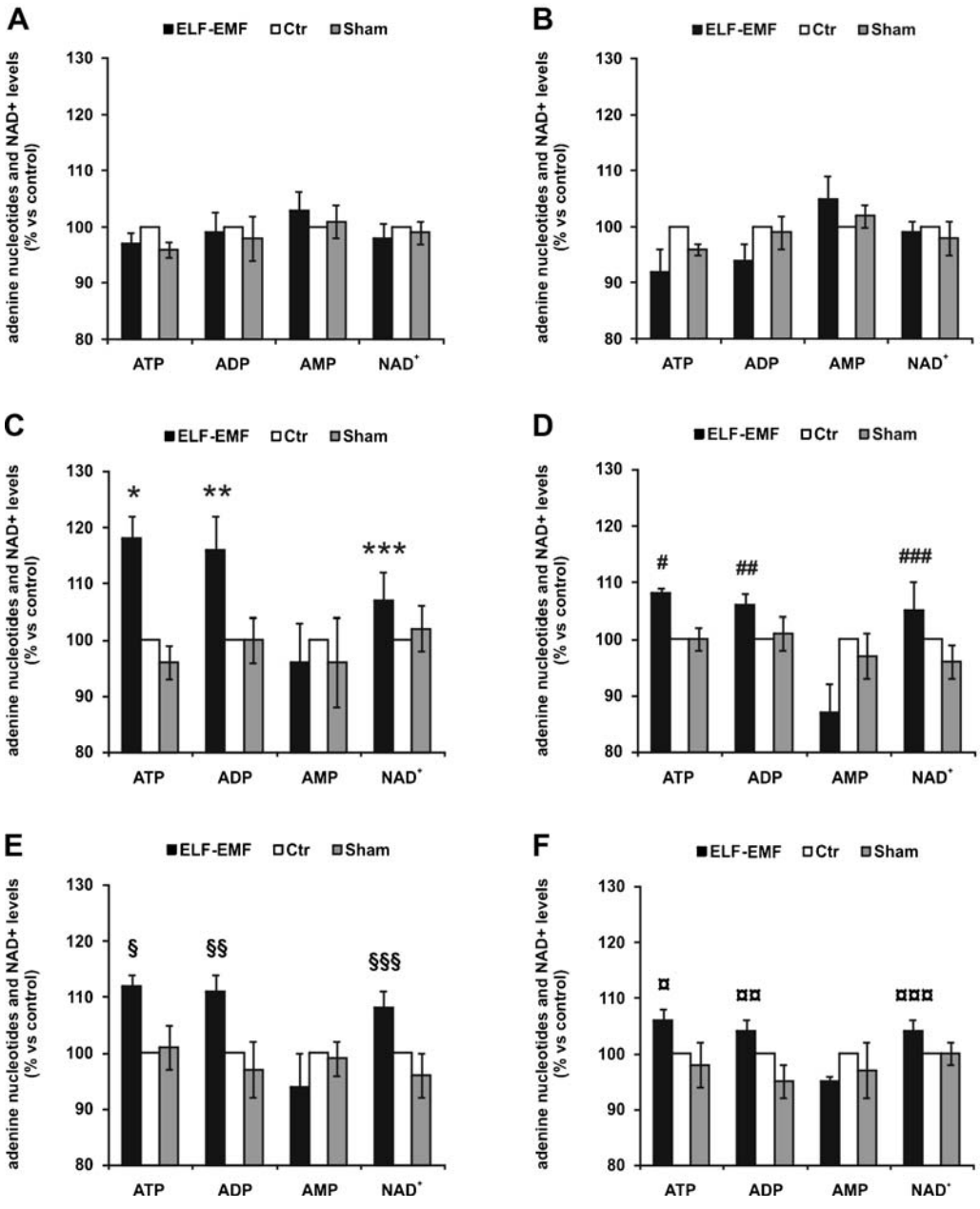

Fig. 7. Effect of mitochondria and glicolysis inhibition on adenine nucleotides and NAD^+ levels in sperm exposed to ELF-EMF (square wave; 5 mT; 50 Hz). Sperm incubated in BWW-LP for 2 h (**A**) or 3 h (**B**) after treatment with 0.5 μmol/L ClCCP to inhibit mitochondrial activity; sperm incubated in BWW-G for 2 h (**C**) or 3 h (**D**); sperm incubated in BWW-G + 5.56 mmol/L DOG for 2 h (**E**) or 3 h (**F**). BWW-LP = BWW–HSA deprived of lactate and pyruvate; BWW-G = BWW–HSA deprived of glucose; EMF = ELF-EMF-exposed groups; Ctr = Control groups; Sham = Sham exposed-groups. Data from 16 different experiments were expressed as a percentage of control cells. $^{*}P < 0.001$, $^{**}P = 0.003$, $^{***}P = 0.005$; $^{\#}P = 0.001$, $^{\#\#}P = 0.006$, $^{\#\#\#}P = 0.009$; $^{\S}P = 0.007$, $^{\S\S}P = 0.008$, $^{\S\S\S}P = 0.003$; $^{¤}P = 0.001$, $^{¤¤}P = 0.005$, $^{¤¤¤}P = 0.004$.

affected in cytochrome oxidase are electrons. In addition, recent studies on the Belousov–Zhabotinski reaction [Blank and Soo, 2001, 2003] have suggested that low frequency EMFs accelerated electron transfer. On the basis of these findings, it is reasonable to assume that the effect induced by ELF-EMF on human sperm motility may be due to an interaction of the magnetic field with the charge transfer reactions occurring in the mitochondrial respiratory chain and electrogenic transport systems, which may allow an increase in energy

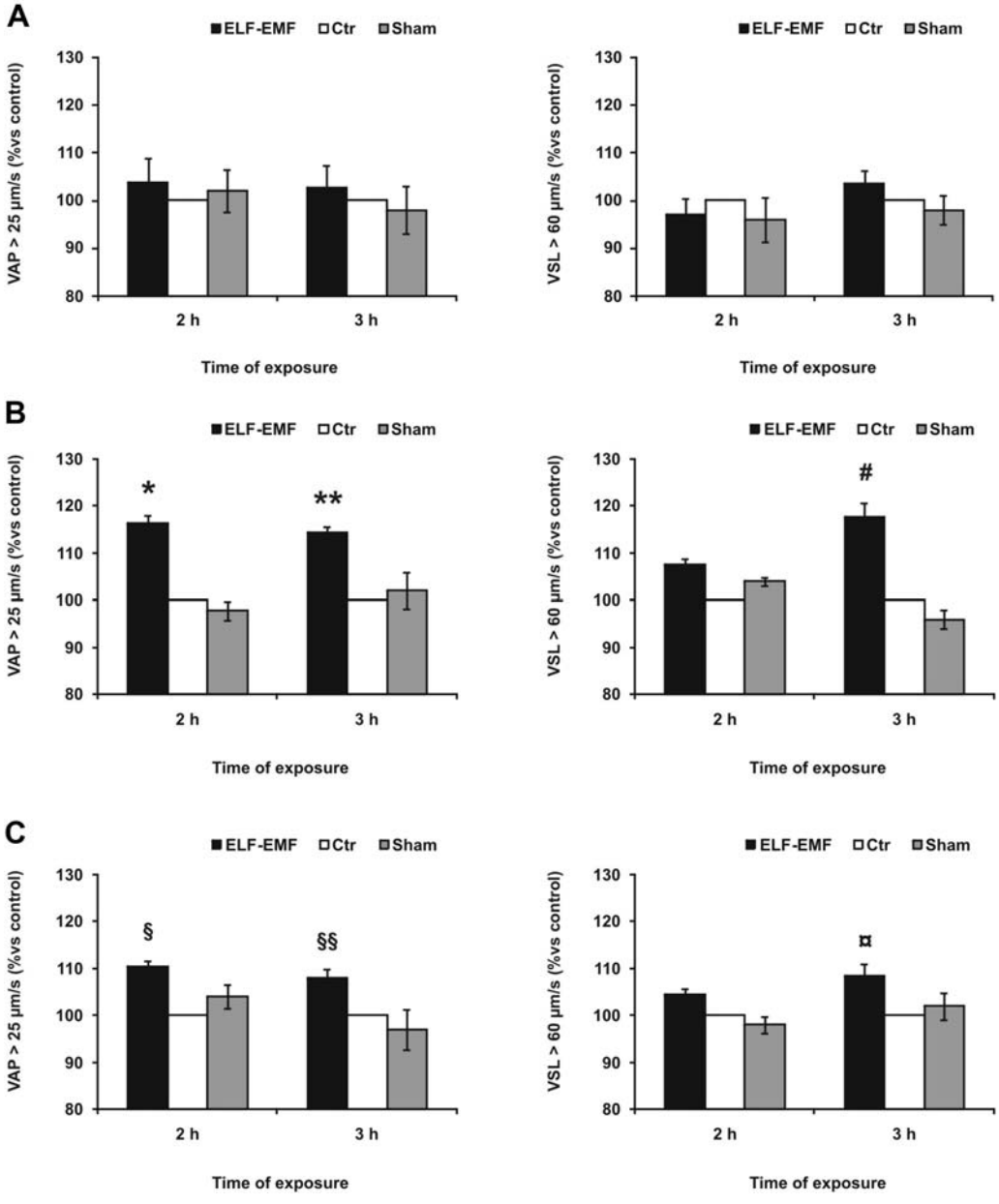

Fig. 8. Effect of mitochondria or glicolysis inhibition on the VAP and VSL in sperm exposed to ELF-EMF (square wave; 5 mT; 50 Hz). **A**: Sperm incubated in BWW-LP after treatment with 0.5 µmol/L ClCCP to inhibit mitochondrial activity; **B**: sperm incubated in BWW-G; **C**: sperm incubated in BWW-G + 5.56 mmol/L DOG to inhibit glicolytic metabolism. BWW-LP = BWW–HSA deprived of lactate and pyruvate; BWW-G = BWW–HSA deprived of glucose; ELF-EMF = ELF-EMF-exposed groups; Ctr = Control groups; Sham = Sham-exposed groups. Data from 6 different experiments were expressed as a percentage of control cells. $^{*}P = 0.001$, $^{**}P = 0.008$; $^{\S}P = 0.004$, $^{\S\S}P = 0.001$; $^{\#}P = 0.01$; $^{¤}P = 0.007$.

conversion flux (the rate of ATP synthesis). In agreement with this possibility, the progressive mitochondrial membrane hyperpolarization caused by ELF-EMF occurs in parallel with a progressive increase in the levels of ATP, ADP and NAD^+.

Unfortunately, we were unable to measure NADH and, therefore, could not calculate the NAD^+/NADH ratio and the total content of NAD ($NADH + NAD^+$) to determine the redox state of the pyridine nucleotides. However, because the NADH is a strong reducing agent

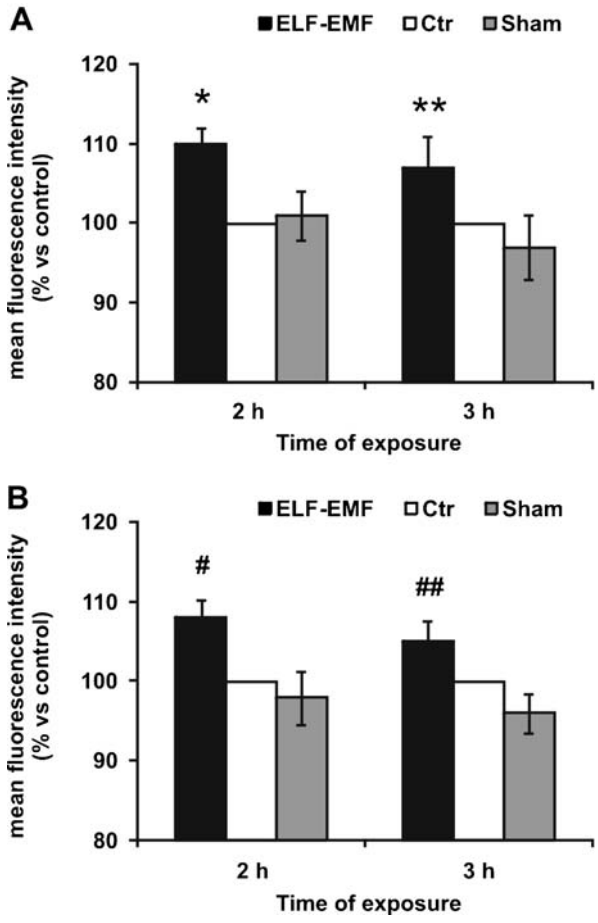

Fig. 9. Effect of ELF-EMF (square wave; 5 mT; 50 Hz) on mitochondrial membrane potential in swim-up selected spermatozoa incubated in (**A**) BWW-G; (**B**) BWW-G + 5.56 mmol/L DOG. BWW-G = BWW−HSA deprived of glucose; ELF-EMF = ELF-EMF-exposed groups; Ctr = Control groups; Sham = Sham-exposed groups. Data from 6 different experiments were expressed as a percentage of control cells. $^{*}P = 0.037$, $^{**}P = 0.008$; $^{\#}P = 0.004$, $^{\#\#}P = 0.011$.

which donates electrons to the respiratory chain, the increase in the levels of NAD^+ may be a consequence of the enhancement of the electrochemical potential difference in the proton across the mitochondrial inner membrane. This, in turn, may be reflected in the increase in the catabolic processes within the mitochondria.

Unlike the total content of NAD, the levels of adenine nucleotides were determined and thus we could calculate the ATP/ADP ratio and the energy charge. The ATP/ADP ratio is the measure of ADP re-phosphorylation [Chulavatnatol and Haesungcharern, 1977], while the energy charge is the result of the balance between the energy-producing and energy-utilizing processes. It has been well established that in cellular systems there is a coupling of ATP supply and energy demand to support biological activity so that the ATP homeostasis is maintained. Considering this, the findings that the ATP/ADP ratios and the energy charge did not co-vary with the ATP and ADP levels indicate that under our experimental conditions, the ELF-EMF treatment does not affect the equilibrium of the cell energy status in human sperm.

Overall, the results reported strongly suggest that mitochondrial activity plays a crucial role in supporting the enhancement of sperm motility caused by ELF-EMF. This possibility is confirmed by the observation that when mitochondrial metabolism was inhibited and the ELF-EMF exposure occurred in the presence of glucose, human sperm did not exhibit any increase in the energy and motility parameters. These findings also indicate that the ATP produced by glycolysis was not involved in mediating the stimulatory effect of ELF-EMF on flagellar movement. The idea that mitochondrial metabolism is essential for the positive effects exerted by ELF-EMF on sperm motility is further strengthened by the observation that when glucose was substituted for pyruvate and lactate, ELF-EMF again caused significant increases in the levels of sperm energy status and flagellar movement.

As pointed out in the Results Section, under this culture condition, gluconeogenesis could be activated to produce glucose for glycolysis using the energy obtained from respiration. In fact, mature mammalian spermatozoa have a fully glycogen metabolism [Ballester et al., 2000; Albarracín et al., 2004] and it has been suggested that when pyruvate and lactate are present as the only energy substrates, sperm could activate gluconeogenesis in the midpiece, producing glucose which is metabolized by glycolysis to provide energy for flagellar movement [Mukai and Okuno, 2004]. Moreover, human sperm could be able to modulate its metabolism according to its energy needs by regulating the glycogen synthase activity [Aquila et al., 2005]. From our results, it seems unlikely that the mitochondrial energy was used to drive gluconeogenesis for glycolitic energy production. In fact, when sperm exposure to ELF-EMF occurred in the presence of mitochondrial substrates and DOG, the kinematics and energy parameters of sperm exposed to ELF-EMF remained significantly higher than those observed in the control cells.

The process of maintaining motility in human sperm is rather complex and involves the integration and crosstalk of several signalling pathways including cAMP/PKA, calcium and phosphorylation/dephosphorylation of proteins [Visconti et al., 1995, 2002; Vijayaraghavan et al., 1997; Salicioni et al., 2007]. Thus, although our data support the hypothesis that

mitochondrial activity can mediate the stimulation of human sperm motility induced by the magnetic field, we cannot exclude the possibility that ELF-EMF could interact with specific pathways that regulate motility and modulate the energy demand. In fact, mitochondria have to make energy conversion meet energy demand and doing this could change the OXPHOS steady state.

In conclusion, even though further research is needed to identify other potential metabolic pathways affected by ELF-EMF to enhance sperm motility, the results presented here demonstrate that the key role in mediating the stimulatory effect exerted by ELF-EMF on human sperm motility is played by mitochondrial metabolism rather than glycolysis. These findings could also provide additional insight into the existing debate on which biochemical pathways play a major role for ATP supplementation in flagellar movement [Miki, 2007; Ruiz-Pesini et al., 2007].

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Low-frequency Pulsed Electromagnetic Field Therapy in Fibromyalgia

A Randomized, Double-blind, Sham-controlled Clinical Study

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Objective: To evaluate the clinical effectiveness of low-frequency pulsed electromagnetic field (PEMF) therapy for women with fibromyalgia (FM).

Methods: Fifty-six women with FM, aged 18 to 60 years, were randomly assigned to either PEMF or sham therapy. Both the PEMF group (n = 28) and the sham group (n = 28) participated in therapy, 30 minutes per session, twice a day for 3 weeks. Treatment outcomes were assessed by the fibromyalgia Impact questionnaire (FIQ), visual analog scale (VAS), patient global assessment of response to therapy, Beck Depression Inventory (BDI), and Short-Form 36 health survey (SF-36), after treatment (at 4 wk) and follow-up (at 12 wk).

Results: The PEMF group showed significant improvements in FIQ, VAS pain, BDI score, and SF-36 scale in all domains at the end of therapy. These improvements in FIQ, VAS pain, and SF-36 pain score during follow-up. The sham group also showed improvement were maintained on all outcome measures except total FIQ scores after treatment. At 12 weeks follow-up, only improvements in the BDI and SF-36 scores were present in the sham group.

Conclusion: Low-frequency PEMF therapy might improve function, pain, fatigue, and global status in FM patients.

Key Words: fibromyalgia syndrome, chronic pain, pulsed electromagnetic fields, randomized clinical trial

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Fibromyalgia (FM) is a chronic pain disorder commonly seen in women and characterized by widespread muscle pain, tenderness, fatigue, nonrefreshing sleep, and other associated symptoms.[1,2] The etiology of FM is unknown and the pathogenesis is not clearly understood, but may involve abnormal levels of peripheral and central nervous system neurotransmitters, dysregulation of the hypothalamic-pituitary-adrenal axis,[3] or oxidative stress/nitric oxide.[4]

There is no standard treatment regimen for FM; therefore current therapy modalities are focused on relieving the symptoms of FM. Analgesics, antidepressants, and exercise are widely used to relieve the symptoms.[3,5,6] In the last decade, patient education, multidisciplinary group programs, and other nonpharmacologic interventions have become important aspects of FM therapy.[5,6] Thus, the use of a pulsed electromagnetic field (PEMF) represents an attractive alternative for patients with FM.

PEMF exposure is approved by the United States Food and Drug Administration for the treatment of problems associated with musculoskeletal disorders, including delayed-union or nonunion fractures, failed joint fusions, and congenital pseudoarthroses.[7–9] Specific joint disorders that have been investigated using this treatment modality include rheumatoid arthritis (RA),[10] osteoarthritis,[11,12] and rotator cuff tendonitis.[13] PEMF induces time-varying ionic currents in tissues, which stimulate changes in cellular calcium and cyclic adenosine monophosphate levels,[14] as well as in the synthesis of collagen, proteoglycans, DNA, and RNA.[15,16] In addition, some of the enzymes and hormones involved in skeletal homeostasis are affected by PEMF and it increases nitric oxide production and levels of reactive oxygen species.[17]

The pathophysiology that produces pain and disability in FM seems to involve a combination of central sensitization and nociceptive input. PEMF can alter pain perception and cognitive processing in both animals and humans. The effect of magnetic field exposure on pain behavior has been investigated in rats, mice, snails, pigeons, and humans.[18–20] Sartucci et al[21] examined the effect of weak, oscillating magnetic fields (MFs) exposure (constant-current rectangular pulses; 0.5 Hz, 0.1 ms in duration, 70 to 29 μT) on human pain perception and pain-related somatosensory evoked potentials (SEPs). After sham treatment, pain thresholds significantly increased, whereas after MFs a slight non-significant decrease in thresholds was found. After both treatments pain-related SEP amplitude was reduced, but this decrease was more evident and statistically significant only after MF exposure. The increase found in thresholds after sham exposure may be due to stress-induced analgesia, and the contrasting behavior recorded after MF exposure might indicate a suppression of stress-induced analgesia. The significant reduction in pain-related SEP amplitude observed after MF exposure provides the first evidence that human SEPs are influenced by MFs. Shupak et al[22] investigated the effect of PEMF exposure on pain and anxiety ratings in RA and FM populations. This study revealed a significant reduction in pain ratings from preexposure to postexposure for both RA and FM patients. These findings provide some initial support for the use of PEMF exposure to reduce pain in individuals with chronic pain. The aim of this study was to evaluate the efficacy of PEMF for the treatment of FM in a randomized, double-blind, sham-controlled trial.

MATERIALS AND METHODS

Participants

Sixty-eight patients with FM were recruited from the musculoskeletal rehabilitation outpatient clinic of the Ankara Physical Medicine and Rehabilitation Research

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Hospital. All participants were initially evaluated for the recruitment, based on an approved protocol. Screening included a medical and psychologic history, as well as physical and laboratory examinations. Criteria for study inclusion were: (1) fulfillment of American College of Rheumatology classification criteria for FM,[23] verified by rheumatologic examination: (a) widespread pain (axial plus upper and lower segment plus left and right side pain for ≥ 3 mo) and (b) tenderness at ≥ 11 of the 18 specific tender point sites; (2) patient-reported visual analog scale (VAS) scores for pain of ≥ 5 cm; (3) aged between 18 and 70 years; and (4) sufficient ability to understand the nature and potential risks of the study.

