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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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 10⁹ 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.

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. 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.

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. According to Dammacco, 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, who described diffuse injury to the cerebral sulci meningeal B-cell follicles entering the cerebral sulci (figure 3, 4).

Bruck 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 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 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. 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 and a view to their possible role as targets of ELF pulsed magnetic fields in the mT range.

The lymphocytes are the precursors of the antibody-producing cells; the plasmacells. A recent immunohistochemical and morphometric analysis conducted by Magliozzi et al 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.

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 dissolving 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-
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Figure 1  1) Ependymal cell; 2) neuron; 3) axon; 4) oligodendrocyte; 5) astrocyte; 6) myelin sheet; 7) microglia cell. From Consejería de Educación 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 3  SEM images of monocytes (×3200) carpeted with microvilli, lamellar villi and blebs. From R. Laschi (ed.) “Patologia Ultrastrutturale”. Editrice Compositori, Bologna 1980: 54.

† 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. Meloni, Zanichelli, Bologna 1991, 2nd edit: 399.

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ing from bone-marrow monocytes. Activated microglial cells (figure 5) 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 5B) 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. This is, however, difficult to achieve as the activation mechanisms of the microglia remain elusive. The dendritic cells (figure 6), which are responsible for presenting the antigen to the T-cells, are extremely important in MS. 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. Interactions among the various cells of the immune system take place through the release of interleukins and through direct membrane interactions. 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. 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, 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: 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 and especially beta 1-b interferon are useful in both RRMS (figure 7A) and SPMS (figure 7 B, C). Recently, the synthetic protein glatiramer acetate has proved efficacious. 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. Immunosuppressive treatments, both with antibiotics and by means of total lymphoid irradiation are also efficacious. However, being highly toxic, they are undertaken only in highly selected cases. 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, amyotrophic lateral sclerosis and multiple sclerosis. ELF magnetic fields of weak and very weak intensity can exert interesting and proven therapeutic effects on the CNS. 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 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. 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. 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
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*[^1]–[^6] some in collaboration with Derpapas[^7], Iacono[^8,^9] and Dann[^10,^11]. 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)^[7,^8] thereby enabling interactions according to Jacobson resonance^[^10] to occur between the applied field and some cerebral functions.

The duration of the applications...
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, A, and B, B) 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 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-

| A1 Very Rapid Complete Resolution | – 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  
| • pretreatment latencies of the visual and auditory evoked potentials  
| • Lhermitte’s sign  
| • acute parkinsonian syndrome  
| A2 Complete Resolution Obtained after Weeks or Months of Treatment | – in 3 pts \(^{55}\) after brief treatment courses  
| • impairment of dual-task performance (talking while walking)  
| • acute exacerbation of various MS symptoms  
| • loss of dream recall on waking  
| • alexia  
| • severe dysarthria  
| • premenstrual exacerbation of MS symptoms  
| • partial cataplexy  
| • long-standing suicidal tendency  
| • sleep-related paralysis  
| B1 Very Rapid Improvement | – in 1 pt \(^{36}\) after the first session  
| • headache and other neuralgia during acute exacerbation of MS  
| • deficit of cognitive functions  
| • various MS symptoms  
| • various MS symptoms  
| • impairment of dual-task performance (talking while walking)  
| • speech impairment  
| • intention tremor and postural tremor  
| B2 Improvement after Weeks or Months of Treatment | – in 7 cases \(^{40,62}\) slow but progressive  
| • various cognitive deficits  
| • body-image perception  
| • severe fatigue  
| • carbohydrate craving  
| • impairment of depth perception with postural instability  
| • in 5 \(^{46}\) and 2 pts \(^{56}\) after one course  
| • in 3 pts \(^{54,67}\) after a few months  
| • in 1 pt \(^{47}\) after two sessions of 20'  
| • in 1 pt \(^{47}\) after two sessions of 30'  
| • in 1 pt \(^{47}\) after two sessions of 45'  
| • in 3 pts \(^{45}\) after 4-5 sessions  
| • in 3 pts \(^{44}\) after brief sessions  
| • in 7 cases \(^{40,62}\) slow but progressive  
| • in 5 \(^{46}\) and 2 pts \(^{56}\) after one course  
| • in 3 pts \(^{54,67}\) after a few months  
| • in 1 pt \(^{47}\) after one treatment course  
| • in 1 pt \(^{47}\) after one treatment course
Observation 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. 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). As the symptoms of MS seem to be conditioned by neurotransmission deficiency, and particularly by serotonin deficiency, 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.

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, while its regulatory effect on circadian rhythms was demonstrated in 1978 by Brown et Al on experimental animals. It should also be mentioned that in 1983 in the vicinity of the hypophysis and pineal gland, a magneto-sensitive organ secreting melatonin, 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).

