THE EFFECT OF MAGNETIC FIELD-EXPOSURE ON THE NERVOUS SYSTEM

BY

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Abstract

The effect of magnetic field exposure on the nervous system

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The influence of magnetic field exposure on the hippocampal-evoked potentials (PS), sciatic nerve compound action potentials (CAP), and animal behavior were studied. Two forms of magnetic field were mainly used: 1) the low intensity (15mT) low frequency (0.16Hz) so called pulsed magnetic field (PMF) and 2) repetitive trans-cranial magnetic stimulation (rTMS). The PMF induced effect was frequency dependent (tested only on CAP), and as its frequency varied from static to 0.5 Hz, respectively, its effect ranged from depression to amplification. The NMDA receptor antagonist did not block the PMF-induced amplification. However, PMF-induced epileptic field potential (EFP) and increased-spontaneous firing in the hippocampal slices were reduced by carbenoxolone (cbx), a gap junction blocker. When the absolute inhibition or facilitation of the second pulse was measured PPF and PPI were significantly increased. Expressed as a percentage of the initial pulse, there may not have been a change in the PPI or PPF. Antidromically evoked PS was increased in hippocampal slices following exposure to PMF and the PMF amplified sciatic nerve evoked CAP. Neither of the cbx, PKA and PKC inhibitors, nor the addition of free radical scavengers to the bath, had any effect on PMF-induced CAP

amplification. In addition, PMF increased short-term depression (STD) significantly. Therefore, one interpretation of the data is that PMF shortened the recovery time of sodium channels from inactivation. It was also found that TEA, a potassium channel blocker, abolished the PMF effect on STD completely and reversed it to facilitation at longer inter-stimulus intervals. Elevated $[K^+]_o$ blocked the PMF-induced CAP amplification, and potentiated the PMF effect on STD. The PMF reversed the action of TTX (10nM) and lidocaine (150μM), but not veratridine (100μM). Veratridine potentiated the effect of PMF on CAP2 inhibition but prevented the increase in CAP1. These results further showed, that PMF exposure changed the conformation of protein complexes of ion channels and transporters further modifying the ionic concentration surrounding the cell membrane and leading to long-term nervous system excitability. In behavioral experiments, PMF impaired the memory and learning processes of mice by inducing a masking effect that superseded the synaptic plasticity needed for these processes to take place. The rTMS had a similar effect at low frequencies (1 to 8 Hz) on memory and learning in mice. At a higher (15Hz) frequency, rTMS facilitated these processes when animals were tested immediately after application, but worsened when they were tested later. Furthermore, while LTP induction probability and amplitude was enhanced in the hippocampal slices obtained from the animals exposed to rTMS at high frequency, the lower frequency of rTMS exposure had the opposite effect. Magnetic field exposure as described in this dissertation, may act through identified mechanisms that change nervous system excitability, and also be utilized by the brain as an intercellular signal. The discovery of the PMF mediatory mechanism shown in the proceeding results should guide into better PMF application in research and in medicine.

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Abbreviations:

AMPA	α-amino-3-hydroxy-5-methylisoxazole-4-propionic acid, an agonist for AMPA receptor.
4-AP	4-aminopyridine – potassium channels blocker.
BL	Baseline.
CAP	Compound action potential.
CNQX	Non-NMDA receptors blocker
Cbx	Carbenoxolone – a non-specific gap junction blocker.
Comm.	Commissural fibers – fibers arriving from the contralateral hippocampus that join with the Schaeffer collaterals.
CS	Conditioning stimulus.
Dpi	Diphenylene iodonium chloride.
EPSP	Excitatory postsynaptic potential – Depolarization of postsynaptic membrane.
GABA	γ-aminobutyric acid major inhibitory neurotransmitter in the brain.
HFS	High frequency stimulation.
IPI	Inter-pulse interval.
LTP	Long-term potentiation – long term enhancement of synaptic efficiency.
LFS	Low frequency stimulation – from 1 to 3 Hz.
NMDA	N-methyl-D-aspartic acid, agonist for NMDA receptor.
NOR	Novel object recognition task.
PKA	Protein kinase A.