Exclusion criteria included ischemic heart disease, arrhythmia, uncontrolled thyroid disease, pregnancy, breastfeeding, cardiac pacemaker, malignancy, tuberculosis, neuropsychiatric disorders (dementia, cerebrovascular disease, alcohol abuse, severe depression, panic disorder, bipolar disorder, or psychosis), and comorbid painful conditions that could confuse the clinical picture, such as inflammatory arthritic conditions or cervical radiculopathy.

Participants had not received physical therapy or made changes in their pharmacologic therapy during the previous 2 months. No new drugs. No supplementary therapies, special diets, or aerobic exercise programs were allowed during the study period. Eight patients were excluded after the medical evaluation and 4 did not sign the consent form. Fifty-six patients with primary FM were studied.

All participants provided written informed consent. The study protocol was approved by the ethics committee of the Ankara Physical Medicine and Rehabilitation Research Hospital.

Randomization

After the baseline assessment and data collection, a computer-generated random number list was used to randomize patients into 2 equal groups, the PEMF or sham group. Randomization was performed using sequential sealed envelopes prepared by an independent physician before enrollment. The sealed envelopes contained a record of the allocation. The researchers and participants were all blind to the group allocation throughout the study.

PEMF Therapy

PEMF was administered to the whole body using a 1.8×0.6 m mat (wave ranger professional, MRS 2000+ Home, Eschestrasse 500, FL-9492 Eschen). This mat produced a PEMF with a mean intensity of 40 μT and frequency ranging from 0.1 to 64 Hz. Each patient lay on the mat for 30 minute per session, twice a day for 3 weeks. The sham intervention was identical to the actual intervention except that the PEMF device was not switched on. This method is particularly suitable for double-blind trials, as application of PEMF therapy does not cause any sensation in the patient. The device used had a specially designed switch concealed at the back that enabled the independent researcher to interrupt the PEMF for the sham group; the "on" sign and the parameters of PEMF therapy were displayed to all patients (sham and PEMF groups) throughout the procedure.

Assessment

All patients were assessed at baseline, at the end of therapy, and after 12 weeks, by the same assessor, who was blinded to treatment. Whenever possible, follow-up was conducted at the same time of day as the baseline assessment, to control for diurnal fluctuation. At each assessment, pain severity was measured on a 100 mm VAS. Both groups completed the Fibromyalgia Impact Questionnaire (FIQ), the Short-Form 36 Health Survey (SF-36), and the Beck Depression Inventory (BDI) at baseline, at the end of therapy, and at the follow-up. In addition, at the end of therapy and after 12 weeks, both the PEMF and sham group responded to the Patient Global Assessment of Response to Therapy (PGART).

Primary Outcome Measures

FIQ

FM-related quality of life was assessed by a validated Turkish version of the FIQ.[24] The FIQ is a 20-item, patient-reported instrument, developed by Burckhardt and coworkers.[25] It consists of 10 subscales, which are combined to yield a total score. Eleven questions are specifically related to physical functioning (PF). The remaining items assess pain, fatigue, stiffness, tiredness on waking, difficulty in working, days when the patient feels good, and symptoms of anxiety and depression. Scores range from 0 to 100, with higher scores signifying greater disease impact. The FIQ is responsive to change and has been translated into many languages.

VAS

The VAS was used to assess subjective pain intensity.[26,27] Patients marked the extent of pain they had experienced during the previous week on a horizontal 100 mm VAS (0 = no pain and 100 = the worst imaginable).

Secondary Outcome Measures

PGART

One of the secondary outcome measures was the patient's global assessment of their impression of improvement. The question asked was "What were the effects of treatment on your complaints?" Patients indicated their answers on a 5-point Likert scale (1 = much better, 2 = better, 3 = slightly better, 4 = no change, and 5 = worse).

BDI

All patients evaluated filled out the BDI, self-reported scale, which evaluates 21 symptoms of depression. For each symptom, patients rate themselves as 0, 1, 2, or 3. The maximum score is 63 and the minimum score is 0. Higher scores indicate greater depression.[28,29]

SF-36 Health Survey

The Medical Outcomes Study SF-36 questionnaire (Turkish version) was used to measure quality of life.[30] The SF-36 includes 1 multi-item scale that assesses 8 health concepts: PF, role limitations-physical, bodily pain, general health (GH), vitality, social functioning, role limitations-emotional, and mental health. SF-36 scale scores derive from 2 summary measures of health status: the physical component summary (PCS) and mental component summary (MCS). The PCS includes scales assessing PF, role limitations-physical, bodily pain, and GH. The MCS includes scales assessing vitality, social functioning, role limitations-emotional, and mental health. Each SF-36 scale is scored using norm-based methods that standardize the scores to a mean of 50 and a SD of 10 in the general population, with higher scores indicating better health.

Scores on the 8 SF-36 scales were further aggregated to produce PCS and MCS scores, which are also measures of health status. The PCS and MCS were also scored using norm-based methods.[31] The validity and reliability study of the Turkish version of SF-36 has been well documented.[32]

Sample Size

The required sample size was determined with a goal of measuring an improvement in VAS pain score with a SD of 2.0, as found in previous studies of FM populations.[32] Power calculations indicated that a sample of 40 patients would provide an 80% ($\beta = 0.20$) chance of detecting a 20% ($\alpha = 0.05$) difference in improvement between the groups.

Statistical Analysis

An intention-to-treat analysis was performed using the last-observation-carried-forward method. The level of significance was set at $P < 0.05$ (2-tailed tests). Groups were compared at baseline using the t test for independent samples for the continuous variables, and the χ^2 test for categorical data. As all outcome variables were normally distributed, analysis of variance with repeated measures was chosen to test the research hypothesis, with a between-patient factor at 2 levels (the 2 groups) and a within-patient factor at 3 levels (assessment time: pretreatment, post-treatment, and follow-up). Independent sample t tests were used to compare the change of scores at treatment completion. A 95% confidence interval (95% CI) was used. The P values and CIs from the comparisons of the means were shown with Bonferroni correction.

To analyze PGART, a score of 1 or 2 was considered clinically important; all other scores and missing values from patients who dropped out, were computed as nonresponsive to treatment. Fisher exact test and the χ^2 test were used to determine differences in rates of improvement between the 2 groups. Data were analyzed using SPSS for Windows, version 11.5 (Chicago, IL).

RESULTS

Demographic Data

A total of 68 patients were screened for inclusion in the study. Of these, 56 patients were enrolled in the study and randomized to 1 of 2 treatment groups: PEMF therapy (28 patients) or sham therapy (28 patients). At baseline, no significant differences were present among the groups regarding age, body mass index, year of education, socio-economic status, disease duration, or total FIQ score. However, the antidepressant intake was higher in the sham group. Mean ages were similar between the groups, and ages ranged from 23 to 60 years. The duration of FM ranged from 2.0 to 6.5 years. The demographics of the study patients are summarized in Table 1.

TABLE 1. Baseline Characteristics of the Study Patients*

	PEMF Group (n = 28)	Sham PEMF Group (n = 28)	P
Age, mean ± SD (y)	42.96 ± 9.57	40.89 ± 6.88	0.35
Body mass index, mean ± SD (y)	25.56 ± 7.16	25.48 ± 4.21	0.96
Duration of FM, mean ± SD (y)	5.6 ± 4.3	5.9 ± 6.0	0.84
Education, %			0.75
< 8 y	78.6	85.7	
8–12 y	14.3	7.1	
> 12 y	7.1	7.1	
Marital status, %			0.75
Unmarried	21.4	25.0	
Married	78.6	75.0	
Work status, %			0.75
Homemaker	75.0	78.6	
Working	25.0	21.4	
Concomitant medications, %			0.35
NSAID	21.4	25.0	
Tricyclic antidepressants	7.1	14.3	
SSRIs	10.7	25.0	
Anxiolytics	3.6	0	
Muscle relaxants	3.6	3.6	
Antiepileptics	3.6	0	
FIQ (total) score, mean ± SD	66.0 ± 12.8	61.9 ± 14.7	0.28
Physical functioning	5.82 ± 1.99	5.11 ± 1.43	
Number of days felt good	7.35 ± 2.09	5.26 ± 1.90	
Ability to do job	7.25 ± 2.10	6.60 ± 2.33	
Pain	7.46 ± 1.97	7.25 ± 1.81	
Fatigue	8.42 ± 1.85	8.82 ± 1.94	
Morning tiredness	8.78 ± 2.42	9.03 ± 1.83	
Stiffness	8.28 ± 2.40	6.14 ± 2.23	
Anxiety	7.14 ± 3.30	8.78 ± 1.59	
Depression	7.28 ± 3.12	7.85 ± 1.60	

*P values were determined by Student's t test or χ^2 test for categorical data.

FIQ indicates Fibromyalgia Impact Questionnaire; FM, fibromylagia; NSAIDs, nonsteroidal anti-inflammatory drugs; PEMF, pulsed electromagnetic field; SSRIs, selective serotonin reuptake inhibitors.

Dropout Rate

Figure 1 shows the flowchart for the study. At the end of 12 weeks, 45 patients were still participating in the study protocol. During the study, 11 patients dropped out: 5 from the PEMF group and 6 from the sham group. The reasons were: no benefit from PEMF or sham treatment (n = 2 and n = 5, respectively), temporary orthostatic hypotension after PEMF treatment (n = 2), death of father (n = 1), and stress at home (n = 1). Seventy-five percent of enrolled patients completed the study, with no significant differences in dropout rates (82.1% and 78.6% in the PEMF and sham groups, respectively).

Efficacy Results

The FIQ scores in the PEMF group showed significant improvements at the end of therapy (fourth week) compared with baseline. The PEMF group had a significantly lower FIQ score than the sham group at the end of therapy. The mean ± SEM change in the FIQ score from baseline to therapy end was -33.51 ± 2.71 (52%) in the PEMF group and -8.65 ± 1.91 (11%) in the sham group, with a between-group difference of -25.46 (95% CI -32.11, -18.80) ($P = 0.000$) (Tables 2, 3; Fig. 2). A significant difference was also observed between the groups at follow-up ($P = 0.000$). PEMF therapy significantly improved VAS pain scores at the end of therapy (Table 2). In the PEMF group, 13 patients achieved 30% improvement, whereas 8 patients achieved 50% improvement on the VAS score after treatment. At follow-up, 6 of the patients in the PEMF group achieved 30% improvement on the VAS scores. In the sham group, 3 patients achieved 30% improvement on the VAS scores after treatment.

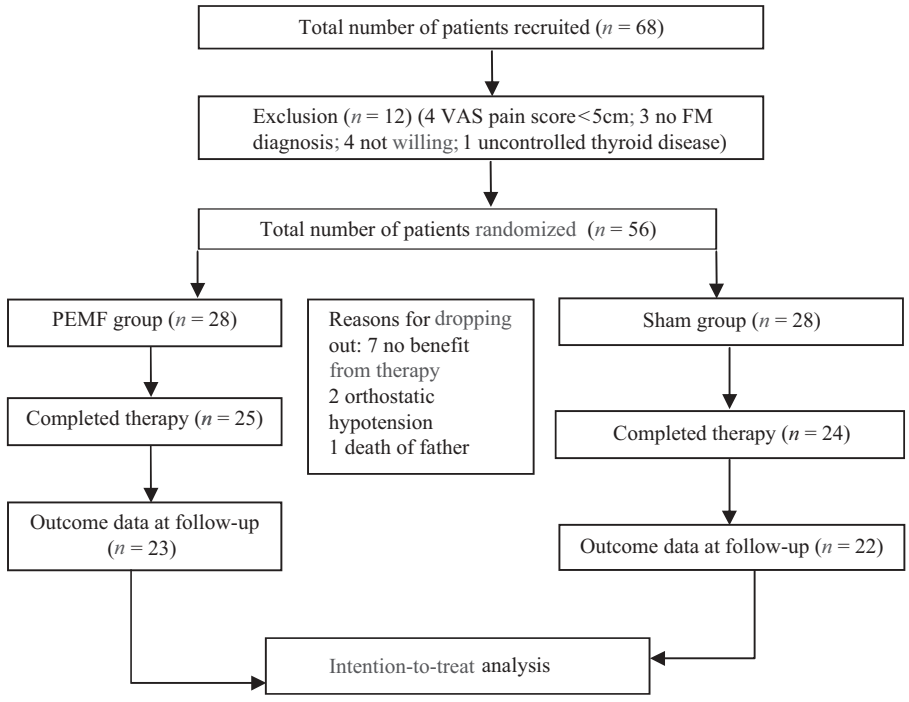

FIGURE 1. Flow diagram for randomized patient assignment. FM indicates fibromyalgia; PEMF, pulsed electromagnetic field; VAS, visual analog scale.

Sixty-four percent of patients from the PEMF group rated themselves as clinically improved after treatment, but 54% of the PEMF patients reported worsening in the PGART at the follow-up. Twenty-one percent of sham patients were responders after treatment and showed no further improvement at the follow-up. There was a significant difference between the groups after treatment ($P = 0.018$), but not at follow-up ($P = 0.538$).

The BDI score at each of the 2 assessment time points showed improvement from baseline in the sham group, but in the PEMF group the BDI scores were only improved after treatment. However, the improvements were not significantly different between 2 groups (Table 3).

Table 3 shows the statistically significant differences in SF-36 pain score from baseline observed in both groups at each of the 2 assessment time points. In all domains except GH, changes in the SF-36 scores from baseline to the end of therapy showed a trend towards greater improvement in both groups.

Side-effects

Two patients in the PEMF group had orthostatic hypotension and were withdrawn from the study. Orthostatic hypotension did not continue after stopping PEMF treatment.

TABLE 2. Result of the FIQ, VAS Pain, BDI, SF-36 Score Outcome Measures After Treatment

	PEMF Group (n = 25)	Sham Group (n = 24)	Between-group Difference at	
	Change, Mean ± SEM	Change, Mean ± SEM	Endpoint (95% CI)	*P*
FIQ total score (range: 0-80)	− 33.51 ± 2.71	− 8.65 ± 1.91	− 25.46 (− 32.11, − 18.80)	0.000
VAS pain (range: 0-100 mm)	− 35.29 ± 2.18	− 4.98 ± 1.28	− 30.31 (− 35.38, − 25.23)	0.000
BDI total score (range: 0-63)	− 4.84 ± 1.52	− 2.33 ± 0.37	− 2.50 (− 5.71, 0.70)	0.123
SF-36 (range: 0-100)				
Physical functioning	13.69 ± 1.13	0.72 ± 0.45	12.96 (10.46, 15.46)	0.000
Role limitations-physical	8.73 ± 1.53	1.15 ± 1.17	7.58 (3.66, 11.50)	0.000
Bodily pain	10.33 ± 1.06	1.68 ± 0.53	8.64 (6.21, 11.07)	0.000
General health	1.04 ± 0.92	0.60 ± 0.49	0.44 (− 1.69, 2.58)	0.679
Vitality	11.95 ± 1.74	3.07 ± 0.42	8.87 (5.19, 12.55)	0.000
Social functioning	12.53 ± 1.20	3.81 ± 0.94	8.71 (5.62, 11.81)	0.000
Role limitations-emotional	13.58 ± 2.11	2.26 ± 1.14	11.32 (6.43, 16.21)	0.000
Mental health	14.89 ± 1.39	3.53 ± 0.99	11.35 (7.87, 14.82)	0.000

BDI indicates Beck Depression Inventory; CI, confidence interval; FIQ, Fibromyalgia Impact Questionnaire; PEMF, pulsed electromagnetic field; SF-36, Short-Form 36; VAS, visual analog scale.

TABLE 3. Changes of Outcome Parameters at Pretreatment, Posttreatment, and Follow-up (Intention-to-treat Analysis)

	n	Baseline	After Treatment	Follow-up
FIQ				
PEMF group	28	66.0 ± 12.8	32.5 ± 14.2**	54.8 ± 14.2**
Sham group	28	61.9 ± 14.7	53.9 ± 12.6**	61.2 ± 13.7
VAS pain				
PEMF group	28	73.3 ± 14.0	38.07 ± 16.9**	59.4 ± 9.8**
Sham group	28	68.4 ± 12.1	63.4 ± 13.8**	67.4 ± 11.8
BDI				
PEMF group	28	39.9 ± 7.5	35.2 ± 16.8*	37.5 ± 15.4
Sham group	28	28.0 ± 13.6	25.6 ± 12.6**	27.1 ± 13.1**
SF-36 pain score				
PEMF group	28	32.0 ± 3.9	42.7 ± 4.4**	32.6 ± 3.3**
Sham group	28	32.3 ± 7.7	33.9 ± 8.1*	31.7 ± 7.3*

Data are mean ± SD. *P* values were obtained using analysis of variance for repeated measures (with Bonferroni correction).

*$P < 0.01$; **$P < 0.001$.

BDI indicates Beck Depression Inventory; FIQ, Fibromyalgia Impact Questionnaire; PEMF, pulsed electromagnetic field; SF-36, Short-Form 36; VAS, visual analog scale.

DISCUSSION

To the best of our knowledge, this is the first randomized, double-blind, sham-controlled study to examine the effect of PEMF therapy in patients with FM. This 12-week trial showed that low-frequency PEMF therapy has beneficial effects in terms of function, pain, fatigue, and global status in patients with FM.

Patients in both the PEMF and sham therapy groups experienced improvement in all outcome measures after treatment. The PEMF group showed significant beneficial effects in FIQ, VAS pain, BDI, and SF-36 pain scores at the end of therapy. The sham group also showed improvement in all outcome measures in the same period.

At the end of therapy, the difference between groups was in favor of PEMF therapy in all outcome measures except the SF-36 GH domain and BDI scores.

In the PEMF group, all the outcomes of the study except BDI scores continued to improve up to week 12. The improvements of the primary outcomes (FIQ, VAS pain) in the sham group were not sustained 12 weeks after treatment ended. However, in this group, only the BDI and SF-36 pain scores were continued until the follow-up.

Shupak et al[22] studied the efficacy of an acute 30-minute MF exposure on pain and anxiety in female RA (n = 13) and FM patients (n = 18) who received either a PEMF or sham-exposure treatment. They found that patients in the PEMF group for both patient populations had significantly reduced VAS scores; however, of the sham-exposed patients, only those in the FM sample had significantly reduced scores. They suggested that, aside from the placebo effect, decreases in pain ratings for patients randomly assigned to the sham group can be attributed to relaxation from being seated in a comfortable chair for 55 minutes during therapy. In this study, the improvements seen in the sham group could be placebo or regression to the mean or reflect the natural history of the disease.[33]

Dunkl and colleagues[34] found that the FIQ was the measure that was most responsive to perceived clinical improvement, and they recommended its inclusion as a primary end point in FM clinical trials. In this study, PEMF significantly improved the FIQ total score. It has been shown that PGART can discriminate treatment effects in FM.[3] We observed that over 64% of completers in the PEMF therapy group reported an improvement in their overall status, whereas only 14% reported worsening. In the sham arm, the most frequent category reported was ''worsening,'' with over 53% of sham patients who completed the trial rating themselves as worse. Low-frequency PEMF may improve many of the symptoms of FM, which is reflected in this outcome measure.

PEMF application was generally well tolerated in this study. There were no treatment-related serious adverse events reported by the patients. Two patients experienced orthostatic hypotension that stopped when treatment was discontinued. There have been no previous reports of orthostatic hypotension during low-frequency PEMF treatment in the literature. In addition, this method of PEMF application can be used easily at home in the treatment of patients with FM.

The clinical rationale for using PEMF therapy for patients with FM is primarily based on empirical observations and interpretation of information from physiologic and clinical studies. Several factors might mediate therapeutic effects, such as alteration in pain perception, increasing pain thresholds and hormone levels, inhibition of inflammatory edema, and vascular changes.[35]

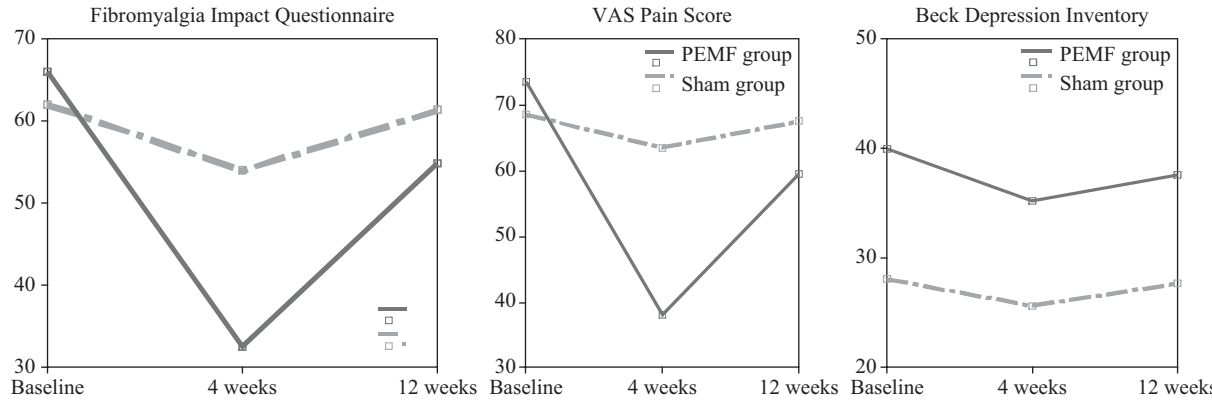

FIGURE 2. Changes in the fibromyalgia impact questionnaire, visual analog scale (VAS) pain, and Beck Depression Inventory, Short Form-36 pain score. PEMF indicates pulsed electromagnetic field.

Antidepressants are widely used to treat symptoms associated with FM.[36–38] Although more trials are needed to explore the efficacy of antidepressants in FM, the evidence supports the use of antidepressants in treating pain and other symptoms associated with the disorder.[39] In this study, despite the fact that antidepressant intake was higher in the sham group; no difference was found between the study groups in baseline pain and disease activity scores. However, the higher antidepressant intake in the sham group might positively affect outcome scores, and this may, therefore, be regarded as a potential limitation of the study. Although the PEMF therapy showed improvements in all postintervention outcomes except BDI scores, and this continued during the follow-up period, at 12 weeks a regression of the beneficial effects of PEMF therapy was observed, compared with the values after treatment. Thus, from our results, it seems that the PEMF therapy provided short-term improvement, and this was supported by the PGART scores of patients at follow-up.