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

<table>
<thead>
<tr>
<th>Authors</th>
<th>Cells</th>
<th>Waveform</th>
<th>Field Strength</th>
<th>Duration</th>
<th>Effect Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marinozzi et al</td>
<td>Hep 2</td>
<td>Three waveforms (see figure 8)</td>
<td>7 mT</td>
<td>1-32 h</td>
<td>Disappearance of microvilli, filopodia and blebs; membrane has rough appearance and evident breaks (see figure 9)</td>
</tr>
<tr>
<td>Paradisi et al</td>
<td>K562</td>
<td>sinusoidal 50 Hz</td>
<td>2.5 mT</td>
<td>24-72 h</td>
<td>Disappearance of microvilli and diffuse blebbing of the membrane (see figure 10)</td>
</tr>
<tr>
<td>Santoro et al</td>
<td>Raji</td>
<td>sinusoidal 50 Hz</td>
<td>2 mT</td>
<td>24, 48, 72 h</td>
<td>Reduced membrane fluidity, disappearance of microvilli, redistribution of actin filaments in the cytoskeleton</td>
</tr>
<tr>
<td>Lisi et al</td>
<td>Raji</td>
<td>sinusoidal 50 Hz</td>
<td>1 mT</td>
<td>13-64 h</td>
<td>Disappearance of microvilli; progressive appearance of deep membrane infolding; redistribution of actin filaments in the cytoskeleton (see figure 11)</td>
</tr>
<tr>
<td>Grimaldi et al</td>
<td>Raji</td>
<td>sinusoidal 50 Hz</td>
<td>2 mT</td>
<td>9-64 h</td>
<td>Disappearance of microvilli and pseudopodia; appearance of roughness and narrow membrane infolding</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Authors</th>
<th>Cells</th>
<th>Field Strength</th>
<th>Duration</th>
<th>Effect Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chionna et al</td>
<td>Lymphocytes</td>
<td>6 mT</td>
<td>24 h</td>
<td>Round cells → irregularly elongated cells; appearance of lamellar microvilli when simultaneously exposed to apoptogenic agents</td>
</tr>
<tr>
<td></td>
<td>U937 cells promonocytes carpeted with microvilli</td>
<td>6 mT</td>
<td>24 h</td>
<td>Numerous lamellar microvilli; rearrangement of F-actin filaments</td>
</tr>
<tr>
<td>Chionna et al</td>
<td>Hep G2 polyhedral cells carpeted with short microvilli</td>
<td>6 mT</td>
<td>24 h</td>
<td>Polyhedral cells → elongated cells; short microvilli → irregular microvilli; distributed at random changes in microfilaments and microtubules</td>
</tr>
<tr>
<td>Dini et al</td>
<td>Various cell types</td>
<td>6 mT</td>
<td>24-48 h</td>
<td>Appearance of irregular lamellar microvilli; time-dependent changes in microfilaments and microtubules</td>
</tr>
<tr>
<td>Teodori et al</td>
<td>Human glioblastoma cells with long microvilli</td>
<td>8 up to 300 mT</td>
<td>300 mT</td>
<td>Loss of long villi; disappearance of surface ripples and furrows; appearance of membrane roughness and blebs</td>
</tr>
</tbody>
</table>
Indeed, a recent study conducted of a normally functioning gland, though not necessarily in the case of a hypofunctioning 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 interhemispheric transcallosal connections 56, 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 and B). 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 hypothalamic 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 Marozzi 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 82. 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 Marozzi 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 Marozzi Effect by ELF Magnetic Fields and Static Magnetic Fields

In 1982, Marozzi 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 8b) 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 Marozzi et Al (henceforth shortened to “the Marozzi effect”) was confirmed in 1993 by Paradisi et Al 86 on human K562 erythroleukaemia cells, by Santoro et Al 87 in 1997 on human lymphoid cells (Raji) and again, on the same cells, by Lisi et Al 88 in 2000 and by Grimaldi et Al 89 in 2004. The experimental conditions used by these authors 82,85,86,87 are summarised in table 2 and differed slightly from those used by Marozzi et Al in that the mag-
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Magnetic 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 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 Marinoszi’s research the blebs were present before exposure to the magnetic field and disappeared after exposure.
Santoro et Al \cite{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 \cite{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 \cite{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 \cite{88,89}, the loss of long villi from the membrane \cite{91}, the disappearance of the regular surface undulations and their substitution by membrane roughness and blebs \cite{91}, and the appearance of irregular microvilli arranged at random \cite{89} or even of lamellar microvilli \cite{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 \cite{88,89,90} known to be endowed with anisotropy of diamagnetic susceptibility \cite{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 \cite{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 \cite{88} or lamellar \cite{88,90} microvilli. This may depend on the more uniform and constant magneto-mechani-
Functional Changes in Cells Undergoing the Marinozzi Effect Due to ELF Magnetic Fields or Static Magnetic Fields

In the studies consulted, 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. and Lisi et al., 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. and Dini et al. also concluded that cell proliferation was only partially modified. According to Chionna et al., 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) but also as an apoptosis-inducing factor in another study. 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.

An important observation was made by Dini et al., 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 in line with the findings of Grimaldi et al. 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 PT, 1993; Lacy-Hulbert et al, 1998; Zhadin MN, 2001. Among these reviews, Walleczek’s 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, the inflammatory response, and the activity of NK cells in the peripheral blood. 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 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 “Ca” uptake); [H] Urdnine uptake and the consequent gene transcript levels; and [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. Non-sinusoidal magnetic fields (square or saw-tooth wave) at both 3 Hz and 50 Hz elicit an increase in [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\textsuperscript{107,108}, but also of the biological conditions of the cells exposed, mitogen-activated cells being more responsive than resting cells\textsuperscript{103,107,108,110}.

To explain this ambivalence of the effects of ELF magnetic fields on the immune system, Marino et Al\textsuperscript{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 101,104,112,113,114.

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 cytoidal, 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 militesla (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”) 11 mentioned in section 2 (macrophages and microglia cells), and also the B-cell follicles found in the meninges of the cerebral sulci 7. 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 60,65, 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 10^10 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. 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 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[81,82,86,89,90,91] and other types of cells[84,85,88,90,91], but not on microglia 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 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[12], 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[15], who used radioactive 45Ca++ 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 that areas of chronic denervation 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. 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 function 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 as the most efficacious (1 h 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 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, Vasilyev et Al, 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, 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 and depression of antibody production with fields of 20-100 mT – a study by Frahm et Al 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, 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 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 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) 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 and 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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