PKC	Protein kinase C.
PMF	Pulsed magnetic field – it has four alternating stages (rising, static, falling and off phases).
PPF	Paired pulse facilitation.
PPI	paired pulse inhibition.
PS	Population spike.
rTMS	Repetitive trans-cranial magnetic stimulation
STD	Short-term depression.
TEA	Tetraethylammonium chloride – potassium channels blocker.
TTX	Tetrodotoxin – sodium channel blocker.
VrD	Veratridine – Sodium channels blocker.

Introduction

Review of magnetic field exposure-induced effect

Although magnetic field interference is part of our lives and its influence on biological systems has been established, its mediating mechanism is still unknown. Magnetic field is naturally present, as the earth's magnetic field or as the sun-radiated magnetic field. In addition to the natural surrounding magnetic field, there are many forms of man-made magnetic fields such as house and office supplies (mobile phones, televisions, and computers), electrical power lines and therapeutic and diagnostic medical devices. The magnetic resonance scanners (MRI) expose people to radio-frequency transmitter fields, time-dependent gradient fields and static magnetic field. The repetitive trans-cranial magnetic stimulation is a tool that is used in research and therapy. In addition, there are many claims that magnets have healing powers for many conditions, pain in particular. Although, the research on magnetic field effect on the human body is remarkable, the mechanism that mediates this effect is still unknown. There are many theories attempting to explain the effect of magnetic field on the human body, yet none of them have explained the mechanism. Magnetic field exposure could vary according to its intensity, frequency, and polarity, and so can its effect. The other source of confusion is the utilization of many models in investigating its influence on biology. For example, static magnetic field would have a depressive effect on the hippocampal evoked population spikes (Wieraszko, 2000) whereas the pulsed magnetic field – at the same intensity and polarity as the static field – had amplification effect on the hippocampal evoked population spikes (Wieraszko 2004). The magnetic field effect depends on the polarity of the field. For instance, electrophysiological recording from cells of the pineal

organ of the guinea pig (Semm et al 1980) showed that the number of spikes was depressed by static magnetic field exposure and then restored when the field was inverted. The static magnetic field was also dependent on the type of cells (Semm et al 1980) since not all cells responded to static magnetic field exposure.

The forms of magnetic field utilized in research could be classified as: static magnetic field (SMF), pulsed magnetic field (PMF), extremely low frequency electromagnetic field (ELFEMF) and high frequency electromagnetic field (HFEF). Static magnetic field could be produced by permanent magnets or by electrical coil. SMF exposure could be applied at wide range of intensities that ranges from nano to few Tesla. SMF can be generated by direct current (DC) only. PMF could be produced by experimentally interrupting the static magnetic field at a specific frequency. In that case, the field would be rectangular with four phases: rising, static, falling, and off (see the material and method section figure 2.2 and 2.3). Repetitive trans-cranial magnetic stimulation (rTMS) is a form of pulsed magnetic field that utilizes a high intensity current that generates a magnetic field of up to 3 Tesla. ELFEMF is a magnetic field that is produced by alternating electrical current (AC) and it is usually at frequencies below 100 Hz. HFEF is a magnetic field produced by mobile phones, and refers to frequencies in the range of 100 Hz – GHz. However, this classification is very simplified and not exhaustive.

Static magnetic field

Probably the most established and the least controversial finding in the area of bio-electro magnetic research, is the use of the earth’s magnetic field by many animals in their homing and navigation (Wiltschko W., 1972; Mouritsen et al 2004; see a review by