As we have previously stated, the PEMF affect pain perception in many different ways. These actions are both direct and indirect. Therefore, we suggest that longer treatment times may lead to better clinical results.

Shupak et al[22] are the only researchers to have investigated the effect of PEMF on pain ratings in FM patients in a randomized clinical trial, so there is no widely accepted agreement on the optimal duration or technique of application. This is another potential limitation to our study. Also, only women were entered in this study, which may not be representative of all FM patients. Further research is needed to optimize the duration and application of PEMF treatment, as well as to identify the mechanisms of treatment action in FM.

The findings of this study support the need for future investigations of PEMF therapy for the treatment of FM. Such studies should explore the duration of the effects of PEMF by performing longer-term follow-up evaluations, and also by using different parameters of stimulation. In conclusion, PEMF therapy may improve function, pain, fatigue, and global status in FM patients and may offer a potential therapeutic adjunct to current FM therapies in the future.

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A Double-Blind Trial of the Clinical Effects of Pulsed Electromagnetic Fields in Osteoarthritis

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ABSTRACT. Objective. Further evaluation of pulsed electromagnetic fields (PEMF), which have been observed to produce numerous biological effects, and have been used to treat delayed union fractures for over a decade.

Methods. In a pilot, double-blind randomized trial, 27 patients with osteoarthritis (OA), primarily of the knee, were treated with PEMF. Treatment consisted of 18 half-hour periods of exposure over about 1 month in a specially designed noncontact, air-coil device. Observations were made on 6 clinical variables at baseline, midpoint of therapy, end of treatment and one month later; 25 patients completed treatment.

Results. An average improvement of 23–61% occurred in the clinical variables observed with active treatment, while 2 to 18% improvement was observed in these variables in placebo treated control patients. No toxicity was observed.

Conclusion. The decreased pain and improved functional performance of treated patients suggests that this configuration of PEMF has potential as an effective method of improving symptoms in patients with OA. This method warrants further clinical investigation. (*J Rheumatol 1993;*20:456–60)

Key Indexing Terms:
OSTEOARTHRITIS PULSED MAGNETIC FIELDS

Pulsed electromagnetic fields (PEMF) have been widely used in the treatment of delayed union fractures for over a decade, and there is a growing body of literature concerning the biological and clinical effects of such low frequency, nonionizing forms of energy.

Clinical responses have been reported in longstanding nonunion of fractures[1], failed arthrodeses[2], avascular necrosis of hips in adults[3,4], and Legg-Perthes's disease in children[5]. Similar devices are being evaluated in the treatment of osteoporosis. A device to generate PEMF installed in a body brace increased the success rate of lumbar fusions in a double blind control study[6]. There are also reports of augmentation of peripheral nerve regeneration and function and promotion of angiogenesis[7]. Patients with persistent rotator cuff tendinitis, refractory to steroid injection and other conventional measures, showed significant benefit compared with placebo treated patients[8]. In clinical studies done over 17 years in over 200,000 patients treated safely with PEMF, no toxic effects have been reported[9].

One of us (RM) developed a unique delivery system of extremely low frequency (ELF) pulsed waves, consisting of a magnetic field generator with an electronic interface to a freely moving air coil. Two delivery systems have been developed, one for peripheral joints and one for the axial skeleton. The most effective magnetic field configuration delivered by these devices was established empirically by treating groups of 20 patients with rheumatic conditions, varying the field variables until the optimal configuration was determined. Uncontrolled observations were then carried out in Europe in 861 patients with various painful rheumatic conditions; data suggested improvement of symptoms in 70-80% of treated patients. Therefore, a carefully controlled, double blind pilot study of possible therapeutic effects of this device was undertaken.

MATERIALS AND METHODS

Patients. All patients met the criteria for the diagnosis of osteoarthritis (OA) published by Altman[10]. Radiographs were available in all but one of the patients with OA; severity grading was done by the criteria of Brandt[11].

We followed the approach to data gathering and analysis suggesting that the impact of a new drug or device on chronic arthritis is best determined by the patients' accounts of their joint pain and their ability to perform activities of daily living (ADL)[12-14].

Patients were required to be older than 18 years of age with persistent arthritic symptoms of at least one year duration, incompletely relieved by conventional treatment including nonsteroidal antiinflammatory drugs (NSAID), other analgesics, and physical therapy modalities. Patients who

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had started any new form of treatment, including NSAID, within one month were also excluded from the investigation. The single most symptomatic peripheral joint was studied (knee in 21, the first carpometacarpal (MCP) or interphalangeal group of joints of the hand in 5 and posttraumatic OA of the ankle in 1.) Patients with OA of isolated MCP or single proximal interphalangeal (PIP) joints, or OA of the spine were not accepted for the study.

All women of child bearing age had to agree to use contraceptives. Other exclusions included the use of a cardiac pacemaker or the presence of any serious, unstable medical illness. Informed consent for entry into a double blind trial was obtained.

Patients who were taking stable daily doses of NSAID were instructed not to change their usual medications during the study period. the use of medications was checked by history at each evaluation point, but no pill counts were done.

Although some patients with other forms of arthritis were evaluated, data are presented here only for the patients who met the published criteria for the diagnosis of OA, since too few patients with other diagnoses were studied to allow conclusions to be drawn.

Treatments. Treatments were administered by the device described above, which produces an extremely low frequency (less than 30 Hz.), varying, pulsed electromagnetic field averaging 10-20 gauss of magnetic energy at a coil current of up to 2 amperes drawn from a power source of 120 volts AC. The pulse phase duration was 67 ms, including 15 micropulses with a pause duration of 0.1 s. The wave duration varied according to the frequency used. The patients rested the joint being treated on a pillow, encircled by the air coil which did not contact the skin. Treatments were given for 30 min; 3-5 sessions were given each week for a total of 18 treatments, the entire treatment period extending over about one month.

The magnetic therapy system used comprised 3 components: the magnetic field generator (MFG), the electronic interface and the air coils of varying dimensions as described above. The MFG produces a low voltage, DC current. The gating circuits employed CMOS logic integrated circuits. The electronic interface carried the current to the air coil, forming a flexible link between the fixed MFG and the air coil. The winding geometry employed in the air coil is formed with a specially designed jig to produce a pure DC homogeneous magnetic field.

The air coil produced a uniform homogeneous magnetic flux throughout the x, y and z axes. The joint under treatment was positioned eccentrically in the air coil, completely within the magnetic field flux. Since the device applied a pure magnetic field through the air coil, no heat was generated, nor did the patients feel any sensation during the treatment.

The magnetic field flux engulfed the joint area; it can penetrate the entire joint area since neither the skin nor other tissues present a barrier to magnetic energy. Since the field consisted of a uniform (homogeneous) flux density, the size of the body part (joint) treated did not affect the quality or quantity of magnetic energy delivered. Therefore, there was little or no variability of dosage to various joints or body parts treated.

Randomization. Upon entry to the study, patients were randomized to receive active PEMF or placebo using a table of 1,000 random digits. A coded master record sheet was kept by an office administrator, and a trained PEMF therapist activated the coil energy of the PEMF device and timed each treatment to 30 min. The ON/OFF control of the MFG was in the "ON" position with the associated red light for all of the patients in both the active and placebo groups during treatments. The PEMF device produces no noise or sensation and therefore the placebo therapy was applied by not energizing the air coil; all patients kept the affected joint in the air coil for the same 30 min period signalled by a laboratory timing clock placed on the generator. In this way, both the patient and the examining physician remained blinded as to whether active PEMF or placebo was being given.

Data collection. A case report form was prepared for each patient which recorded their enrollment eligibility criteria, pertinent clinical history, baseline medications, and examination. Baseline hematocrit and white blood cell counts (WBC), serum electrolytes, creatinine, and an erythrocyte sedimentation rate (ESR), as well as a urinalysis, were done to exclude any concurrent illness and to monitor for any changes. These tests were repeated at the end of the treatment period. A radiograph of the joint to be treated was taken unless one had been obtained within the preceding 4 months.

Evaluations by the patient and the physician observers were made at 4 points during the study: baseline, midway through the treatment series, at the end of the treatment period, and 4 weeks after treatment was completed. At each evaluation the patient's opinion about joint symptoms was recorded along with the physician's assessment of the patient's symptoms and clinical signs. Data were collected for overall severity of pain, difficulty performing the activities of daily living (ADL) identified as the most troublesome by the patient before therapy was begun, pain generated by those specific activities of daily living, and the worst discomfort experienced by the patient in the previous week. A standard visual analog pain scale was marked by the patient to indicate the total amount of pain, pain with ADL and worst discomfort in the past week; the scale had no markings but was 100 mm long and the patient's mark was measured from the zero point. The difficulty in performing the specific ADL identified as most troublesome were quantitated on a scale of 1 to 5 using explanatory adjectives of "none, slight, moderate, severe, or extreme" to assist patients in their evaluations.

Data were also recorded for a rheumatologist's evaluation of pain on joint motion and tenderness of the joint being treated to firm palpation. At each observation after baseline, the physician made an "overall assessment of improvement" on a 5-point scale (worse or no change = 1, slight but insignificant change = 2, moderate improvement = 3, excellent improvement = 4, and complete disappearance of symptoms in affected joint = 5).

Statistical methods. Data were analyzed for change from baseline to each observation point for each patient by nonparametric matched pair 2-tailed t tests using the Wilcoxon signed rank test, for both treated and control groups. Instat (Graphpad Software Co.) was used for statistical calculations. The physicians' overall assessment of improvement was analyzed at the end of the treatment period and one month later, comparing treated and placebo groups, also using 2-tailed t tests.

RESULTS

Fifteen patients with OA were randomized into the active treatment group, 12 into the placebo treatment group. Two patients, after agreeing to randomization, withdrew from the study before the start of active treatment and asked for unblinded, active treatment; one (randomized to active treatment) withdrew because of transportation difficulties; one (randomized to the placebo group) was hospitalized because of a hernia; both of these patients had been evaluated at baseline, but not after treatment had begun and were not included in the data analysis. Five patients did not appear for the evaluation one month after completion of treatment; 4 had received active treatment, one, in the placebo group, had been hospitalized for community acquired pneumonia. All data for each patient were included in the analysis, including the last observation point; the numbers evaluated at each point are given in Table 1.

The 2 groups of patients did not differ significantly in respect to age, sex, race, body weight, or number of years with symptoms. The active treatment group included 11 with OA of the knee, 3 of the hand (PIP and MCP joints); 1 had OA of the ankle. The placebo group included 10 with OA of the knee, 2 of the hand. The dropouts all had OA of the knees.

At baseline there were no significant differences between

Table 1. *Numbers of patients with OA observed at each phase of the study*

	Numbers of Patients	
	Treated	Placebo
Entered into study	15	12
Evaluated at middle of treatment	14	11
Evaluated at end of treatment	14	11
Evaluated at one month followup	10	10

the active treatment and the placebo groups in any of the subjective patient variables or the physician examination variables evaluated.

Improvement occurred in each variable followed in the treated group, and data analyzed as matched pairs showed significant differences for the data for each variable at the midpoint of treatment, the end of treatment and one month after treatment. The patients who were followed showed continued improvement during the month after completion of treatment (Table 2A).

The placebo treated group showed some improvement from baseline in each variable, the change not reaching statistical significance for any of the observations (Table 2B).

The actively treated group averaged 34% improvement in the mean value for each variable evaluated at the midpoint of therapy, and 36% at the end of treatment; by one month after treatment ended, improvement averaged 47%; Figure 1A shows the percentage improvement in each of the 6 variables observed for the treated patients. Among the patients in the placebo group, improvement averaged 8% at the midpoint, 10% at the end of treatment, and 14% one month later (Figure 1B).

The overall assessment of improvement by the physician observer at the midpoint, end of treatment and one month after completion of treatment for treated and placebo groups is shown in Table 3.

The degree and frequency of improvement for upper extremity and lower extremity joints was similar.

No patient reported any increase in the use of their usual medications during the period of observation. Two patients, both in the treatment group, reported discontinuation of usual medications (ibuprofen, 800 tid in one, and pentazocine, 50 mg prn in the other); no placebo patient reported any change in medication.

Laboratory data at the end of treatment showed no changes in any tests including CBC, ESR, serum electrolytes, BUN, creatinine, and tests of liver status. No patient reported symptoms suggestive of toxicity nor was any toxicity observed by the physician evaluators.

The study was not designed for crossover analysis of an active treatment phase for the placebo treated patients; at the completion of observations, however, placebo patients were informed of the nature of their "treatment" and offered the opportunity to have active treatment in an unblinded fashion, and 7 did so. New baseline and followup observations were

Table 2. *Observations on actively treated and placebo-treated patients at each point. Figures are numbers of patients observed at each point (n) mean for groups*

Table 2A. Actively treated patients

	Baseline	Midpoint	End of Treatment	1 mo Later
n =	15	14	14	10
Overall severity of pain (scored on 10 cm visual analog scale)				
Mean	7.65	4.28	3.80	3.55
SEM (±)	0.6405	0.88	0.82	1.08
p =		0.0215	0.0023	0.0052
Difficulty score with most troublesome ADL (scale: 1 to 5)				
Mean	4.27	3.25	3.07	2.80
SEM (±)	0.20	0.25	0.31	0.29
p =		0.0098	0.0020	0.0078
Pain with most troublesome ADL (scale: 1 to 5)				
Mean	3.90	2.89	3.14	2.40
SEM (±)	0.12	0.22	0.29	0.27
p =		0.002	0.0313	0.0078
Worst discomfort in previous week (scored on 10 cm visual analog scale)				
Mean	8.14	5.36	5.03	4.03
SEM (±)	0.58	0.74	0.83	0.94
p =		0.0046	0.0009	0.0059
Pain on joint motion by MD exam (scale: 1 to 5)				
Mean	3.47	2.07	2.29	1.85
SEM (±)	0.21	0.29	0.38	0.26
p =		0.002	0.0195	0.0117
Joint tenderness by MD exam (scale: 1 to 5)				
Mean	3.57	2.29	2.00	1.40
SEM (±)	0.33	0.29	0.29	0.22
p =		0.0034	0.0024	0.0039

Table 2B. Placebo-treated patients

n =	12	11	11	10
Overall severity of pain (scored on 10 cm visual analog scale)				
Mean	8.07	7.23	7.32	7.10
SEM (±)	0.75	0.55	0.47	0.35
p =		> 0.3	> 0.6	> 0.5
Difficulty score with most troublesome ADL (scale: 1 to 5)				
Mean	3.88	3.77	3.59	3.65
SEM (±)	0.15	0.18	0.24	0.26
p =		> 0.6	> 0.4	> 0.6
Pain with most troublesome ADL (scale: 1 to 5)				
Mean	3.92	3.77	3.50	3.45
SEM (±)	0.15	0.23	0.20	0.26
p =		> 0.6	> 0.2	> 0.2
Worst discomfort in previous week (scored on 10 cm visual analog scale)				
Mean	8.18	7.16	6.65	6.82
SEM (±)	0.59	0.63	0.79	0.82
p =		> 0.4	> 0.3	> 0.3
Pain on joint motion by MD exam (scale: 1 to 5)				
Mean	3.25	2.86	2.82	2.60
SEM (±)	0.28	0.38	0.42	0.42
p =		> 0.3	> 0.4	> 0.2
Joint tenderness by MD exam (scale: 1 to 5)				
Mean	3.00	2.73	2.95	2.45
SEM (±)	0.44	0.36	0.32	0.26
p =		> 0.8	> 0.9	> 0.8

SEM = standard error of the mean, and p value is for change from baseline for that variable.

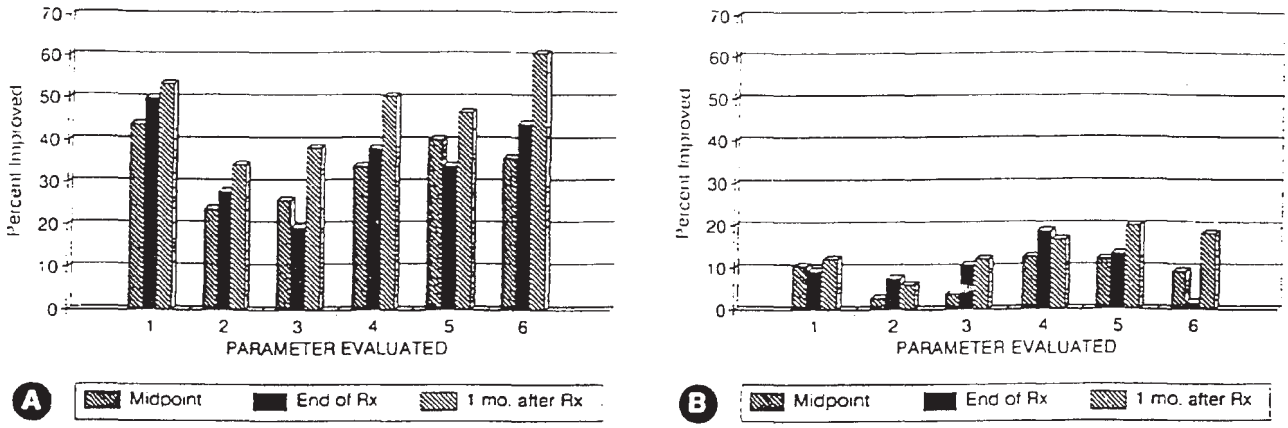

Fig. 1. Percent improvement, defined as difference between baseline value and value at each specific observation point, divided by baseline value (times 100). Numbered observations shown included (1) Overall severity of pain. (2) Difficulty performing ADL identified as the most troublesome by the patient before therapy was begun. (3) Pain generated by the most troublesome ADL. (4) The worst discomfort experienced in affected joint area in the past week. (5) Pain on motion of the treated joint detected by the examining physician. (6) Tenderness of study joint detected by the examining physician. 1A: Treated patients; 1B: Placebo patients.

Table 3. *Assessment of improvement by observing physician at midpoint of treatment, end of treatment and one month after completion of treatment. p Value is for difference between treated and placebo groups*

	Midpoint	End of Treatment	1 mo Later
Treated patients			
Mean	2.71	2.71	3.30
SEM (±)	0.27	0.37	0.45
Placebo patients			
Mean	1.73	1.86	1.75
SEM (±)	0.27	0.47	0.34
p =	0.0175	0.1611	0.0134

made on these patients. As a group, these patients showed improvement during the active treatment phase but, in view of the small numbers involved, statistically significant changes that occurred did not occur in all of the variables followed.

Radiographs were graded as to severity of the OA. There were too few cases in the treated group to permit meaningful statistical analysis of the response according to radiological criteria of severity. It is possible to say, however, that 5 patients with radiologic grade 3 and 4 disease obtained good or excellent responses according to physician assessment at the last observation, and thus that advanced disease does not preclude symptomatic benefit from this form of therapy.

DISCUSSION

The results of our prospective double blind study of PEMF treatment show beneficial effects in the amelioration of symptoms, subjective improvement in functional ability and decrease in objective findings in a small group of patients with OA. The benefit seemed to continue for at least the first month after completion of treatment. Furthermore, no toxicity was observed.

This application of PEMF therapy is not similar to other physical modalities of treatment, such as ultrasound, TENS, diathermy, moxibustion, etc. The PEMF generated by the device used in our study differs from the device used in the treatment of unhealed fractures in that it generates a lower frequency (< 30 Hz vs 72 Hz), as well as differing in pulse and wave form characteristics. The extremely low frequency pulsed magnetic fields used in these studies, as well as those used in laboratory experiments, are too weak to work through a mechanism such as thermal effect, dielectric breakdown, particle displacement or electrophoresis. Mechanisms which have been suggested include some form of induced resonance of outer shell electrons, an effect on cell membrane receptors or on other endogenous processes, such as an effect on ion flux, but these suggested mechanisms lack experimental substantiation[15-18]. Evidence exists that pulsed magnetic fields can modulate the actions of hormones, antibodies, and neurotransmitters at surface receptor sites of a variety of cell types[15]. Effects on fibroblast, chondrocyte and osteocyte metabolism and lymphocyte functions have been reported. Augmentation of mRNA and protein synthesis has been reported in several tissue culture symptoms[16,17,19-25].

Since the factors responsible for the pain in patients with OA are varied and often uncertain in an individual patient, an attempt to delineate the mechanism of pain relief brought about by this form of therapy in relation to known biological effects of pulsed magnetic fields would be purely speculative.

This form of nonionizing radiation is not known to have

any deleterious clinical effects, despite the variety of metabolic changes that have been demonstrated in laboratory experiments. On the basis of the findings in this pilot study, further investigation of clinical effects of pulsed magnetic fields in OA is warranted.

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INTERMITTENT BURST-FIRING WEAK (1 MICROTESLA) MAGNETIC FIELDS REDUCE PSYCHOMETRIC DEPRESSION IN PATIENTS WHO SUSTAINED CLOSED HEAD INJURIES: A REPLICATION AND ELECTROENCEPHALOGRAPHIC VALIDATION[1]

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Summary.—14 patients who reported chronic depression more than one year after closed head injuries were exposed to weak (1 microTesla), burst-firing magnetic fields either across the temporal lobes or over the left frontal lobe. The treatment was for 30 min. once per week for 6 wk. The reduction in depression scores after 5 wk. of treatments and after 6 wk. of no treatment (follow-up) accommodated 54% of the variance for both groups. The changes in depression scores did not differ significantly between the two groups (temporal vs frontal). Following treatment, the frequency of complex partial epileptic-like experiences decreased significantly only for the 7 who received the bilateral stimulation over the temporal lobes. Quantitative bipolar electroencephalographic measurements over the occipital, prefrontal, and temporal regions showed increased power within the 16-Hz to 18-Hz range 6 wk. after termination of treatment for those 7 patients who received the burst-firing magnetic fields bilaterally over the temporal lobes but not over the left prefrontal region.