Jonhsen and Lohmann 2005). There are many ingenious mechanisms proposed to explain how animals might sense the different parameters of the earth's magnetic field. Biophysicists have proposed that migratory birds use the photoreceptor system to detect the magnetic field (Ritz et al 2002). There is evidence of the involvement of the radical pair reaction in that detection process. The radical pair reaction starts with photon absorption that leads to the transfer of electrons from one molecule to another; and that in turn results in a pair of molecules each with unpaired electrons. The unpaired electrons have particular spins, which could be affected by the interaction with the external magnetic field (Grissom 1995; Edmond 2001). The magnetic field was found to interact with the bird's visual system assisting them with their spatial orientation. There are however, other findings from animals like turtles; salmon and lobsters, which demonstrate their ability to orient themselves under complete darkness (Walker et al 1997). Therefore, other kinds of magnetic sensory systems were proposed. In the rainbow trout, Walker and colleagues (1997) have identified a magnetic sensory system that is located in the trout's nose. The magneto receptor cells in the trout's nose were associated with learned responses to magnetic fields. A magnetite based magneto receptor may be accounted for such responses. Magnetite (Fe_3o_4) is a crystal that has one or several domains (Edmond 2001). The magnetic field can generate a torque force on the magnetite crystal similar to that seen on the magnetic compass. If we hypothetically assumed that a cluster of magnetite crystals is located in the membrane of neurons, then the attraction and the repelling forces between the crystals will depend on the direction of the external magnetic field. These forces would deform the cell membrane and cause

opening or closing of ion channels as well as changing the conformation of certain enzymes and modifying their function (Jonhsen and Lohmann

2005). This magnetite-based system is clearly identified in the magneto-tactic bacteria and algae (Blakemore et al 1982, 1986; Balkwill et al., 1980; Frankel and Blakemore, 1988). In addition, magnetite has been found in the nervous system of honeybees, pigeons, bobolinks, tuna, and others (Kirschvink et al., 1981). Recently magnetite was found in the human hippocampus (Kirschvink et al., 1992; Dunn et al., 1995; Schultheiss-Grassi et al., 1999; Schultheiss-grassi and Dobson 1999; Dobson 2001, 2002).

Another proposed mechanism is electromagnetic induction. Magnetic field can be detected by induction mechanisms in animals that could detect the electrical field (Walker et al., 1997). Elasmobranches, such as sharks, skates and rays have an electrical field detector (Edmond 2001). This electrical field detector (ampullae of Lorenzini) can measure small currents induced by magnetic fields.

In the most widely reported research, static magnetic field has a depressive effect on the activity of neurons. Static magnetic field exposure refers to the steady state of the magnetic field and also the relation between objects being exposed when the field does not change in time. In almost all of the cited studies below, the authors were using different intensities of static magnetic field with an unspecified polarity.

Single cell recording from the pineal body of the guinea pig showed depression of the spontaneous activity of cells in response to low magnitude static magnetic field (Semm et al., 1980). Not all cells have the same response to static magnetic field. Some cells responded by showing depression of their activity while others showed no response

to the same static magnetic field intensity and polarity. In the same study cells with their spontaneous firing were silenced by one polarity of SMF exposure. Inverting the field polarity returned their spontaneous firing rate to control level (Semm et al., 1980). Static magnetic field (11 mT) exposure blocked the electrically evoked action potential recorded from cultured neurons (McLean et al.,1991, 1995). The exposure to 12 mT static magnetic field was associated with reduction in the cat's striate cortex excitability, which was expressed as a decrease in evoked potentials amplitude (Rosen and Lubowsky, 1986). The exposure to 123 mT static magnetic field decreased the spontaneous discharge frequency recorded from the cat's lateral geniculate body, and changed the discharge pattern (Rosen and Lubowsky, 1990). The exposure of isolated murine neuromuscular junction to static magnetic field at an intensity of 123 mT, led to miniature endplate potential inhibition, which was dependent on the duration of the exposure (Rosen 1993). Single channel recording revealed that cell exposure to static magnetic field at an intensity of 120 mT, inhibited calcium channel activation in GH3 cells (Rosen, 1996) and significantly increased the activation time constant of sodium channel (Rosen, 2003). Rosen has proposed that static magnetic field may partially realign diamagnetically anisotropic molecules within the cell membrane thus changing the properties of the channels. Furthermore, the SMF effect on many other preparations was tested using variety of SMF intensities and polarities. In cultured hippocampal rat cells, exposure to SMF led to transient increase of activator protein - 1 binding to DNA (Hirai et al., 2002).