Whereas Transcranial Magnetic Stimulation (TMS) involves focal applications of very strong (1 Tesla) simply shaped magnetic fields over the skull (Wassermann & Lisanby, 2001), transcerebral magnetic (TCM) field treatments involve the applications of temporally complex magnetic fields at field strengths about one million times less intense. Baker-Price and Persinger (1996) reported that six weekly applications of a burst-firing magnetic field (1 microT) across the temporal lobes significantly reduced the psychometric depression of patients who had sustained closed head injuries. The effect size of applying the burst-firing field, whose structure was derived from the firing pattern of amygdaloid neurons, was similar to that reported for Transcranial Magnetic Stimulation. More recent studies involving normal volunteers indicated that bitemporal application of this field once per week for three weeks, but not for three consecutive days, elevated mood (Tsang, Persinger, & Koren, 2002) in normal volunteers.

The present study was designed to discern if transcerebral stimulation with the effective burst-firing magnetic fields over the left prefrontal region

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would be as potent as the application bilaterally over the temporal lobes. We selected the left prefrontal region for comparison because the major cerebral correlates of clinical depression are hypofunction within the prefrontal regions (Pearlson & Schlaepfer, 1995; Videbech, 2000). Application of repetitive transcranial stimulation (rTMS) over the left prefrontal region has been associated with increases in regional cerebral blood flow (Speer, Kimbrell, Wassermann, Repella, Willis, Herscovitch, & Post, 2000).

Over the years more than half of all of the patients we have assessed (approximately 1,000) who were depressed and reported pain during the years following "mild" brain injuries had not responded to antidepressant medications prescribed by their family physicians or psychiatrists. We suspect the refractory profile may reflect the procedures by which antidepressant medications are selected. Drugs developed for marketing are usually based on the results of clinical trials involving patients whose depression was secondary to sociogenic (loss of a loved one, ontogenetic progression) or endogenous sources.

Our hypothesis has been that a substantial proportion of patients who experience depression and pain subsequent to a closed head injury (with or without loss of consciousness) do not respond to antidepressant medications because the symptoms are due to elevated metabolic and electrical activity within the mesiobasal (hippocampus and amygdala) portions of the temporal lobes rather than a generalized decreased metabolic activity within the (particularly left) prefrontal lobes. However, the clinical manifestations of this "dominating" limbic effect upon even a normal prefrontal function would be similar to "hypofunction." We (Bureau & Persinger, 1995) had found that the incidence of limbic motor seizures in rats had been reduced following 20 pairings of subclinical dosages of lithium and pilocarpine with the same burst-firing pattern as that employed in the present study.

On the basis of our previous study (Baker-Price & Persinger, 1996) and the anticipated effect size of the treatment, we reasoned that a sample of 14 patients (seven per group) would be sufficient for any differences between applications of the fields over the temporal lobes or left prefrontal lobe to achieve statistical significance with an effect size that would be practical for clinical application. For humane reasons we decided before the experiment to reduce our probability of obtaining statistical significance but to increase the likelihood of benefit to the patients by not including a sham-field group. Consequently, the focus of the study was based on the potentially greater effectiveness of one site of application of the field compared to the other.

Method

Subjects

A total of 14 male and female patients (ages 25 through 45 years) who

had experienced chronic depression (that did not respond to antidepressants) following a closed head injury were referred for treatment by local professionals or volunteered in response to a published notice in a local newspaper. Four of the subjects reported they experienced a cessation of consciousness following the mechanical impact. The average time between the brain trauma and the testing was two years. Seven of the patients were randomly assigned to receiving the same treatment reported by Baker-Price and Persinger (1996) across the temporal lobes while another seven received the same magnetic field patterns over the left prefrontal region.

Procedure

The basic design involved meeting the patient once per week for 6 wk. (six sessions). The protocol had been approved by the university's Human Ethics Committee, and the volunteers signed informed consents that emphasized they could discontinue the treatment at any time. Because our goal was to produce permanent rather than postsessional improvements, the psychometric tests and electroencephalographic measurements were completed at the beginning of a session before the transcerebral application of the 1 microTesla magnetic fields. After the end of the sixth session the patient did not return for measurements and was not contacted by the clinician (first author), except for a reminder of the follow-up session, until six weeks had elapsed. At that time final measurements were collected. Each subject was then given a brief report of progress.

At the beginning of each week (before the transcerebral magnetic field treatment) and 6 wk. after the final treatment (follow-up), each subject completed the Beck Depression Inventory (Beck & Steer, 1987). At the beginning of the first week, at the last treatment (6 wk. later), and 6 wk. after that the person completed Roberts' questionnaire for the Epileptic Spectrum Disorder (Roberts, Varney, Hulbert, Richardson, Springer, Sheperd, Swan, Legrand, Harvey, & Stuchen, 1990). During the 6 wk. of the treatment three of the subjects who had been receiving the left prefrontal treatment did not return for the last two or three sessions.

At Session 1 (baseline), Session 6 (after five treatments), and Session 7 (6 wk. follow-up, after 6 wk. of no treatment or contact with the clinician), silver electrodes were attached by EC2 electrode cream over the frontal (F7, F8), temporal (T3, T4), and occipital (O1, O2) lobes. Once the canisters were attached, bipolar activity was recorded (filter set at 10 Hz) for 10 min. by a P79 Grass electroencephalographic instrument. The canisters containing the solenoids were then held by Velcro over either the temporal lobes or the left frontal region. Electroencephalographic measurements were completed for 10 min. while the fields were not activated.

During the treatment the patient sat within a comfortable chair that

was housed in a darkened acoustic chamber. The fields were delivered through two small canisters (each containing four small solenoids) through which a burst-firing magnetic field was presented once every 3 sec. for 30 min. once per week for 6 wk. The point duration for each of the 259 values that composed the burst-firing (see Persinger, Tiller, & Koren, 2000 for a pictorial representation) was 3 msec. The configuration of the circuit was such that the fields were generated between the pairs of solenoids and the burst-firing pattern was rotated to each homologous pair of solenoids (one in each canister) every 0.5 sec. (1 cycle = 2 sec.).

The records for each of the three channels were later examined for dominant frequency in the following manner. A ruler was placed along the record at the midpoint between the peaks and troughs. The numbers of excursions more than one-third above the midline of the activity of the pen recordings were recorded manually by the first author for each second of the 600 successive (10-min.) readings).

For each subject the numbers of seconds in which appeared specific 1-Hz frequency increments, between 9 Hz and 27 Hz, were counted for each channel (occipital, temporal, frontal) for each subject for the three sessions. Multivariate analyses of variance were completed for each of these sets of 1-Hz increments of frequencies as a function of the session (baseline, 6 wk. after treatment, and 6 wk. after cessation of treatment) and the application position of the solenoids: bilateral temporal lobes versus left prefrontal region.

To extract a variant of "power" for each frequency, the total numbers of seconds containing each 1-Hz increment for each session was divided by the total numbers of seconds counted for that session, for each channel. This calculation also minimized the excessive contribution from any single subject. Two-way analyses of variance, with one level repeated (session) and one between-groups treatment (position of magnetic field) were also completed for each of the 19 1-Hz frequency bands. All analyses involved SPSS software on a VAX computer.

Results

Two-way analysis of variance with one level repeated (first measure, sixth measure) and one between-subject factor (left prefrontal vs bilateral) for the Beck Depression scores showed no significant difference between the region of application of the field ($F_{1,9} = .11$, $p > .05$) but a significant difference over the time of the treatment ($F_{1,9} = 10.37$, $p < .01$; $\eta^2 = 53\%$ of the variance); the interaction between application geometry and sessions was not statistically significant. The means and standard deviations for the first and second Beck Depression scores were 19.7 ($SD = 8.6$) and 14.1 ($SD = 5.2$), respectively. The M and SD for the scores at follow-up were 15.1 ($SD = 7.6$).

Post hoc analysis using *t* tests for correlated data indicated that the depression scores were significantly lower after the five treatments (Week 6) and 6 wk. after no treatment (the follow-up) than the baseline levels.

Although the results of the analyses with the complex partial epileptic-like signs were not significant statistically with the full analyses, the seven patients who had received the 6 wk. of treatment with the bilateral fields showed significantly ($F_{1,9}=8.91$, $p=.01$; ω^2 estimate = 48% of variance explained) less frequent signs ($M=2.0$, $SD=1.1$) than those who received the left prefrontal treatment ($M=4.8$, $SD=1.9$). These group differences were not present ($M=4.6$, $SD=3.3$; $M=5.3$, $SD=2.7$, respectively) before the treatment began. At the time of the 6-wk. follow-up the scores for the patients who received the frontal and temporal treatments were $M=2.1$ ($SD=1.6$) and $M=4.0$ ($SD=1.8$), respectively. The results are also shown in Fig. 1 (insert). Covariance for the concomitant depression scores before the analyses did not change the strength of the group difference ($F_{1,8}=6.37$, $p<.05$; $\eta^2=41\%$ of the variance explained).

The results of the two-way analysis of variance for the numbers of seconds containing the various 1-Hz bands of activity yielded no statistically

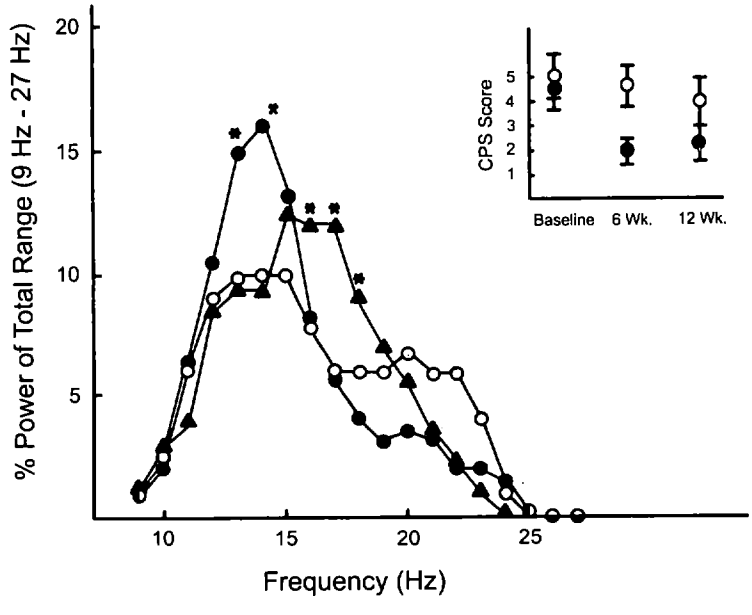

FIG. 1. Percentages of power within 1-Hz frequency increments between 9 Hz and 27 Hz over the temporal lobes during baseline (o), after 6 wk. of treatments (•), and after 6 wk. of no treatment (12 wk. follow-up) (▲). Inset: mean score for complex partial epileptic-like signs for patients who received the burst-firing magnetic fields either over both temporal (• , $n=7$) lobes and only the left prefrontal (o , $n=4$) region.

significant differences between the groups who received the two field applications ($Fs_{1,9} < 1.00$) and no statistically significant interactions ($F_{2,18} < 1.00$) between session and the position of the applied magnetic field.

For the "power measurements," two-way analyses of variance with one level repeated (baseline, 6 wk. later, 12 wk. later) and one not repeated showed statistically ($p < .05$) significant (all dfs = 2,20) differences between sessions (η^2 estimates in parentheses) for the 13-Hz ($\eta^2 = 23\%$), 14-Hz ($\eta^2 = 41\%$), 17-Hz ($\eta^2 = 30\%$), 18-Hz ($\eta^2 = 23\%$), and 19-Hz ($\eta^2 = 20\%$) bands only.

The results are shown in Fig. 1. *Post hoc* analyses indicated statistically significant increases in the percentages of activity over the temporal lobes only within the 13-Hz to 14-Hz bands for both prefrontal and bitemporal treatments following the five treatments compared to the baseline measurements and the measurements 6 wk. after the termination of treatment. On the other hand, the significant differences in the percentages of activity within the 17-Hz to 19-Hz band over all three lobes was primarily due to the elevated amount of activity in this range during the follow-up period after 6 wk. of no treatment for the patients who received the bitemporal magnetic fields compared to those who received the fields applied over the left prefrontal region. The measures for this range of activity did not differ significantly between groups for the baseline or after 6 wk.

To discern if the changes in scores for the complex partial epileptic-like signs and the Beck Depression Inventory were quantitatively associated with the electroencephalographic scores, Spearman *rho* correlations were calculated between the measures of power for the 13-Hz and 14-Hz band (Fig. 1) over the temporal lobe after 6 wk. of treatment and for the measures of power for the 17-Hz, 18-Hz, and 19-Hz band (Fig. 2) recorded during the follow-up. Values of Spearman *rho* were significant between the severity of depression (Beck scores) before treatment and the amount of power in the 12-Hz (.64, $p < .05$), but not for 13-Hz (.52, ns), and 14-Hz (.53, ns) bands.

These values for the association between the power measures for these increments and the depression scores after 6 wk. of treatment were –.38, –.42, and –.27, respectively (ns). The *rho* coefficients between the scores for complex partial signs for the subjects who received the treatment over the temporal lobes only and the significant bands measured during the follow-up were 16 Hz (.03), 17 Hz (–.61, $p = .07$), 18 Hz (–.40, ns), and 19 Hz (–.85, $p < .01$). There were no significant correlations between the psychometric scores during the follow-up and the power for any 1-Hz band except for 18 Hz ($rho = .74$, $p = .02$).

Discussion

The psychometric results of this study replicated those reported by Bak-

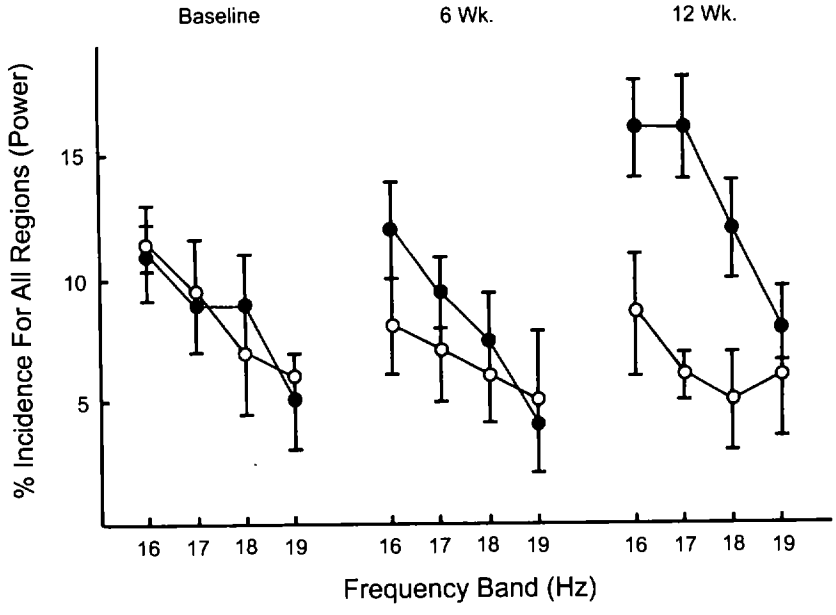

FIG. 2. Percentage of transcerebral power (frontal, temporal, and occipital) within the 1-Hz increments between 16 Hz and 19 Hz during baseline, after 6 wk. of treatment, and after 6 wk. of no treatment (12 wk. follow-up) for patients who received the treatment over the left frontal (o) or bilateral temporal (•) region.

er-Price and Persinger (1996). Subjects who were exposed to the treatment showed a significant reduction in depression by Week 6 after five 30-min. exposures to the burst-firing magnetic fields. The treatment accommodated more than 50% of the variance in the psychometric measures.

We also found that exposure to treatment was followed by significant decreases in the weekly incidence of complex partial epileptic-like experiences. Like the Beck Depression scores, the attenuation of complex partial epileptic-like signs was still evident when the subjects returned for testing 6 wk. later after 6 wk. of no treatment.

There were no statistically significant differences in the depression scores for the patients who received the applications of the burst-firing magnetic fields across the temporal lobes versus the left prefrontal regions. However, there was a statistically significant interaction between the frequency of the complex partial epileptic-like experiences and the position of the applied fields. Those subjects who received the bilateral temporal applications showed a conspicuous reduction in the frequencies of these experiences after five treatments, and this was still evident even after six weeks of no treatment. This reduction did not occur for the four patients who received the prefrontal applications.

For humane reasons we decided not to run a sham-field group in the present study. Instead, we compared the application geometry of the fields. We assumed that, if the bitemporal magnetic fields were most potent, their effects should still be evident and statistically significant even when compared with the left prefrontal application. Although this approach would reduce the *size* of the effect of the magnetic field treatment (relative to the inclusion of a sham-field group), as clinicians we felt that the patients receiving the prefrontal applications would at least have some relief. All of these patients had been treated by psychiatrists or physicians without relief. Many of these patients were experiencing the type of futility that precedes self-destructive behaviors. It is interesting that three of the patients who had been randomly assigned to the prefrontal arrangement did not complete the study. This may suggest that the treatment was not beneficial. That issue needs study.

Two electroencephalographic results were notable in this study. The first involved a significant increase in the relative power of frequencies between 16 Hz and 19 Hz following bitemporal treatments but not prefrontal treatments. This increase did not appear until the follow-up. If this increase is considered in context of the reduction in complex partial epileptic-like signs that occurred by the end of the treatment and was still evident during the follow-up examination, then it is possible that this treatment increased the percentage of cerebral activity within the low beta range.

Tebano, Cameroni, Gallozzi, Loizzo, Palazzino, Pezzini, and Ricci (1988) reported that patients who sustained mild to moderate head injury 3 to 10 days before the measurements showed a reduction in the mean power for fast beta activity (20 Hz to 36 Hz) and fast alpha activity (10.5 Hz to 13.5 Hz) but an increase in the mean power of slow alpha (8 Hz to 10 Hz). This effect was noted whether the patient had or had not sustained a suspension of consciousness. In the present study application of the burst-firing magnetic fields over either the prefrontal or the temporal lobes increased the "power" within the 13-Hz to 14-Hz band over the temporal lobes.

Because the increase in power within the 13-Hz to 14-Hz band over the temporal lobes occurred for both groups, we cannot exclude the possibility that some nonspecific factor may have been responsible for the improvement. However, the increase in power within the 17-Hz to 19-Hz range was specific to the patients who received the application of the fields across both temporal lobes but not over the left prefrontal lobe. That this effect was an artifact of field application is not possible because the electroencephalographic measurements were not taken at the time of the field application. That this increase was due to entrainment is unlikely. Spectral analyses (fast Fourier transform) of the burst-firing pattern directly from the software that

generated the magnetic field showed primary peaks around 29 Hz, 33 Hz, and 42 Hz.

There were smaller peaks in the spectral power of the burst-firing field around 14 Hz to 15 Hz and 18 Hz to 19 Hz. One cannot exclude the possibility that between the first and the sixth sessions permanent modifications occurred within aggregates of neurons mediating the specific cortical frequencies reflected in electroencephalographic activity. Zhongqi, Gang, Cuiyun, Zhiuan, Guozhen, Yan, Yao, and Shaozhang (1998) have shown that 16-Hz electromagnetic fields but neither 3-Hz nor 31-Hz magnetic fields within a specific intensity window can significantly affect the influx of calcium ions within cerebral tissue. If this assumption be correct, then treatment may have facilitated the learning, which is defined as the more or less permanent change in behavior with experience of less clinical and more adaptable behaviors.

The interstimulus interval between treatments by bitemporal application of the burst-firing magnetic fields may be critical. An intuitive decision to increase the frequency of applications, for example, to daily treatments, may not be optimal. Tsang, *et al.* (2002) exposed normal volunteers to the same complex magnetic field employed in the present study. Relative to sham-field applications, the burst-firing applications reduced scores for psychometric depression only when applied for 30 min. once per week for 3 wk. Applications for 30 min. per day for three successive days were not effective.

With respect to efficacy the temporal structures of complex magnetic fields can be considered similar to the spatial (molecular) structures of pharmacological agents. The temporal relationship of the duration of the field with the interstimulus interval for the application of complex magnetic fields might be considered comparable to the dosage of a drug. Pharmacological wisdom clearly shows that an optimal effect with a particular dosage of an antidepressant or anxiolytic compound does not always increase simply by increasing the amount of drug. In fact, for some psychotropic drugs, particularly antidepressants and anxiolytics, larger amounts may eliminate improvements. The optimal prophylaxis of some anticoagulant drugs, such as aspirin, may only require consumption once per week.

A similar "optimal dosage" manifested as "optimal intervals" of interstimulus exposures, may occur for transcerebral stimulation by weak complex magnetic fields. It would be analogous to the powerful differences between massed-practice vs spaced-practice for some types of learning. More permanent changes in behavior are favoured by spaced-practice relative to massed-practice.

That the response of the brain to electrical stimulation is strongly influenced by the interstimulus interval has been shown for kindling. According to Goddard, Dragunow, Maru, and Macleod (1986), kindling from the amyg-

dala did not occur with interstimulus intervals of 5 or 10 min. but required intervals between 20 min. and 60 min. Assuming a functional impact of 10 sec. from a single kindling stimulus, the ratios for the stimulus duration divided by the interstimulus interval would be .04, .02, respectively, for the ineffective procedures and .008 and .003, respectively, for the effective procedures.

The duration of our exposures has been about 30 min. For daily treatments, the ratio for this stimulus duration divided by the interstimulus interval would be about .02. For weekly treatments, these ratios would be about .004. If there be some "scale invariance" for the impacts of electromagnetic fields within the brain, similar to Weber's law, then isolation of the interstimulus interval may be essential to elicit optimal clinical effects.

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Short-Term Efficacy of Pulsed Electromagnetic Field Therapy on Pain and Functional Level in Knee Osteoarthritis: A Randomized Controlled Study

Diz Osteoartiritinde Pulse Elektromanyetik Alan Tedavisinin Ağrı ve Fonksiyonellik Üzerine Kısa Dönemde Etkisi, Randomize Kontrollü Çalışma

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Abstract

Objective: We aimed to determine the efficacy of pulsed electromagnetic field therapy on pain and functional level in knee osteoarthritis when compared to therapeutic ultrasound (US) and controls.

Material and Methods: Forty-five patients with knee osteoarthritis (mean age: 63.5±10.2 years) were randomly assigned to three groups. The first group received pulsed electromagnetic field therapy (frequency: 2 Hz, 100 Hz, 25 Hz consecutively, 35 minutes/session), the second group received therapeutic US (frequency: 1 MHz, power: 1.5 watt/cm^2 continuously, 10 minutes/session) and the third group served as the no- treatment control group. Evaluations were done at baseline and at the end of the treatment (third week). Assessment parameters were pain, stiffness and functional level scores of the Western Ontario and McMaster Universities (WOMAC) questionnaire and pain severity evaluated by Visual Analog Scale (VAS) (0-10).

Results: VAS (p=0.005), WOMAC pain score (p=0.001), WOMAC joint stiffness score (p=0.027) and WOMAC functional level score (p=0.003) significantly improved in the first group. VAS (p=0.001), WOMAC pain scores (p=0.008), WOMAC stiffness scores (p=0.012) and WOMAC functional level (p=0.004) scores significantly improved in the second group as well. No change was observed in any assessment parameter in the third group (p>0.05). There were differences between groups regarding the percent change in VAS scores (p<0.001), WOMAC pain scores (p<0.001), WOMAC joint stiffness scores (p=0.013) and WOMAC functional level scores (p<0.001) after the treatments.

Conclusion: Both the pulsed electromagnetic field and therapeutic US were significantly more effective than no treatment. The pulsed electromagnetic field may be applied as an effective and alternative therapy approach in knee osteoarthritis.

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Key words: Pulsed electromagnetic field, knee, osteoarthritis, ultrasound

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Özet

Amaç: Diz osteoartiritinde pulse elektromanyetik alan tedavisinin ağrı ve fonksiyonel düzey üzerine etkisini ultrason tedavisi ve kontrolle karşılaştırarak araştırmaktır.

Yöntem ve Gereçler: Kırkbeş diz osteoartiriti olan hasta randomize olarak üç gruba ayrıldı (ortalama yaş= 63.5 ±10.2 yıl). Birinci gruba pulse elektromantetik alan tedavisi (frekans: sırasıyla, 2 Hz, 100 Hz, 25 Hz, 35 dakika/seans), ikinci gruba ultrason tedavisi (frekans: 1 MHz, güç:1,5 watt/cm^2 devamlı,10 dakika/seans) uygulandı. Üçüncü grub kontrol grubu oldu. Değerlendirmeler başlangıçta ve tedavi sonunda (üç hafta sonra) yapıldı. Değerlendirme değişkenleri, Western Ontario ve McMaster Üniversiteleri Anketi'nin (WOMAC) ağrı, eklem sertliği ve fonksiyonel düzey skorları ve görsel ağrı skalalasına (GAS) göre (0-10) ağrı şiddeti idi.

Bulgular: Birinci grupta, GAS skoru (p=0.005), WOMAC-ağrı skoru (p=0.001), WOMAC eklem sertliği skoru (p=0.027), WOMAC fonksiyonel düzey skoru (p=0.003) açısından anlamlı iyileşme kaydedildi. İkinci grupta da WOMAC ağrı (p=0.008), WOMAC eklem sertliği (p=0.012), WOMAC fonksiyonel düzey skorları (p=0.004) ve GAS (p=0.001) skorlarında anlamlı düzelme saptandı, Üçüncü grupta hiçbir değerlendirme parametresi açısından anlamlı değişiklik gözlenmedi (p>0.05). Gruplar arasında değerlendirme parametrelerinin tedavi sonundaki yüzde değişimleri açısından GAS skoru (p<0.001), WOMAC ağrı skoru (p<0.001), WOMAC eklem sertliği skoru (p=0.013) ve WOMAC fonksiyonel düzey skoru (p<0.001) açısından fark saptandı.

Sonuç: Hem pulse elektromanyetik alan tedavisi hem töropatik ultrason tedavisi kontrol grubuna göre anlamlı olarak daha etkili bulunmuştur. Pulse elektromanyetik alan tedavisi diz osteoartriti tedavisinde etkili bir alternatif tedavi yaklaşımı olarak uygulanabilir.

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Anahtar sözcükler: Pulse elektromanyetik alan, diz, osteoartrit, ultrason

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Introduction

Osteoarthritis (OA) is the most common rheumatologic disease and commonly affects the large weight-bearing joints, such as the hips and the knees (1). Decrease in the content of aggrecan and collagen, and increase in collagenases result with the breakdown and loss of the cartilage of the effected joint (2). Degeneration and inflammation of the cartilage can stimulate new bone outgrowths to form around the joints. These degenerative changes lead to joint pain, swelling and stiffness (1).

Since no treatment can stop osteoarthritic process, the treatment of knee OA (KOA) has been focused on symptom relief and function improvement. Physical agents such as superficial and deep heat, cold, electrotherapy and exercises have been used alone or in combination for many years (3, 4). However, an opthimal therapy for the management of KOA has not been developed yet. Therapeutic US is one of the most prefered physical agent for the treatment of KOA in routine daily clinical practice although it is not supported by clinical trials and not recommended by EULAR and OARSI guidelines. It is a kind of diathermy (deap heat) delivered by high-frequency sound waves (5). It relieves pain, decreases muscle spasm, increases collagen extensibility and accelerates metabolic processes (6) by providing temperature elevations up to 4 -5 degree at depths of 8 cm and by micromassage effect (7).

Pulsed electromagnetic field (PEMF) is rarely prefered in the treatment of KOA in clinical practice. The PEMF was initially used in the early 1970s for the treatment of soft tissue injuries (8). Its most accepted effect is promoting bone and cartilage repair, particularly in case of delayed healing such as non-union fractures (8). But it has also been used in the treatment of the non-fracture musculoskeletal conditions (9- 14). The action of PEMF is based on creating small electrical fields in tissue and thereby promoting biological effects (15). Furthermore, PEMF has some advanteges such as non-contact with the skin, has few contrendications and complications, has no detectable thermal effect and it does not take time of the therapist. So it is suggested that, PEMF should be an alternative and attractive therapy choice for KOA. There are few studies investigated the effect of PEMF in KOA. The previous studies were either animal studies (2, 16) or sham controlled studies which reported positive effects (17-19). To our knowledge, the efficacy of PEMF in KOA has not been investigated by comparing it to any physical agent yet. The aim of this study is to investigate the effect of PEMF on pain intensity and functional level of the patients with KOA by comparing to that of therapeutic US therapy and to that of no ttreatment control group.

Material and Methods

Sample size

A power analysis indicated that a sample size of 45 patients would provide 80% power at an alpha level of 0.05 (effect size: 0.506).

Patient population

Fiftyfive patients, who admitted with knee pain lasted for at least 3 months were evaluated. Patients were assessed by one of the two authors by history and detailed physical examination. Laboratory tests (whole blood count, C-reactive protein, erythrocyte sedimentation rate, rheumatoid factor and routine biochemical tests) were assessed to rule out secondary OA. All patients were initially questioned for age, sex, weight and height. The diagnosis of KOA was based on the American Collage of Rheumatology criteria (20). Patients who had not responded adequately to treatment with nonsteroidal anti-inflammatory drugs, had grade II-III Kellgren-Lawrence (21) scores and had no limitation in range of motion were included to the study. Exclusion criteria were as follows: 1.secondary OA, 2. contraindications for PEMF and US therapy such as tuberculosis, pregnancy, malignancy, cardiac pacemaker or any implanted electrical device, atrophic skin or scar tissue on the knee region, bleeding disorders, insensitivity, edema and ischemia, 3. unable to understand the questionnaires. Furthermore patients were excluded from the study if they had been on pyhsical therapy programme or had recieved intraarticular injections in the previous 6 months, had undergone an operation for any knee pathology previously. Eight patients were excluded from the study. Two patients did not participate because of inconsiderable reasons so 45 patients completed the study. All participants gave written informed constant. The study was approved by the ethics commitee of the university.

Randomization

Patients were randomly allocated to three groups by sequential assignment, according to their application turns. First group (1st, 4th, ...43rd) recieved PEMF therapy, second group (2nd, 5th, ...44th) recieved US therapy, third group (3rd, 6th, ...45th) recieved no treatment. Figure 1 shows the randomization and follow-up process of the groups. Neither the assessors nor the patients were blinded.

Therapy protocols: PEMF group

PEMF therapy was applied by a magnetotherapy device (BodyMag, manufactured by Eltech S.r.l, Treviso, Italy). Both knee of the patients were put in the middle portion of the big cycle solenoid applicator in supine position. The fre-

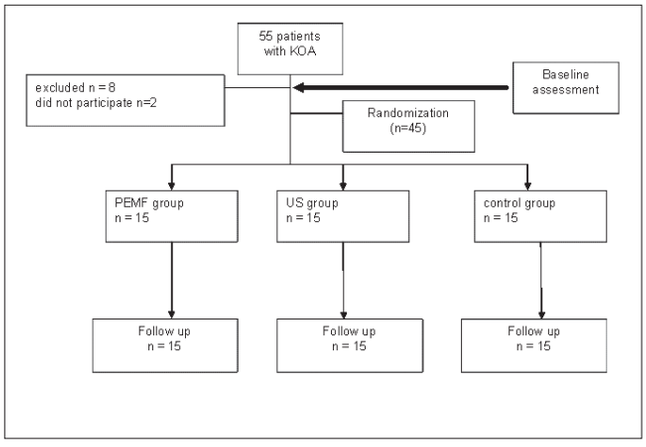

Figure 1. Flow-diagram of the patients

quency, intensity and application duration/session were selected according to the recommendations of the manufacturer. PEMF was applied in a frequency of 2 Hz, 100 Hz and 25 Hz, consecutively. Intensity variated between 2mT and 10 mT during the application. A therapy session lasted for 35 minutes and 15 sessions performed during 3 weeks (5 sessions/week).

US therapy group

The skin was coated with an acoustic gel not containing any pharmacologically active substance. US was then applied to the superomedial and lateral parts of the knee by the same therapist stroking the applicator in circular movements. The transducer head was applied to the knee at right angles to sustain maximal absorption of the ultrasound energy. Continuous ultrasonic waves with 1 MHz frequency and 1.5 watt/cm^2 power were applied with a 3 cm diameter applicator ultrasound equipment (Chattanooga, TN, USA.). US therapy lasted for 10 minutes/session.

Third group served as control. Only the third group was allowed to receive paracetamol when needed during the study.

Outcome measures

Severity of joint pain, joint stiffness and physical function levels were evaluated as outcome measures. Assessment tools were Visual Analoge Scale (VAS) (from no pain=0 to unbearable pain=10) and Western Ontario and McMaster Universities (WOMAC) questionnaire. WOMAC is a validated, disease specific and sensitive measurement of symptom related to KOA (22). It has 3 parts which measures pain, stiffness and physical function. The validity of Turkish version of WOMAC questionnaire has been well documented (23). WOMAC scores were recorded on a Likert scale of 0 -4, where 0= no pain/ limitation and 4=very severe pain/limitation. Maximum scores for stiffness, pain and physical function were 8, 20 and 68 respectively.

Statistical analysis

Statistical tests were performed by SPSS program (version 11.0). All data was expressed as mean±standard deviation or median (minimum-maximum). Demographic characteristics were compared by χ^2 test and ANOVA. Kruskal Wallis test was used in order to compare the differences between changes of scores during time among the groups. For the significant differences according to Kruskal Wallis variance analysis, Mann-Whitney U test was used to analyze which group is different from the other in terms of those parameters. Bonferroni correction was applied for all possible multiple comparisons. Differences within groups were analyzed by Wilcoxon signed rank test.

Results

All of the patients had bilateral KOA. The right knee was evaluated in all patients. 45 patients (63.5±10.2 yrs of age) completed the study. Demographic characteristics are presented in Table 1. No complication has been noted in group I and II. There were no significant differences with respect to age, gender, body mass index, and Kellgren-Lawrence scores among groups. All outcome parameters improved significantly within group I and group II. No change was observed in any outcome measure within group III (Table 2). There were differences among groups regarding the percent change of VAS scores ($p<0.001$), WOMAC pain scores ($p<0.001$), WOMAC joint stiffness scores ($p=0.013$) and WOMAC functional level scores ($p<0.001$) after the treatments (Table 3). There were no differences between first and second group regarding the improvements in VAS, WOMAC pain, joint stiffness and functional level scores (Table 3).

Discussion

In the present study, PEMF therapy has been found to be effective in reducing pain and stiffness and improving functional level in KOA.

There are a few studies which investigated the effectiveness of PEMF in KOA. Benefical effects of PEMF have been presented by either clinical studies (17-19) or animal experiments (2, 16) but its efficacy has not been compared to that of another physcial agent yet. Jacobson et al. (19) found 46% improvement in pain reduction in a

Table 1. The demographic properties and baseline parameters of the patients (mean±SD)

		Group I (PEMF) n=15	Group II (US) n=15	Group III (control) n=15	P
Age (years)		65.8±10.3	63.1±13.6	62.0±6.0	0.656
Gender	Female	n=10	n=13	n=12	0.400
	Male	n=5	n=2	n=3	
Weight (kg)		71.6±8.2	68.5±16.0	68.5±5.1	0.462
Height(cm)		165.0±8.7	162.5±6.8	161.7±3.3	0.755
BMI (kg/m^2)		0.43±0.01	0.42±0.1	0.42±0.02	0.987
Kellgren-Lawrence score	Grade 2	n=7 (47%)	n=7 (47%)	n=10 (67%)	0.397
	Grade 3	n=8 (53%)	n=8 (53%)	n=5 (33%)	

PEMF: Pulsed electromagnetic field, VAS visual analog scale, WOMAC: Western Ontario Macmaster Questionnaire, SD: Standard deviat'on

Table 2. The scores of outcome parameters (median) (minimum-maximum) and changes within and between groups

	Group I (PEMF) (n=15)			Group II (US) (n=15)			Group III (control) (n=15)			P**
	Pre-treatment	Post-treatment	P*	Pre-treatment	Post-treatment	P*	Pre-treatment	Post-treatment	P*	
VAS	5 (2-10)	3 (0-6)	0.005	7 (5-10)	2 (0-6)	0.001	7 (4-9)	5 (2-10)	0.344	<0.0001
WOMAC-pain	7 (2-16)	4 (0-8)	0.001	9.5 (1-17)	4.5 (0-11)	0.008	7 (5-9)	8 (5-9)	0.059	<0.0001
WOMAC-stiffness	4 (0-7)	1 (0-5)	0.027	3.5 (2-11)	2 (0-7)	0.012	4 (2-7)	3 (2-6)	0.609	0.013
WOMAC-functional level	27 (6-42)	16 (0-26)	0.003	31 (6-41)	11.5 (0-26)	0.004	25 (17-35)	24 (18-30)	0.675	<0.0001

PEMF: Pulsed electromagnetic field, VAS visual analog scale, WOMAC: Western Ontario Macmaster Questionnaire, *within groups, **between groups

Table 3. P values representing the differences among groups

		VAS-percent change	WOMAC-pain-percent change	WOMAC-stiffness-percent change	WOMAC-functional level-percent change
Group I (PEMF)	Group II (US)	0.183	0.979	0.536	0.244
	Group III (control)	0.008	<0.000	0.005	0.001
Group II (US)	Group III (control)	<0.000	0.001	0.003	0.001

VAS: visual analog scale, WOMAC: Western Ontario Macmaster Questionnaire, *among groups

similar sample size of the patients with KOA. Nicolacis et al. (18) also used WOMAC, and reported that PEMF reduced pain and improved daily living activities. Fischer et al. (15) and Thamsborg et al. (17) reported similar results on pain reduction even for the long-term. The results of the present study are similar to those of recent studies. However the frequency, duration of each session and total number of sessions are different from each other among these studies.

The PEMF has been shown to increase upregulation of gene expression for aggrecan, type II collagen synthesis (24, 25) and TGFβ (26-28). TGFβ stimulates the aggrecan and collagen synthesis, suppresses the pro-enzyme forms of collagenase and interleukin-1 (29), which may result with pain reduction. The opthimal frequency, intensity and duration required for the completion of these biological effects and for total recovery in human tissues, are unknown. Recent studies have suggested that PEMF activates cellular signaling process rapidly within few minutes (30, 31) and signaling is largely blunted after 30 minutes. So, 35 minutes/session may be sufficient for this process. Further histopathological analysis are needed to find out the opthimal dosage and duration.

In the present study, the improvements in pain relief, joint stiffness and functional level in the PEMF group have not been found superior to those of the US therapy group. Our US application form has been recommended for the restricted movements (32) and pain relief (33). Improvement in stiffness level of PEMF group should be due to enhanced blood circulation in the periarticular compartment. PEMF has been shown to activate synthesis of nitric oxide (34) which may enhance blood flow. Further studies should analyze long-term results in severe OA with restricted movements.

The effect of US therapy on pain relief in KOA has been documented by several studies (35-37). The US therapy has been compared to other physical agents such as shortwave diathermy, galvanic or interferancial current (36, 38). According to a meta-analysis, most of them have been reported that therapeutic US is effective but not superior to other physical agents (37). In the present study, PEMF has been compared to US therapy and similar results for pain relief have been found. There are several limitations of these recent studies such as heterogenous groups, untrastfull validty and reliability of the outcome measures, or lack of a control group. Only one study compared therapeutic US to that of placebo in KOA (38). US therapy has been found effective on pain and stiffness level but they have found no difference compared to sham (37). Similar to the others (35, 36), dosages were unclear in their study, too.

In the present study, both US and PEMF have been found effective compared to control group. The control group did not recieve sham either for US or PEMF. So, the placebo effect of both therapies should not be taken into consideration in the present study.

In conclusion, both therapy approaches were considered effective and PEMF should be used in higher rates in routine clinical practice as an alternative therapy method. For a more definitive answer on the use of PEMF in KOA, larger randomized studies are needed.

Conflict of Interest
No conflict of interest is declared by authors.

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Study protocol

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Pulsed electromagnetic fields after arthroscopic treatment for osteochondral defects of the talus: double-blind randomized controlled multicenter trial

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Abstract

Background: Osteochondral talar defects usually affect athletic patients. The primary surgical treatment consists of arthroscopic debridement and microfracturing. Although this is mostly successful, early sport resumption is difficult to achieve, and it can take up to one year to obtain clinical improvement. Pulsed electromagnetic fields (PEMFs) may be effective for talar defects after arthroscopic treatment by promoting tissue healing, suppressing inflammation, and relieving pain. We hypothesize that PEMF-treatment compared to sham-treatment after arthroscopy will lead to earlier resumption of sports, and aim at 25% increase in patients that resume sports.

Methods/Design: A prospective, double-blind, randomized, placebo-controlled trial (RCT) will be conducted in five centers throughout the Netherlands and Belgium. 68 patients will be randomized to either active PEMF-treatment or sham-treatment for 60 days, four hours daily. They will be followed-up for one year. The combined primary outcome measures are (a) the percentage of patients that resume and maintain sports, and (b) the time to resumption of sports, defined by the Ankle Activity Score. Secondary outcome measures include resumption of work, subjective and objective scoring systems (American Orthopaedic Foot and Ankle Society – Ankle-Hindfoot Scale, Foot Ankle Outcome Score, Numeric Rating Scales of pain and satisfaction, EuroQol-5D), and computed tomography. Time to resumption of sports will be analyzed using Kaplan-Meier curves and log-rank tests.

Discussion: This trial will provide level-1 evidence on the effectiveness of PEMFs in the management of osteochondral ankle lesions after arthroscopy.

Trial registration: Netherlands Trial Register (NTR1636)

Background

Osteochondral defects (ODs) of the talus often have a severe impact on the quality of life of the patients. The patients are usually young and athletic; most are male (62%) in the third decade of their lives after a traumatic ankle sprain [1]. The primary treatment of a symptomatic OD consists of arthroscopic debridement and microfracturing [2]. This treatment yields 87% good or excellent results [3]. However, it can take up to one year to obtain improvement of clinical symptoms. Moreover, it is a great challenge to achieve early resumption of sports, which is the main goal of many of these young patients. In a series published in 2007, 26 "high-demand" athletic patients with an OD returned to sports at a mean of 15 weeks after debridement and microfracturing [4]. If we could shorten this period, we would considerably improve the quality of life in these active patients.

A potential solution to obtain this goal is the application of pulsed electromagnetic fields (PEMFs). Bassett in the 1960s and 1970s introduced and improved the clinical use of this treatment modality [5,6]. Since then, PEMFs have been applied increasingly, including their use in the treatment of osteoarthritis and (non-united) fractures [7,8]. They are designed as a portable PEMF generator, which consists of electromagnetic fields with an on-off effect of pulsing. This produces athermal effects that suppress inflammation, promote tissue healing, and relieve pain [9]. In vitro and in vivo studies have shown that PEMFs act as adenosine A2a agonists, leading to an increase of Transforming Growth Factor β-1, thereby improving bone development, reducing cartilage damage and increasing chondrocyte proliferation [10-21]. These results clearly indicate improved regeneration of bone and possibly cartilage in a scientific setting.

Clinically, its favorable effects are less obvious. PEMF as a solitary treatment for osteoarthritis of the knee has been repeatedly investigated, with conflicting results [7,22-25]. Although the effect of PEMFs on osteoarthritis of the knee seems equivocal, their value in the additional treatment of other bony and cartilaginous pathologies is promising. PEMFs have been proven as a successful method in fracture healing, especially in the case of non-union [8,26,27]. PEMF-treatment also favors the recovery of patients after arthroscopic treatment of chondral lesions in the knee, and reduces the use of non-steroidal anti-inflammatory drugs [28]. To our knowledge, sport resumption with the use of PEMFs has not been investigated. Based on the above data, we believe that PEMFs may act on ODs by improving bone regeneration and suppressing inflammation evoked by surgery.

When the above results are combined, it seems justified to state that additional PEMF-treatment may contribute to the management of ODs. Our study question is: "Does treatment with PEMFs compared to sham device lead to earlier resumption of sports in a higher percentage of patients with an osteochondral defect of the talus after arthroscopic debridement and microfracturing?".

Methods/Design

Study design and informed consent

The study is designed as a double-blind, randomized, placebo controlled, multicenter trial, which is in accordance with the Declaration of Helsinki [29]. The methodology will follow CONSORT (Consolidation of Standards of Reporting Trials) guidelines [30,31]. Five centers in the Netherlands and Belgium will participate. Approval has been obtained from the local Medical Ethics Committees in the participating centers (MEC 08/236). Written informed consent for participation in the study will be obtained from all patients at study entry. An information letter notifying the patients' participation will be sent to their general practitioners.

Randomization

The participants will be randomized to receive either active PEMF-treatment or sham device, stratified for participating center, body mass index (≤/> 25 kg/m^2) [32,33], and diameter of the defect on computed tomography (CT) (≤/> 10 mm) [1]. Randomization will be performed in randomly allocated blocks of two or four patients using ALEA, a validated web-based computer program [34]. The provider of the PEMF-devices (IGEAmedical, Carpi, Italy) will supply an equal number of active and sham devices identified by code numbers which correspond to the randomization program. Treatment allocation will be managed by an independent, unblinded research assistant (IS), who will not be involved in patient care or assessment. Patients and treating physicians as well as medical assessors will be blinded to the allocation of treatment. The code numbers will not be broken until all patients have completed the study.

Inclusion criteria

- Patients with a symptomatic OD of the talus who are scheduled for arthroscopic debridement and microfracture [2]
- OD diameter < 15 mm on CT (in three dimensions: medial-lateral, anterior-posterior and superior-inferior)
- Ankle Activity Score (AAS) ≥ 4 before symptoms (Table 1) [35]
- Age 18 years or older

Exclusion criteria

- Concomitant OD of the tibia

Table 1: Ankle Activity Score by Halasi et al. [35]

		Ankle Activity Score[a]		
Category	Sports and Activities	T	C	R
10	American football	10	9	8
	Basketball	10	9	8
	Gymnastics	10	9	8
	Handball	10	9	8
	Rugby	10	9	8
	Soccer	10	9	8
9	Hockey	9	8	7
	Korfball	9	8	7
	Martial arts: judo, karate, kung fu, taekwondo	9	8	7
	Orienteering	9	8	7
	Rhythmic gymnastics	9	8	7
	Volleyball	9	8	7
8	Boxing	8	7	6
	Freestyle snowboarding	8	7	6
	Ice hockey	8	7	6
	Tennis	8	7	6
	Wrestling	8	7	6
7	Aerobics, fitness	7	6	5
	Badminton	7	6	5
	Baseball	7	6	5
	Cross-country running (running on uneven ground)	7	6	5
	Modern pentathlon	7	6	5
	Squash	7	6	5
	Surfing, windsurfing	7	6	5
	Table tennis	7	6	5
	Track and field: field events	7	6	5
	Water skiing	7	6	5
6	Dancing	6	5	4
	Fencing	6	5	4
	Floorball	6	5	4
	Mountain and hill climbing	6	5	4
	Nordic skiing	6	5	4
	Parachuting	6	5	4
	Softball	6	5	4
	Special professions and working activities[b]	6		
5	Diving	5	5	4
	Scuba diving	5	5	4
	Skating, in-line skating	5	5	4
	Track and field: track events (running on even ground)	5	5	4
	Triathlon	5	5	4
	Weightlifting, body building	5	5	4
	All competitive sports of categories 4 and 3 with seasonal conditioning	5		
	Heavy physical work	5		
4	Alpine skiing and snowboarding	4	4	4
	Bowling/curling	4	4	4
	Golf	4	4	4
	Mountain biking/bmx	4	4	4
	Power lifting	4	4	4
	Sailing	4	4	4
	Physical work	4		
3	Cycling	3	3	3
	Equestrian	3	3	3
	Motorsports, technical sports	3	3	3
	Rowing, kayaking	3	3	3
	Shooting, archery	3	3	3
	Water polo and swimming	3	3	3
	Able to walk on any uneven ground	3		

Table 1: Ankle Activity Score by Halasi et al. [35] *(Continued)*

2	No sports, everyday activities not limited	2
1	Able to walk on even ground, but everyday activities limited	1
0	Unable to walk, disabled because of ankle problems	0

[a]T, top level (international elite, professional, national team, or first division); C, lower competitive levels; R, recreational level (participation should be considered only if it exceeds 50 hours per year).
[b]Special professions include ballet dancer, professional soldier, special rescue worker, stuntperson, and so forth.
If multiple options are applicable, the highest level is chosen.

- Ankle osteoarthritis grade 2 or 3 [36]
- Ankle fracture < 6 months before scheduled arthroscopy
- Surgical treatment of the index ankle performed < 1 year before scheduled arthroscopy
- Concomitant painful or disabling disease of the lower limb
- Rheumatoid arthritis
- Pregnancy
- Implanted pacemaker
- Participation in concurrent trials
- Participation in previous trials < 1 year, in which the subject has been exposed to radiation (radiographs or CT)
- Patients who are unable to fill out questionnaires and cannot have them filled out
- No informed consent

Device description

PEMFs are applied using a portable generator attached to the ankle (Figure 1). The coil in the active treatment device generates a peak magnetic field intensity of 1.5 mT, supplied by an electric pulse frequency of 75 Hz [37]. The sham devices do not differ from active devices in shape, color, weight, and in acoustic or visual signaling. Neither the active nor the sham device produces noise or sensation and they are entirely indistinguishable. The only difference is the generated magnetic field; the sham device produces a negligible peak of less than 0.05 mT, supplied by the minimal current necessary to power the device indicators.

Standard treatment and investigational treatment

All surgical procedures will be performed using a standardized technique [2]. Briefly, the ankle joint is approached by arthroscopy using an anterior or posterior approach. The OD is identified with a probe and debrided with a curette and bonecutter shaver. All unstable bone and cartilage are removed. After full debridement, the subchondral bone is perforated with a microfracture awl, with intervals of approximately 3 mm. At the end of the procedure a pressure bandage is applied.

After surgery the protocol-based rehabilitation program, guided by a physiotherapist, will be equal in both groups. It will be initiated with partial (eggshell) weight bearing on crutches, as tolerated, and progressed to full weight bearing over a period of six weeks. During this period active non-weight-bearing and partial weight-bearing sagittal range of motion exercises are encouraged, i.e., 15 minutes twice daily. After this six week' period, resumption of sports will be permitted as tolerated, and will not be directed by the clinician.

In both groups the investigational treatment (active PEMF-treatment or sham device treatment) will start within three days after surgery. It will be applied four hours daily (in one or two sessions) for a period of 60 days [37,38]. The patients' compliance will be monitored by a clock inside the device that records the hours of stimulation.

The prescription of nonsteroidal anti-inflammatory drugs will be avoided due to their negative effect on bone regeneration [39]. The use of paracetamol will be allowed up to a maximum dose of 4 g/d and will be discontinued one week before the visits at baseline, 1 month, 2 months, 6 months and 1 year.

Outcome measures

The combined primary outcome measures are:

(a) the number of patients that resume and maintain sports during 12 months follow-up, and

(b) the time to resumption of sports, defined by the AAS.

Secondary outcome measures are:

- time to resumption of work,

- American Orthopaedic Foot and Ankle Society – Ankle-Hindfoot Scale (AOFAS-AHS),

- Foot and Ankle Outcome Score (FAOS),

- quality of life (EuroQol-5D),

- pain (Numeric Rating Scale),

- satisfaction (Numeric Rating Scale),

- computed tomography, and

- adverse events.

Definitions

Primary outcome measures

Because there is no consensus as to defining what actual resumption of sport is – as Saxena and Eakin stated [4] – we define time to resumption of sports as the time after arthroscopy (weeks) until initiation of any sport with a minimum level of the pre-symptoms level minus 1 point on AAS, and maintained for at least 30 days. If a patient's activity level decreases to below the minimum level within 30 days after sport resumption, the resumption date will not be counted. To evaluate the level of sport activity, we will use the AAS that has been developed and validated by Halasi and associates [35]. This 10-point score is based on the type and level of sport or work, with 0 points indicating the lowest activity and 10 points indicating the highest activity (Table 1).

Secondary outcome measures

Resumption of work is defined as the ability to perform normal work exercises without any deficits in work quality [40]. The AOFAS-AHS is a frequently used combined objective-subjective 100-point scale which devotes 40 points to pain, 50 points to function, and 10 points to alignment [41]. The subjective part was recently validated [42]. The FAOS is a subjective 42-item questionnaire assessing five subscales: pain, other symptoms, activities of daily living, sports, and quality of life. All items are scored on a Likert-scale, and each of the five subscales is transformed to a score of 0 (worst) to 100 (best). The original English version has been validated [43], and the Dutch translation is currently being validated in our institution. The EuroQol (EQ-5D) is a validated and extensively used general health questionnaire to measure quality of life [44,45]. It comprises five dimensions: mobility, self-care, usual activities, pain/discomfort and anxiety/depression. Each dimension is marked as either no problems, some problems, or severe problems, which results in a 1-digit number expressing the level selected for that dimension. The digits for five dimensions are combined in a 5-digit number describing the respondent's health state. The numeric rating scale (NRS) for pain consists of an 11-point scale (0 – 10) which represents the whole spectrum of no pain up to the worst pain imaginable [46]. Pain at rest and pain when running will be measured. Patients' satisfaction will be measured using a NRS where 0 indicates no satisfaction and 10 indicates maximally possible satisfaction.

Figure 1
The application of pulsed electromagnetic fields on the ankle, generated in the green coil and attached with the elastic band (I-ONE, IGEAmedical, Carpi, Italy).

To objectively assess bone repair we will obtain multislice helical CT-scans of the affected ankles at baseline and one year after surgery (Figure 2). CT-scanning has been proven to be accurate in the detection and follow-up of ODs of the talus, regarding location and extent as well as healing of the defect [47,48]. The scanning protocol will involve "ultra high resolution" axial slices with an increment of

0.3 mm and a thickness of 0.6 mm, and multi-planar coronal and sagittal reconstructions of 1 mm [49]. The scans will be analyzed twice by a single physician, blinded to both treatment allocation and clinical outcome, measuring completeness, thickness, and level of the subchondral plate (i.e., flush, depressed, or proud) [50]. Additionally, bone volume filling of the defect after one year will be measured, and graded as good (67% to 100%), moderate (34% to 66%), or poor (0% to 33%) [51].

Adverse events

Any (serious) adverse event during the trial period will be recorded. Adverse events are defined as any undesirable experience occurring to a subject during a clinical trial, whether or not considered related to the investigational treatment, e.g. infection, numbness, or paraesthesia. A serious adverse event (SAE) is any undesirable experience associated with the use of the investigational treatment that results in death, is life threatening (at the time of the event), requires hospitalization or prolongation of existing inpatients' hospitalization, or results in persistent or clinically relevant disability or incapacity. All SAEs will be reported to the central Medical Ethics Committee according to their requirements. Patients suffering from a SAE will stop their PEMF- or sham-treatment.

Data collection

For each randomized patient a specially designed digital Case Report Form (CRF) will be completed. The CRF consists of a sequential set of instructions with provision for data recording. Internet-based remote data capture will be used for entering, managing and validating data from the investigative sites. For this purpose Oracle Clinical will be used, a program designed to meet industry regulations, including FDA 21CFR Part 11 Rule (March 20, 1997), ICH; Good Clinical Practice: Consolidated Guideline (May 9, 1997) and FDA Guidance for Industry "Computerized Systems Used In Clinical Trials" (May 10, 1999).

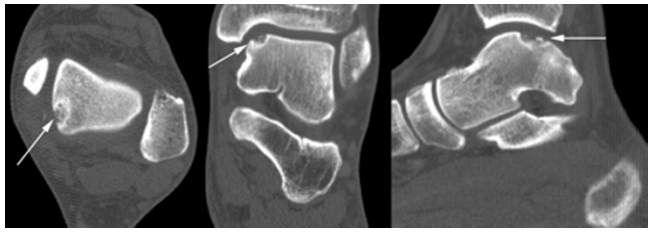

Figure 2
Preoperative computed tomography (axial, coronal, and sagittal slices) of the left ankle of a 25-year-old female showing a typical osteochondral defect located on the posteromedial talar dome (arrows).

All randomized patients are identified by a Patient Identification Number (PIN) in combination with a center number. Trial personnel will not pass names outside the local hospitals. The investigator will ensure that patients' anonymity is maintained. On CRFs or other documents submitted to the coordinating center, patients will not be identified by their names but by a PIN in combination with a center number. The subject identification code list will be safeguarded by the investigator.

Data acquisition and follow-up

Participating patients will be assessed at the following time points (Table 2):

1. *Preoperatively*: information letter, informed consent, baseline characteristics (age, gender, weight, height, affected side, duration of symptoms, past medical history, smoking status), type of sport and profession, AAS (2×: before symptoms and at preoperative assessment), AOFAS-AHS, FAOS, EQ-5D, NRS pain (2×: at rest and when running), CT: size, localization and classification of the OD (Table 3) [52]

2. *1–2 weeks postoperatively*: check compliance, (S)AEs, wound inspection (healing, signs of infection)

3. *1 month postoperatively*: check compliance, (S)AEs, resumption of work, EQ-5D, NRS pain (at rest) and satisfaction

4. *2 months postoperatively*: check compliance, (S)AEs, resumption of sport and work, AAS, AOFAS-AHS, FAOS, EQ-5D, NRS pain (at rest and when running, if applicable) and satisfaction, wound inspection, stop PEMF- or sham-treatment

5. *6 months postoperatively*: resumption and maintenance of sport and work, AAS, EQ-5D, NRS pain (at rest and when running)

6. *1 year postoperatively*: resumption and maintenance of sport and work, AAS, AOFAS-AHS, FAOS, EQ-5D, NRS pain (at rest and when running) and satisfaction, (S)AEs, CT: subchondral plate and bone volume filling

Recording sport resumption

To assess the resumption of sports and work, the patients will keep a diary that will be supplied at inclusion. Every time they perform sports they will record the type of sport and activity level (i.e., professional, competitive, or recreational) in this diary. They will also record the resumption of work, as defined above. This diary will be used for the monitoring of resumption and maintenance of sports and activity levels. At the postoperative visits the patients will

Table 2: Patient assessment.

	Physician									Patient			
	Baseline characteristics*	Sport resumption	Work resumption	AAS	AOFAS-AHS	CT	Wound inspection	Compliance	Adverse events	FAOS	EQ-5D	NRS pain	NRS satisfaction
Preoperative	X			X	X	X				X	X	X	
1–2 weeks							X	X	X				
1 month			X					X	X		X	X	X
2 months		X	X	X	X		X	X	X	X	X	X	X
6 months		X	X	X							X	X	
1 year		X	X	X	X	X			X	X	X	X	X

* Baseline characteristics include age, gender, weight, height, affected side, duration of symptoms, type of sport and profession, past medical history, smoking status, and size, localization and classification of osteochondral defect on computed tomography.
AAS = Ankle Activity Score; AOFAS-AHS = American Orthopaedic Foot and Ankle Society – Ankle-Hindfoot Scale; CT = Computed Tomography; FAOS = Foot and Ankle Outcome Score; EQ-5D = EuroQol questionnaire; NRS = Numeric Rating Scale.

be asked to check their diaries and report their sport activities and work resumption, to be filled out on the CRF. At one year the diary will be collected for assessment and confirmation of resumption dates.

Sample size

Our sample size calculation is based on the combined primary endpoints (a) number of patients that resume and maintain sports during 12 months follow-up, and (b) the time to resumption of sport. Based on our experience it is expected that 50% of patients will resume and maintain sports within one year after the surgical intervention. Offering additional PEMF-treatment, we aim to improve this outcome to 75%. Of the patients who resume to sport the mean time to return to sports after debridement and microfracturing is 15 (standard deviation, 4 weeks) [4]. We consider a 20% reduction in time to return to sports as clinically relevant, i.e., 3 weeks. A sample size of 30 patients in each group (60 patients in total) will have 80% power to detect a joint difference (control group proportion of 0.50 versus treatment proportion of 0.75; control group mean of 15 weeks versus treatment group mean of 12, assuming a common standard deviation of 4), using a Fisher's combination test with a 0.05 two-sided significance level. In reported clinical trials with this device 9% to 13% of included patients dropped out [28,38]. Therefore, 34 patients will be included in each treatment group (68 patients in total).

Table 3: Computed tomography classification of osteochondral defects of the talus [52].

Grade	Description
I	Compression
II	Partially fractured but undisplaced
III	Completely fractured but undisplaced
IV	Displaced fracture
V	Radiolucent (fibrous) defect

Statistical methods

The following baseline characteristics will be summarized using descriptive statistics: number of patients, gender, age, affected side, duration of symptoms (months), prior ankle surgery, body mass index (kg/m^2), trauma, smoking, size of lesion (mm), classification, duration of PEMF- or sham-treatment (hours), AAS, NRS pain, FAOS, AOFAS-AHS, and EQ-5D. Continuous data will be presented as mean and standard deviation if normally distributed, or as median and range in case of skewed distribution.

The main analysis of this trial consists of a comparison between the treatment groups of the primary outcomes: number of patients who resume and maintain sports and the time to resumption of sport (of the patients who resume sport). The number of patients who resume sport will be analyzed using the two group X^2 test, whereas the difference in mean weeks to sport resumption will be ana-

lyzed by a two group Student's *t*-test. Both *p*-values will be combined using the Fisher's combination test. Kaplan-Meier survival curves and the log-rank test will also be used for comparing time to resumption of sport. The stratification variables will be included in the primary analysis.

The repeated datastructure of the secondary outcomes (AAS, AOFAS-AHS, FAOS, EQ-5D, NRS-pain, and NRS-satisfaction) will be analyzed with linear mixed models, including a time-treatment interaction effect. The number of adverse events and time to resumption of work will be analyzed using a X^2 test or log-rank test, when appropriate. Analyses will be based on the intention-to-treat principle and performed in SPSS. Statistical uncertainty will be quantified via 95% confidence intervals.

Participating centers and inclusion time

Centers that will participate and their estimated annual inclusions are:

1. Academic Medical Center, Amsterdam, the Netherlands (Prof. Dr. C.N. van Dijk and Dr. G.M.M.J. Kerkhoffs): 24 patients

2. Erasmus MC, University Medical Center Rotterdam, the Netherlands (Dr. D.E. Meuffels and Dr. R. Heijboer): 20 patients

3. Stedelijk Ziekenhuis Roeselare, Belgium (Dr. P.R.N. d'Hooghe): 8 patients

4. Diaconessenhuis, Leiden, the Netherlands (Dr. R. Krips): 8 patients

5. Algemeen Ziekenhuis Sint Lucas, Brugge, Belgium (Dr. G. van Damme): 8 patients

It will take an estimated year to include 68 patients. With one year follow-up, this trial will take an expected two years to be performed.

Quality assurance

A clinical research associate from our Clinical Research Unit will monitor the trial. All centers will be monitored twice: after the fourth included patient after two months follow-up and after the last patient's last visit. Monitoring will consist of 100% check informed consent procedure, registration of adverse events, completeness of the trial master file, and verification of source data (primary outcome in 10% sample).

Public disclosure and publication policy

This trial has been registered in the Netherlands Trial Register (NTR1636). Publication will be in accordance with the basic principles of the International Committee of Medical Journal Editors on publication policy [53]. The writing committee will consist of the following people: C.J.A. van Bergen, L. Blankevoort, R.J. de Haan, and C.N. van Dijk. All principal investigators at the participating centers will have the opportunity to scientifically contribute to the manuscript, and, if so, will be listed as an author. If they do not wish to contribute to the manuscript, they will be acknowledged in the order of the number of participants randomized. Other individuals who make substantial contributions to the trial will be acknowledged at the discretion of the writing committee.

Discussion

This paper describes the rationale and study protocol for conducting a double-blind, randomized controlled trial on the effectiveness of PEMF in the rehabilitation of ankle arthroscopy for ODs of the talus.

The primary outcome measure focuses on sport resumption. This is a difficult measure – as several authors wrote previously [4,54,55] – since a univocal definition does not exist. By clearly defining sport resumption, we aim at providing evidence of any relevant differences between active and passive PEMF-treatment. Moreover, if our definition shows to be useful in the present study, it can be used for the design of future trials.

Regarding the treatment of ODs of the talus, we consider bone regeneration more important than cartilage regeneration. Cartilage is not innervated; the patient's pain probably arises from the bony lesion [56]. Additionally, a differentiation can be made between ODs localized in the ankle joint and those localized in the knee joint. Most studies concerning ODs of the knee focus on cartilage repair rather than bone repair [57,58]. This seems reasonable since the knee joint is less congruent and the ODs are usually localized in high-load-bearing areas. Moreover, the knee joint is more susceptible to osteo-arthritis than the ankle joint [59,60]. The ankle joint, however, has different biomechanical properties. The joint is more congruent and talar articular cartilage is thinner than distal femoral cartilage [61]. The load-bearing contact surface of the ankle joint is somewhat larger [62-64], and the OD is often smaller. Hence, the remaining intact surface of the talar dome is usually sufficiently large to bear the loads; contact surface pressures do not significantly change with talar defects up to 15 mm in diameter [65]. Combining these properties, we believe treatment of ODs in the ankle joint should primarily aim at repair of the subchondral bone, and secondarily at coverage by fibrocartilaginous tissue. In this respect, PEMF-treatment may be particularly suitable for ODs of the talus since its bone-healing capacity has been proven [8,26,27,66].

This trial will contribute to the knowledge of the effectiveness of PEMF, and may improve health care of patients

with an OD. Given the modality's relatively simple technology and ease of use, it has high potential to provide a safe and effective additional treatment option for ODs of the talus.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

All authors were involved in the design of the trial. CvB, under supervision of LB and NvD, was responsible for writing this paper, and will act as trial coordinator. RdH was closely involved in the design of the trial and preparation of the manuscript, in particular of the statistical sections. IS assisted in the design of the trial, including the chosen outcome measures. DM, PdH, RK, GvD, and NvD will perform the arthroscopic procedures and will participate in patient inclusion and assessment. All authors have read the manuscript, provided comments on the drafts, and approved the final manuscript.

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Pulsed Magnetic Field Therapy in Refractory Neuropathic Pain Secondary to Peripheral Neuropathy: Electrodiagnostic Parameters—Pilot Study

Michael I. Weintraub and Steven P. Cole

Context. *Neuropathic pain (NP) from peripheral neuropathy (PN) arises from ectopic firing of unmyelinated C-fibers with accumulation of sodium and calcium channels. Because pulsed electromagnetic fields (PEMF) safely induce extremely low frequency (ELF) quasirectangular currents that can depolarize, repolarize, and hyperpolarize neurons, it was hypothesized that directing this energy into the sole of one foot could potentially modulate neuropathic pain.* Objective. *To determine if 9 consecutive 1-h treatments in physician's office (excluding weekends) of a pulsed signal therapy can reduce NP scores in refractory feet with PN.* Design/setting/patients. *24 consecutive patients with refractory and symptomatic PN from diabetes, chronic inflammatory demyelinating polyneuropathy (CIDP), pernicious anemia, mercury poisoning, paraneoplastic syndrome, tarsal tunnel, and idiopathic sensory neuropathy were enrolled in this nonplacebo pilot study. The most symptomatic foot received therapy. Primary endpoints were comparison of VAS scores at the end of 9 days and the end of 30 days follow-up compared to baseline pain scores. Additionally, Patients' Global Impression of Change (PGIC) questionnaire was tabulated describing response to treatment. Subgroup analysis of nerve conduction scores, quantified sensory testing (QST), and serial examination changes were also tabulated. Subgroup classification of pain (Serlin) was utilized to determine if there were disproportionate responses.* Intervention. *Noninvasive pulsed signal therapy generates a unidirectional quasirectangular waveform with strength about 20 gauss and a frequency about 30 Hz into the soles of the feet for 9 consecutive 1-h treatments (excluding weekends). The most symptomatic foot of each patient was treated.* Results. *All 24 feet completed 9 days of treatment. 15/24 completed follow-up (62%) with mean pain scores decreasing 21% from baseline to end of treatment (*P = *0.19) but with 49% reduction of pain scores from baseline to end of follow-up (*P < *0.01). Of this group, self-reported PGIC was improved 67% (n = 10) and no change was 33% (n = 5). An intent-to-treat analysis based on all 24 feet demonstrated a 19% reduction in pain scores from baseline to end of treatment (*P = *0.10) and a 37% decrease from baseline to end of follow-up (*P < *0.01). Subgroup analysis revealed 5 patients with mild pain with nonsignificant reduction at end of follow-up. Of the 19 feet with moderate to severe pain, there was a 28% reduction from baseline to end of treatment (*P < *0.05) and a 39% decrease from baseline to end of follow-up (*P < *0.01). Benefit was better in those patients with axonal changes and advanced CPT baseline scores. The clinical examination did not change. There were no adverse events or safety issues.* Conclusions. *These pilot data demonstrate that directing PEMF to refractory feet can provide unexpected short-term analgesic effects in more than 50% of individuals. The role of placebo is not known and was not tested. The precise mechanism is unclear yet suggests that severe and advanced cases are more magnetically sensitive. Future studies are needed with randomized placebo-controlled design and longer treatment periods.*

Key Words: *Neuropathic pain—Pulsed magnetic field therapy—Peripheral neuropathy.*

Symptomatic peripheral neuropathy is often a painful and progressively disabling condition that traditionally is refractory to treatment.[1] Complex mechanisms and etiologies exist that adversely influence both myelinated and unmyelinated fibers leading to symptomatology. From a pathophysiological standpoint, neuropathic pain is believed secondary to ectopic firing of nociceptive afferent unmyelinated C-fiber axons that are undergoing degeneration.[2] Microneurography has demonstrated that ectopic depolarization is caused by dysregulated expression of sodium and calcium channels.[3,4] Pharmacotherapy is the cornerstone

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approach in the management of neuropathic pain yet currently there are no specific treatments that reverse or arrest progressive peripheral neuropathy.[1] Thus, the search for reliable and new therapeutic strategies is appealing. Because substantial evidence exists that pulsed electromagnetic fields (PEMF) safely induce small electrical eddy currents within the body that can depolarize, repolarize, and hyperpolarize neurons, it was hypothesized that this energy directed to the soles of one foot could potentially influence neuropathic pain scores.[5-12]

STUDY DESIGN

Twenty-four consecutive patients with feet symptomatic from peripheral neuropathy due to diabetes, pernicious anemia, chronic inflammatory demyelinating polyneuropathy (CIDP), mercury poisoning, tarsal tunnel, paraneoplastic syndrome, and idiopathic sensory neuropathy were enrolled in this study between July and November 2002 and met the following criteria: a) neuropathic symptoms of numbness, tingling, burning or pain on a daily basis; b) failure to standard therapies, that is, tricyclics, analgesics, antiepileptics, opioids, acupuncture, neurotrophic vitamins, and so on; and c) ability to keep visual analog scores (VAS) of pain for the duration of the study. Adjectives and numbers appeared on the form for the patients to correlate their pain intensity. Patients were excluded who had mechanical implanted devices or who were pregnant. The most symptomatic foot in each patient received therapy and was studied.

PRIMARY OUTCOME MEASURES

Primary outcome measures were VAS (0-10) scores tabulated daily through the treatment period and also with follow-up scores within 15 days. This would be compared to 1 week of baseline pretreatment scores (VAS). Additionally, at the end of the treatment period, patients would respond to a standardized Patient's Global Impression of Change (PGIC)[13] questionnaire with 7 options describing their response to treatment. Subgroup analysis (VAS scores, nerve conduction velocities [NCV] changes, current perception thresholds [CPT] scores), based on severity and response, would be compared. Secondary endpoints were examination changes.

Baseline electrophysiological tests, that is, NCV, attempted to quantify the severity of the neuropathy and depict if this was axonal or demyelinating. Forced-choice quantified sensory testing (QST) measured by neurometer were performed at baseline to determine the degree of dysfunction. This is a portable, constant sine wave stimulator applied through surface electrodes at 3 frequencies (5 Hz, 250 Hz, and 2000 Hz), and a forced-choice method is used to determine the minimum amplitude of detection. The CPTs are measured with units equivalent to 0.01 mAmperes (mA). The scores were generated as CPTs from 0-10.

This is an open, nonplacebo study with protocol approved by the Phelps Hospital Investigational Review Board (IRB). After a complete description of the study, written informed consent was obtained prior to enrollment. No new analgesics were allowed; however, patients could remain on their current regimens.

DEVICE

Pulsed signal therapy (PST), a variant of PEMF, has been previously described.[14-16] The device generates a pure magnetic field output signal that employs direct current with unidirectional biological frequencies below 30 Hz. The wave form is quasirectangular with measured field strengths generally below 2 mT or 20 gauss. The system is controlled through a pulsed unidirectional magnetic DC field with multiple output frequencies implemented via a free-wheeling diode to optimize the induction characteristics. Various frequency/ amplitude combinations are switched over automatically and are transmitted under continuous control during the treatment period. Induction of treatment takes place during the first 10 min followed by a combination of pulsed signals that deliver the therapy over the remaining 50 min. A 1-h duty cycle timecard is inserted, which starts the induction and subsequent treatment process. This is noiseless and nonthermal. The most symptomatic foot is placed comfortably inside a closed circuit coil for 1 h on 9 consecutive days, excluding weekends (Saturday/Sunday). A time card is inserted, which starts the 10-min induction process followed by 50 min of treatment. The quasirectangular waveforms have a frequency below 30 Hz and a strength below 20 gauss (2 mT). Various patented frequency/ amplitude combinations are automatically sequenced.

MASKING

The investigator (MIW) was not blinded. All patients were informed that this was an open-label trial of active magnetic stimulation. There were no placebo controls. Participants came to the office of MIW for the above treatments.

STATISTICAL ANALYSIS

One-way repeated measures analyses of variance (ANOVA) was used to assess changes in pain scores over the course of the study at baseline, end of treatment, and at end of follow-up. Reductions in pain scores from baseline to end of treatment and from baseline to end of follow-up were tested with a priori contrasts. An intent-to-treat ANOVA was conducted in which the last recorded pain score during treatment was substituted for missing follow-up scores for the patients who completed treatment but did not complete follow-up. For all tests, a p value of 0.05 or less was considered to indicate statistical significance. The Statistical Package of the Social Sciences (Ver. 10.0) was used to analyze the data (SPSS, Inc., 233 South Wacker Drive, Chicago, IL 60606).

FUNDING

There was no funding for this study. Two PST portable devices with duty time cards were provided on loan by Bio Magnetic Therapy Systems, Inc. (Boca Raton, FL). The authors had complete independence regarding study design, data analysis, and manuscript preparation.

DEMOGRAPHIC VARIABLES

All of the 24 feet that were enrolled in the study completed treatment. Of these 24 feet, 15 completed follow-up. Of the 10 female feet and 5 male feet that completed treatment and follow-up, ages ranged from 41 to 85 (M = 67.32, SD = 13.44) and duration of symptoms ranged from 1.33 to 15 years (M = 6.41, SD = 3.78). Etiology of peripheral neuropathy was tabulated to be diabetes mellitus (6), pernicious anemia (2), hypothyroidism (2), tarsal tunnel (3), mercury poisoning (1), prostate cancer (1), hemochromatosis (1), CIDP (2), and idiopathic sensory neuropathy (6).

Nerve conduction studies were performed on 19 patients of which axonal changes (#11) were noted in 58% and demyelinating changes (#8) were noted in 42%. CPT was performed in 11 cases; 5 had advanced scores (9-10), 3 had severe scores (7-8.99), and 3 had mild scores (0-6).

RESULTS

All 24 feet completed 9 days of treatment. However, 9 feet (38%) completed treatment but did not complete follow-up. For the 24 feet enrolled in this study, patient ages ranged from 41 to 85 (M = 67.29 ± 12.43) and duration of symptoms range from 1.33 to 15 years (M = 6.32 ± 3.50).

A repeated measures analysis of variance (baseline, end of treatment, end of follow-up) based on the 15 feet that completed treatment and follow-up demonstrated a statistically significant reduction in pain scores, $F(2,28) = 7.25$, $p < 0.01$, eta-squared = 0.34. Mean pain scores decreased 21% from baseline (6.47 ± 2.64) to end of treatment (5.13 ± 2.59), $p = 0.19$ and decreased 49% from baseline to end of follow-up (3.33 ± 1.78), $p < 0.01$. Self-reported change in overall pain (PGIC) from baseline to end of treatment was collected from patients for the 15 feet. Improvement from baseline was reported for 10 (67%) feet, and no change was reported for 5 (33%) feet.

An intent-to-treat analysis (baseline, end of treatment, end of follow-up) based on all 24 feet demonstrated a statistically significant reduction in pain scores, $F(2,26) = 7.26$, $p < 0.01$, eta-squared = 0.24. Mean pain scores decreased 19% from baseline (6.26 ± 2.44) to end of treatment (5.08 ± 2.57), $p = 0.10$, and decreased 37% from baseline to end of follow-up (3.96 ± 2.27), $p < 0.01$.

Following the above primary analyses, patients were grouped according to their baseline ratings of pain. There were 5 patients (Serlin classification) who reported ratings of 4 or less, which corresponded to mild pain. The remaining 19 patients had baseline scores of 5 to 6 (moderate pain) or 7 to 10 (severe pain). Of the 19 feet with moderate or severe pain that completed treatment, 11 completed treatment but did not complete follow-up. An intent-to-treat analysis (baseline, end of treatment, end of follow-up) based on all 19 feet demonstrated a statistically significant reduction in pain scores, $F(2,18) = 15.83$, $p < 0.01$, eta-squared = 0.47. Mean pain scores decreased 28% from baseline (7.21 ± 1.69) to end of treatment (5.21 ± 2.37),

$p < 0.05$, and decreased 39% from baseline to end of follow-up (4.37 ± 2.29), $p < 0.01$. For the 5 patients with mild pain, there was an 83% increase in mean pain scores from baseline (3.01 ± 03) to end of treatment (5.50 ± 3.32) and a 9% decrease of pain scores at end of follow-up (2.75 ± 1.50). None of the changes for the mild cohort was statistically significant.

SAFETY

There were no adverse events reported.

DISCUSSION

During the past 2 decades, enormous progress has been made in studying the role of magnetic energy on biological systems. Time-varying magnetic fields have been successfully applied to stimulate nerve regeneration in vitro and in vivo.[17-19] Neurite outgrowth has been demonstrated in cell cultures exposed to EMF[8,17-19] and also optical electromagnetic energy.[20] Time-varying weak PEMF of low frequency (3 Hz-3K Hz) had been used in orthopedics and sports medicine, rheumatology, and so on. There appears to be a specific encoding of different tissues to signal amplitude and frequency spectrum. Thus, the rational development of directing time-varying magnetic fields to the sole of the foot is a logical step in attempting to modulate the peripheral ectopic firing afferent C-nociceptors. From an anatomical standpoint, the primary afferent neurons (unmyelinated C-fibers and small A-delta nociceptors) are located in the epidermis and dermis and therefore are easily influenced by cutaneous application of PEMF. Ectopic firing C-fibers with accumulation of sodium channels in area of injury appear to be the principal cause for acroparesthesiae and neuropathic pain.[2-4,21,22] We speculate that the observed antinociceptive effects may be explained by either repolarization or hyperpolarization induced by ELF despite the fact that the specific magnetic flux density at the target area is not known.[23] It is also plausible that the pain reduction in VAS scores is secondary to placebo effect. The results cannot be generalized until a randomized, double-blind placebo-controlled trial is performed. The rationale for PEMF is based on the recognition that injured tissue loses quasirectangular energy and that since time-varying magnetic fields induce small electric currents, it potentially can restore this energy deficit. The waveforms generated are also quasi-rectangular, biphasic, and asymmetric and have a strength of 20 gauss (2mT) or below and a frequency at or about 30 Hz. Irrespective of the precise mechanisms, direct or indirect (electrical or magnetic therapy), interruption and suppression of the afferent signal traffic of the C-fiber's firing pattern is modulated producing an antinociceptive effect. The relative contribution of electrical versus magnetic energy cannot be clarified. Because the intracellular signaling pathway is influenced short term in more than 50% of cases for a 9-day period, the optimum duration and magnitude of energy directed to the soles needs to be considered in a dose/responsive manner.

Despite severe neuropathic pain symptoms, peripheral nerve retains the capacity for recovery of function as long as the nerve cell body remains viable.[28] Although shortcomings of this study include absent placebo controls and biological markers, the fact that more than 50% improvement in a refractory condition occurred is provocative with only 9 treatments. It is noteworthy that when patients were stratified according to severity (VAS, NCV, CPT), those feet that were moderate-severely symptomatic were more magnetically susceptible compared with mild symptomatology. This disproportionate response has been previously noted in several other pharmacological and magnetic studies, suggesting that a moderate amount of neuronal dysfunction must be present to get analgesic benefit.[23,25-27] This observation also provides novel insights about the neuronal circuitry and suggests that a pathophysiological link may exist for differential therapeutic strategies.

In conclusion, the pilot data are provocative for their short-term antinociceptive reduction of neuropathic pain. Precise mechanisms of interaction do not resolve for the role of placebo. Future trials are required with a randomized, double-blind placebo-controlled design utilizing larger cohorts, more prolonged stimulation time, that is, 2-3 months, and so on. This will determine if this modality will be useful in the clinical settings.

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Clinical Note

TREATMENT WITH ELECTROMAGNETIC FIELDS REVERSES THE LONG-TERM CLINICAL COURSE OF A PATIENT WITH CHRONIC PROGRESSIVE MULTIPLE SCLEROSIS

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It is estimated that 10–20% of patients with multiple sclerosis (MS) have a chronic progressive (CP) course characterized by an insidious onset of neurological deficits followed by steady progression of disability in the absence of symptomatic remission. To date no therapeutic modality has proven effective in reversing the clinical course of CP MS although there are indications that prolonged treatment with picotesla electromagnetic fields (EMFs) alters the clinical course of patients with CP MS. A 40 year-old woman presented in December of 1992 with CP MS with symptoms of spastic paraplegia, loss of trunk control, marked weakness of the upper limbs with loss of fine and gross motor hand functions, severe fatigue, cognitive deficits, mental depression, and autonomic dysfunction with neurogenic bladder and bowel incontinence. Her symptoms began at the age of 18 with weakness of the right leg and fatigue with long distance walking and over the ensuing years she experienced steady deterioration of functions. In 1985 she became wheelchair dependent and it was anticipated that within 1–2 years she would become functionally quadriplegic. In December of 1992 she began experimental treatment with EMFs. While receiving regularly weekly transcortical treatments with AC pulsed EMFs in the picotesla range intensity she experienced during the first year improvement in mental functions, return of strength in the upper extremities, and recovery of trunk control. During the second year she experienced the return of more hip functions and recovery of motor functions began in her legs. For the first time in years she can now initiate dorsiflexion of her ankles and actively extend her knees voluntarily. Over the past year she started to show signs of redevelopment of reciprocal gait. Presently, with enough function restored in her legs, she is learning to walk with a walker and is able to stand unassisted and maintain her balance for a few minutes. She also regained about 80% of functions in the upper limbs and hands. Most remarkably, there was no further progression of the disease during the 4 years course of magnetic therapy. This patient's clinical recovery cannot be explained on the basis of a spontaneous remission. It is suggested that pulsed applications of picotesla EMFs affect the neurobiological and immunological mechanisms underlying the pathogenesis of CP MS.

Keywords: Multiple sclerosis; electromagnetic fields; chronic progressive multiple sclerosis; clinical course

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It is estimated that 10–20% of multiple sclerosis (MS) patients have a chronic progressive (CP) course (Smith & Scheinberg, 1985; Whitaker & Benveniste, 1990). The CP course is characterized by an insidious onset of slowly progressive disability in which remissions do not occur (Kraft et al., 1977; Smith & Scheinberg, 1985). CP course tends to have a later age of onset (Noseworthy et al., 1983) and typically first presents with motor disturbances involving gait and limb weakness (Minderhoud et al., 1988). CP MS is associated also with a higher incidence of cognitive deficits (Heaton et al., 1985; Beatty et al., 1989; Franklin et al., 1989). In patients with CP MS, onset of the disease before age 35 years and the occurrence of symptoms primarily of a sensory nature are associated with a more favorable prognosis (Smith & Scheinberg, 1985). On the other hand, early onset of motor signs including weakness, spasticity, or cerebellar symptoms is associated with a poorer prognosis. Pyramidal or cerebellar signs present within 5 years of the disease onset are felt to be a strong indicator of subsequent poor prognosis which appears slightly worse for men than for women (Leibowitz et al., 1969; Kurtzke et al., 1977; Smith & Scheinberg, 1985). Presently, no pharmacologic treatment modality has demonstrated long term beneficial effects on the core symptoms of MS and there is no available specific therapeutic modality effective for CP MS (Bauer, 1978; Hughes, 1991; Rudick et al., 1992). In addition, no therapeutic modality has shown long term beneficial effect in reversing the course of the disease in patients with CP MS (Giesser, 1985; Ellison et al., 1994). Transcortical application of picotesla range electromagnetic fields (EMFs) is a safe and highly effective modality for the symptomatic treatment of MS (Sandyk, 1992; Sandyk & Derpapas, 1993; Sandyk & Iacono, 1993; Sandyk & Dann, 1994; 1995; Sandyk, 1995 *a*; *b*; Sandyk, 1996; Sandyk, in press *a*) and is presently the only modality which has demonstrated symptomatic improvement and reversal of the clinical course of the disease in patients diagnosed with CP MS.

CASE REPORT

This 40 year old woman pharmacist presented in December of 1992 with a history of CP MS with resultant spastic paraplegia, loss of trunk control, profound weakness of the upper extremities with loss of hand functions, neurogenic bladder, loss of bowel control with incontinence, impaired cognitive functions and mental depression. Additional symptoms included intermittent difficulties with breathing, chronic fatigue, extreme sensitivity to heat and mental stress and swelling with rubor of the legs, feet and hands. Her initial symptoms emerged at age 18 when she began to experience weakness of the right leg and fatigue with long distance walking. Over the ensuing years she gradually developed increasing

disability with spastic paraparesis, ataxia of gait, weakness in the upper limbs, and loss of bladder and bowel control. An MRI scan obtained in 1987 revealed multiple subcortical and upper spinal demyelinating lesions. Pattern Reversal VEP studies obtained in April of 1993 showed prolonged P100 latencies bilaterally, greater on the left than on the right (117 ms and 115 ms, respectively) suggesting delayed conduction in the prechiasmal segments of the central visual pathways. Since 1985 she has been confined to a wheelchair. She had very limited functions of her arms and hands being unable to cut her food, comb or wash her hair or dress herself. Upon stress or increasing fatigue her hands would claw. Being unable to move and shift her body position during sleep was a major distressing symptom. Her prognosis was considered extremely unfavorable and it was anticipated that within 1–2 years she would become quadriplegic and require full time care.

In December of 1992 the patient began experimental treatment with transcortical applications of picotesla intensity EMFs. Since then she has received weekly treatments which are administered in a quiet and artificially illuminated room that is magnetically unshielded. EMFs are administered extracranially via an array of 24 coils embedded in a plate which, during treatment, is placed over the patient's cranial vertex. The EMFs are administered using the Sandyk Electromagnetic Stimulator^{SM,TM} which emits an AC pulsed EMF of 7.5 picotesla flux density. A session comprises two successive treatments each of 25 minutes duration separated by an interval of 15 minutes. A 4.2 Hz sinusoidal wave is employed in the first treatment and a 4.2 Hz trapezoidal wave is administered in the second application. During the first year the patient reported improvement in mental functions particularly in mood, memory and concentration. "I am in control of my emotions and I don't cry as often as I used to." Her dream recall was restored and in the summer of 1993 she began reading books for the first time in 10 years. She also experienced improvement in motor functions predominantly increased muscle strength in the upper limbs with recovery of trunk control "enabling me to sit up straight and conduct myself as a healthy person would, rather than sitting in a slumped over position." During the second year she experienced the return of more hip functions and motor recovery began in her legs. Spasticity in the lower limbs was reduced about 50% and for the first time in years she can now initiate dorsiflexion of her ankles and actively extend her knees voluntarily. She has even started to show signs of redevelopment of reciprocal gait. A VEP study repeated in June of 1995 showed normal P100 latencies in both eyes (107 ms on the left side and 105 ms on the right side). Presently, with enough function restored in her legs, she is learning to walk with a walker and is able to stand unassisted and maintain her balance for a few minutes. She continued to regain strength in her upper extremities and is now able to cut her food again, comb and wash her hair, dress herself and cook for her family. Her bladder and bowel control have

improved considerably and with the use of laxatives she now maintains normal bowel functions. Her level of energy has improved considerably and her tolerance to stress has improved as well. Over the past year she noted return of her ability to move and shift her body position during sleep. Most importantly, however, the progressive course of her disease has been reversed since the initiation of treatment with EMFs with no clinical evidence of further progression of symptoms.

DISCUSSION

This patient's clinical recovery cannot be explained on the basis of a spontaneous remission. Since the onset of her first neurologic symptoms at the age of 18 she experienced an insidious and steady deterioration of motor, autonomic, cerebellar and cognitive functions with no remission of symptoms and was classified with CP MS. Recovery of symptoms during magnetic therapy occurred slowly initially involving predominantly mental and cognitive functions and subsequently affecting motor skills with improved strength in the upper limbs, trunk and lower extremities. As part of the recovery process she regained greater personal independence and was able to cut her food, wash and comb her hair, dress herself and prepare meals for her family. She regained sufficient balance to stand unassisted and over the past year was working with her physical therapist on ambulating with a walker. Over the past 4 years the patient experienced no further deterioration of symptoms indicating that during this period the progressive course of the disease has been reversed by treatment with EMFs.

The pathogenesis of CP MS and specifically the neurobiological and immunological mechanisms underlying the relentless progression of symptoms in this group of MS patients remain unknown (Weiner & Hafler, 1988). Specifically, it is not known whether these patients represent a subcategory of disease related to different biological or immunological mechanisms or whether they might, in fact, have had subclinical attacks. Schuller et al. (1973) suggested that in the early active stage of MS the lesions result directly from infection but that in the latter, often progressive, stages, damage to the nervous system results from an autoimmune process directed against myelin tissue. Minderhoud et al. (1988) suggested that relapsing-remitting MS shows some characteristics of an autoimmune process, but the cause of CP MS remains unclear. Biochemically, CP MS is associated with a more extensive loss of serotonin (5-HT) neurons indicated by the findings of significantly lower CSF 5-HIAA levels in CP patients compared to relapsing-remitting patients (Sonninen et al., 1973). CSF 5-HIAA levels were also lower in bedridden patients compared to controls and ambulatory MS patients. These neurochemical changes are relevant to the pathogenesis and

symptomatology of MS since 5-HT mechanisms have a key role in spinal and supraspinal regulation of motor control as well as integration of sensory, autonomic, cognitive, affective and endocrine functions (Barasi & Roberts, 1973; Baumgarten & Lachenmayer, 1985; Jacobs, 1991; 1994; Jacobs & Fornal, 1993; Sirvio et al., 1994; Wallis, 1994). In addition, 5-HT is known to play an important role in the modulation of the immune system and the integrity of the blood brain barrier (BBB) (O'Brien et al., 1962; Jankovic et al., 1970; Essman, 1978; Westergaard, 1980; Hall & Goldstein, 1981; Devoino et al., 1987; Sharma & Dey, 1986 *a*; *b*). It has been suggested that the BBB is a target of the pathological process in MS and disruption of the permeability of the BBB is thought to be a critical factor in the pathogenesis and progression of the disease (Broman, 1964; Berger & Sheremata, 1983; Koopmans et al., 1989; Poser, 1986; 1992). Loss of 5-HT mediated control of BBB permeability may allow continued leakage of inflammatory cells into the CNS causing destruction of the adjacent tissues with resultant progression of symptoms. The pineal gland also influences the integrity of the BBB through the 5-HT system (Jankovic et al., 1970; Sandyk, in press *b*) and may have a critical role in the pathogenesis of MS and its clinical course. In fact, pinealectomy enhances the development of experimental allergic encephalomyelitis (EAE), a T-cell mediated autoimmune disease, which is widely considered as an animal model of MS (Jankovic et al., 1970).

The mechanisms by which chronic pulsed applications of picotesla EMFs induced recovery of this patient's neurologic deficits and reversal of her clinical course remain unknown. In experimental animals pulsed application of EMFs was reported to enhance neurite outgrowth and regeneration of peripheral nerves, transected spinal cord, and optic nerve (Sisken et al., 1989; Rusovan et al., 1992; Borgens et al., 1981; Politis et al., 1988). Stimulation has been reported within a board range of frequencies and field amplitudes (Rusovan et al., 1992). It has been demonstrated that cerebral neuronal activity is associated with the generation of picotesla EMFs (Cohen, 1972; Okada et al., 1987; Kyuhou & Okada, 1993) suggesting that these fields reflect a mode of neuronal communication. Low-frequency EMFs may affect neuronal functions by changing transmembrane signal transduction processes (Luben, 1991). A model proposed by Adey (1981) implicated a role for Ca^{++} in 'membrane amplification' as a possible basis for susceptibility of central neurons to weak magnetic fields. EMFs may affect the release of synaptic transmission, the level of amino acid neurotransmitters and the concentrations of cAMP by changing the permeability of Ca^{++} and K^{+} channels (Kaczmarek & Adey, 1974; Bawin & Adey, 1976; Blackman, 1988; Rudolph et al., 1988; Zecca et al., 1991; Trabulsi et al., 1996). The modulation of intracellular Ca^{++} concentrations by EMFs may trigger long lasting amplification or depression of synaptic efficiency and neuronal membrane excitability (Gareri et al., 1995; Trabulsi et al., 1996). Pulsed EMF

have also been shown to affect the secretion of several hormones including pineal melatonin (Stoupel et al., 1983; Welker et al., 1983; Wilson et al., 1986; 1989; 1992; Semm, 1992) which affects neurotransmitter functions (Anton-Tay et al., 1968; Sugden, 1983; Erlich & Apuzzo, 1985; Miguez et al., 1994), neuronal excitability (Bindoni & Rizzo, 1965; Roldan & Anton-Tay, 1968; Nir et al., 1969;Pazo, 1979; Albertson et al., 1981; Zeise & Semm, 1985), myelin production and nerve regeneration (Relkin & Schneck, 1975; Relkin et al., 1973) and neuroimmunomodulation (Angeli et al., 1988; Pierpaoli & Maestroni, 1988; Maestroni, 1993). Melatonin secretion is diminished in patients with MS during periods of exacerbation of symptoms (Sandyk & Awerbuch, 1992; 1993), a factor which may promote immunodysregulation and ongoing breakdown of the integrity of the BBB. Intermittent, pulsed applications of picotesla EMFs is thought to boost circadian melatonin secretion, increase 5-HT transmission, stabilize neuronal excitability, diminish T-cell mediated inflammatory responses, and promote nerve regeneration all of which may contribute to symptomatic improvement and inhibition of the progression of the disease.

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The Scientist http://the-scientist.com/2011/09/01/puncturing-the-myth September 2011 » Thought Experiment

Puncturing the Myth

Purinergic signaling, not mystical energy, may explain how acupuncture works.

By Geoffrey Burnstock | **September 1, 2011**
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According to traditional Chinese medical theory, acupuncture points are situated on meridians along which *qi*, the vital energy, flows. However, I have proposed a less mysterious neurophysiological mechanism to explain the beneficial effects of this 2,000-year-old practice (*Medical Hypotheses,* 73:470-72, 2009). In particular, my hypothesis is based on the surprising finding that a hitherto unknown extracellular signalling system exists between cells, including nerve cells.

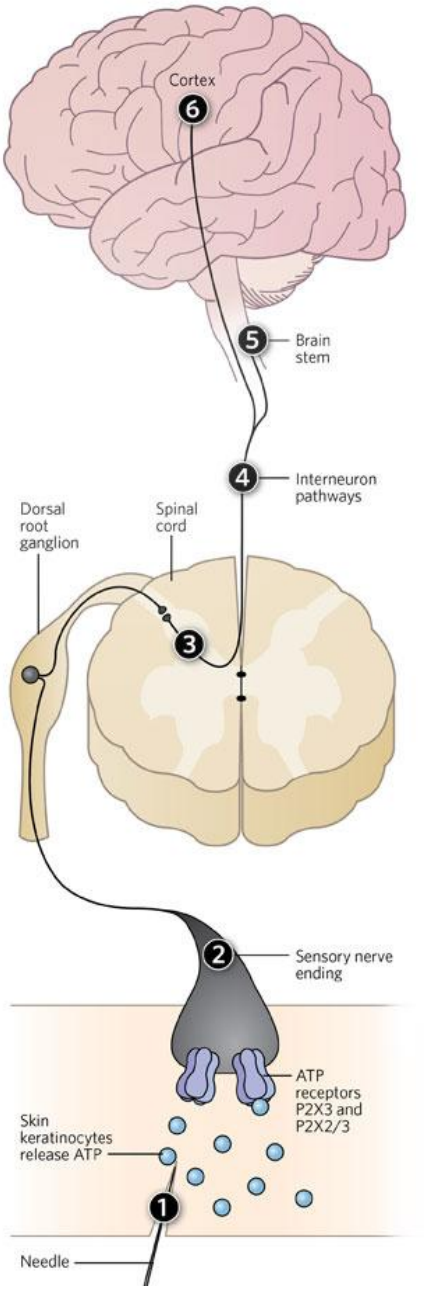
Adenosine 5'-triphosphate (ATP) is well established as an intracellular energy source that powers biochemical processes. In 1972 I proposed that ATP has another biochemical role: it acts as an extracellular signalling molecule between cells. The messages carried by ATP are received on the surface of cells by specific receptors, which I termed purinoceptors, because ATP belongs to a group of chemicals known as purines. Six years later, two families of purinoceptors were identified—P1 receptors for adenosine, the breakdown product of ATP, and P2 receptors for ATP. The purinergic signaling concept was rejected by many for two decades. It wasn't until the early 1990s, when the chemical and molecular structure of the plasma membrane receptors for ATP was characterized and other downstream members of this primitive signalling pathway were identified, that the concept of purinergic signalling between cells became widely accepted, and it is now a rapidly expanding field of physiological and pathophysiological study.

Two intriguing hints prompted me to consider that inserting and twisting a needle might release ATP from the skin and form the physiological basis for the effects of acupuncture: 1) Initially it was thought that the ATP acting as an extracellular signalling molecule was merely a by-product released when cells were damaged or dying. 2) A paper published 34 years ago reported that ATP injected into the human skin stimulated sensory neurons (*Pain*, 3:367-77, 1977).

It is now clear that ATP can be released from many cell types (e.g., osteoblasts and endothelial, epithelial, and glial cells) in response to gentle mechanical stimulation that does not damage the cells. ATP is also released in response to heat and electrical currents—techniques used today in conjunction with acupuncture to enhance its effect. Recent evidence has also confirmed the 1977 finding that sensory nerve terminals in the skin are activated by ATP. In this way, messages can be relayed from the skin via interneurons in the spinal cord to the brain stem. Furthermore, the well-established reduction of pain by acupuncture may be explained by the possibility that the binding of ATP to purinoceptors on sensory nerve endings in the skin activates a signaling pathway which ultimately modulates pain perception in the brain's cortex. Acupuncture's inhibition of pain may also involve the release of endorphins.

The ATP-activated sensory nerves also lead to modulation of the activity of brain-stem neurons controlling autonomic nervous system functions of gut, lung, urogenital, and cardiovascular systems—all of which have been treatment targets for traditional acupuncture procedures. There is published evidence for the release of ATP from keratinocytes, the major cell type in the skin, during mechanical stimulation. Similarly, ATP is released from urothelial cells lining the bladder and

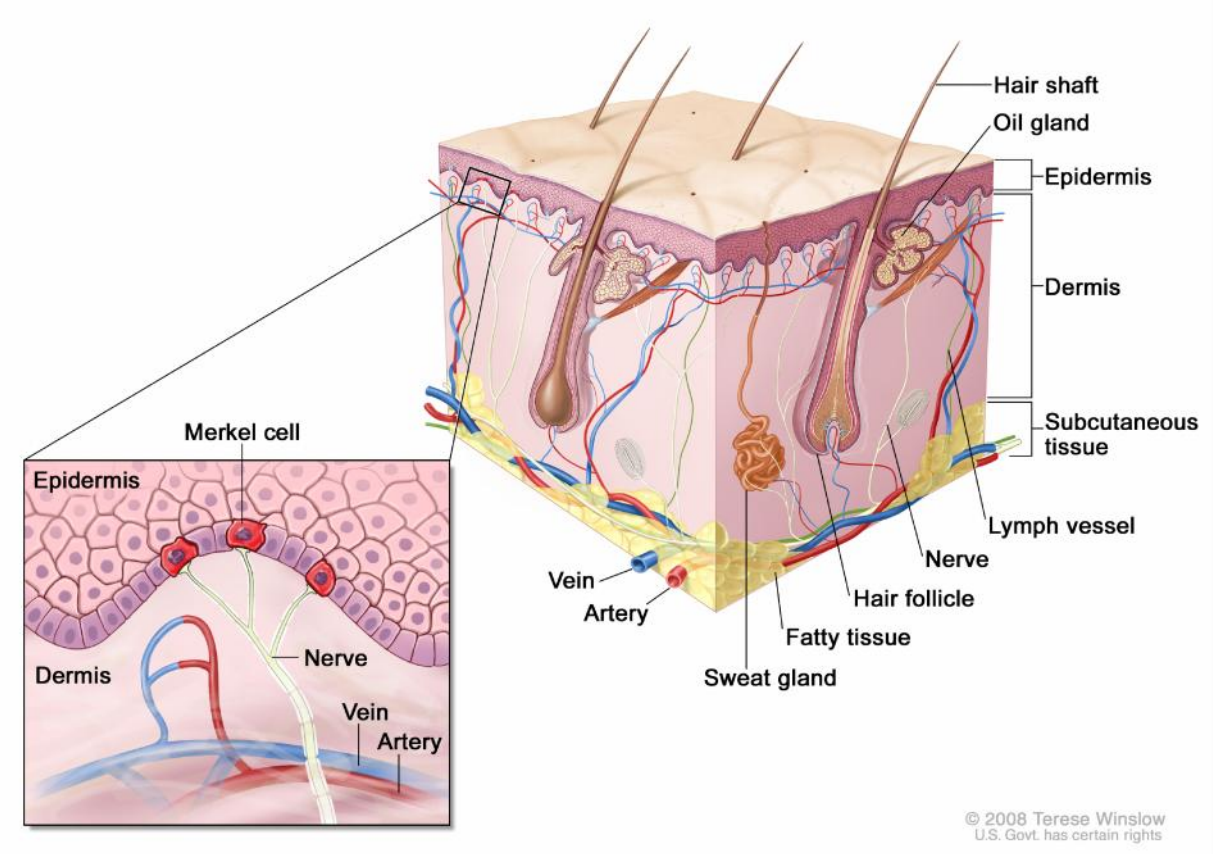
ureter in response to stretch, and receptors for ATP are present on suburothelial sensory nerves, ready to relay messages to the pain centers in the central nervous system. In addition, release of ATP in response to mechanical stimulation (changes in blood flow) from endothelial cells that line blood vessels leads to vasodilatation. And further, ATP is released from epithelial cells lining the airways in response to stretch, leading to activation of ATP receptors on sensory nerves, in turn resulting in the activation of reflexes that protect the lung against hyperventilation. Immunohistochemical studies have shown that the specific ATP receptor subtypes, P2X3 and P2X2/3, are located on sensory nerve endings in the skin. The same subtypes are also especially abundant in the tongue, another site where acupuncture needles are placed. An isolated preparation of tongue tissue showed that the increased electrical activity in lingual general sensory nerves in response to mechanical stimulation could be mimicked by injecting ATP into the preparation and blocked by injecting antagonists to the P2X3 receptor subtype. The cell bodies of the sensory nerve endings that supply the skin are located in sensory ganglia, which then connect with neurons in the dorsal spinal cord. A series of interneurons then mediate modulatory pathways to the brain stem and hypothalamus, which are the nervous control centers for the activities of visceral organs. (See illustration.)



ACUPUNCTURE AND PURINERGIC SIGNALING

Insertion and twisting of the needles employed in acupuncture mechanically deforms the skin, leading to the release of ATP by skin keratinocytes (1). ATP binds to specific receptors located on sensory nerve endings in the skin known as P2X3 and P2X2/3 (2). The signaling message is then relayed via dorsal root ganglia to the spinal cord (3) and subsequently through interneuronal pathways (4) to the brain stem (5) which contains motor neurons that control the functions of gut, lung, heart, arteries and Reproductive organs, all major targets for acupuncture. Signals also travel to pain centers in the cortex, delivering a message to inhibit pain (6).

Merkel cells or **Merkel-Ranvier cells**



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Merkel cells or **Merkel-Ranvier cells** are oval receptor cells found in the skin of vertebrates that have synaptic contacts with somatosensory afferents. They are associated with the sense of light touch discrimination of shapes and textures. They can turn malignant and form the skin tumor known as Merkel cell carcinoma.

There is evidence that they are derived from neural crest.[1] More recent experiments in mammals have indicated that they are in fact epithelial in origin.

Merkel cells are found in the skin and some parts of the mucosa (*stratum germinativum*) or also known as stratum basale of all vertebrates. In mammalian skin, they are clear cells found in the *stratum basale* (at the bottom of sweat duct ridges) of the epidermis approximately 10 µm in diameter. They also occur in epidermal invaginations of the plantar foot surface called rete ridges.[2] Most often, they are associated with sensory nerve endings, when they are known as Merkel nerve endings (also called a Merkel cell-neurite complex). They are associated with slowly adapting (SA1) somatosensory nerve fibers.

Friedrich S. Merkel referred to these cells as *Tastzellen* or "touch cells" but this proposed function has been controversial as it has been hard to prove. However, genetic knockout mice have recently shown that Merkel cells are essential for the specialized coding by which afferent nerves resolve fine spatial details.[3] Merkel cells are sometimes considered APUD cells because they contain dense core granules, and thus may also have a neuroendocrine function.

The origin of Merkel cells has been debated for over 20 years. Evidence from skin graft experiments in birds implies that they are neural crest derived, but experiments in mammals now demonstrate an epidermal origin.[4][5]

Med Hypotheses. 2009 73(4):470-2.
http://www.ncbi.nlm.nih.gov/pubmed/19628336

Acupuncture: a novel hypothesis for the involvement of purinergic signalling.

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Source

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Abstract

The hypothesis is summarised schematically in Fig. 1. **It is proposed that mechanical deformation of the skin by needles and application of heat or electrical current leads to release of large amounts of ATP from keratinocytes, fibroblasts and other cells in skin**; the ATP then occupies specific receptor subtypes expressed on sensory nerve endings in the skin and tongue; the sensory nerves send impulses through ganglia to the spinal cord, the brain stem, hypothalamus and higher centres; the brain stem and hypothalamus contain neurons that control autonomic functions, including cardiovascular, gastrointestinal, respiratory, urinogenital and musculo-skeletal activity. Impulses generated in sensory fibres in the skin connect with interneurons to modulate (either inhibition or facilitation) the activities of the motoneurons in the brain stem and hypothalamus to change autonomic functions; specifically activated sensory nerves, via interneurons, also inhibit the neural pathways to the pain centres in the cortex.

Med Hypotheses. 2010 75(2):162-8.

Multifunctional Merkel cells: their roles in electromagnetic reception, finger-print formation, Reiki, epigenetic inheritance and hair form.

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Source

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Abstract

Merkel cells are located in glabrous and hairy skin and in some mucosa. They are characterized by dense-core secretory granules and cytoskeletal filaments. They are attached to neighboring keratinocytes by desmosomes and contain melanosomes similar to keratinocytes. They are excitable cells in close contact with sensory nerve endings but their

function is still unclear. In this review, following roles are attributed for the first time to the Merkel cells: (1) melanosomes in Merkel cells may be involved in mammalian magnetoreception. In this model melanosome as a biological magnetite is connected by cytoskeletal filaments to mechanically gated ion channels embedded in the Merkel cell membrane. The movement of melanosome with the changing electromagnetic field may open ion channels directly producing a receptor potential that can be transmitted to brain via sensory neurons. (2) Merkel cells may be involved in finger-print formation: Merkel cells in glabrous skin are located at the base of the epidermal ridges the type of which defines the finger-print pattern. Finger-print formation starts at the 10th week of pregnancy after the arrival of Merkel cells. Keratinocyte proliferation and the buckling process observed in the basal layer of epidermis resulting in the epidermal ridges may be controlled and formed by Merkel cells. (3) Brain-Merkel cell connection is bi-directional and Merkel cells not only absorb but also radiate the electromagnetic frequencies. Hence, efferent aspects of the palmar and plantar Merkel nerve endings may form the basis of the biofield modalities such as Reiki, therapeutic touch and telekinesis. (4) Adaptive geographic variations such as skin color, craniofacial morphology and hair form result from interactions between environmental factors and epigenetic inheritance system. While environmental factors produce modifications in the body, they simultaneously induce epigenetic modifications in the oocytes and in this way adaptive changes could be passed onto the next generations.

Merkel cells are multisensorial cells that can receive almost all environmental stimuli including electromagnetic and ultraviolet radiations, temperature, humidity and food type and they seem to transfer the environmental information to oocytes by affecting nuclear receptors in oocytes. (5) Hair form is categorized as straight, wavy and spiral. Merkel cells found at the bulge region of hair follicles may determine the hair form with their different paracrine secretions related to hair cycle producing variations between populations. In conclusion, Merkel cells are multifunctional cells which may close the gap between orthodox medicine and complementary medicine such as acupuncture and Reiki.

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