The effect of SMF on E. coli was expressed as a change in the chromatin conformation (Belaev et al., 1997; Binhi et al., 2001) and as an increase of antibiotic resistance (Stansell et al., 2001) showing therefore, that biological molecules are able to

absorb the free energy from their surroundings, and respond by changing their conformation (proteins), with functional consequences.

The exposure of renal culture cells to SMF led to a gradual decrease in apoptosis and proliferation and a gradual increase in the number of cells with a necrotic morphology (Buemi et al., 2001). Similar treatment of cultured astrocyte cells stimulated a gradual increase in proliferation, apoptosis and in the number of necrotic cells. The authors concluded that static magnetic field effects depend upon the cell type (Buemi et al., 2001).

Erythrocyte was found to orient with their disk parallel to strong (4 – 8 T) SMF direction (Higashi, et al., 1993). Erythrocyte sedimentation rate (ESR) was enhanced by strong SMF (6.3 T) (Iino, 1997). SMF increased the inter-membrane adhesive area by changing the orientation of anisotropic erythrocyte (Iino and Okuda, 2001).

The effect of SMF on the pineal gland is well documented in the literature. The exposure of pineal gland in homing pigeons and guinea pigs to static magnetic field reduced serotonin and melatonin secretions at night time (Semm, 1983). That decrease in the pineal gland activity was correlated with the reduction of the analgesic effect of morphine in rats (Kavalier et al., 1984). Furthermore, mice exposed to MRI (strong static magnetic filed combined with radio frequency pulsing) showed the attenuation in the pineal gland activity and altered analgesic response to morphine (Ossenkopp et al., 1985). Moreover, exposing rats to MRI inhibited the beta adrenergic activation of the pineal gland (LaPorte, et al., 1990). In albino rats, exposure to static magnetic field markedly inhibited melatonin synthesis (Olcese and Reuss, 1986). In addition, rats that were exposed to static magnetic field had a 38% depression in the level of nocturnal pineal

cAMP (Rudolph 1988; Rudolph and Pastermak 1999). In pigeons, the electrical activity of the pineal gland was modified by inversion of the polarity of the static magnetic field (Demaine and Semm, 1985). As a continuation of these studies, it was found that exposing the retina to static magnetic field altered the pineal gland activity and this phenomenon was species dependent (Stehle, et al., 1988). Although the effect of static magnetic field on the pineal gland of many species including humans was clearly demonstrated (Reiter and Richardson, 1992; Reiter, 1993; 1994; Jaite and Zmyslony, 2000), the biophysical mechanism of the SMF action has not been proposed (see a review on this issue by Reiter 1998).

Electrophysiological recording from CA1 that was stimulated through Schaffer collateral in the hippocampal slices exposed to static magnetic field showed reduction in the evoked response (Trabulsi et al., 1996; Wieraszko 2000). This effect was intensity dependent. The exposure to static magnetic field of low intensity showed biphasic response (reduction followed by amplification) of the population spikes recorded from the CA1 sub-field. In the case of slightly stronger static magnetic field, it showed only the reduction phase (Wieraszko 2000). In this study, blocking the intracellular calcium channels eliminated the second phase.

There is a growing body of data that could justify the use of the static magnetic field as a therapeutic tool. Double blind randomized clinical trials revealed that following the SMF exposure pain responses were reduced significantly in patient with post-polio syndrome who reported muscular or arthritic-like pain (Vallbona, et al., 1997) and in chronic pelvic pain (Brown, et al., 2002). Moreover, SMF therapy was tested in another double blind study for its therapeutic effect in treating rheumatoid arthritis (Segal, et al.,

2001). It was also found that SMF decreased pain related to the arthritic pathology (Segal, et al., 2001). Similar treatment decreased chronic low back pain (Holcomb, et al., 2000; Langford and McCarthy, 2004). On the other hand, one study showed that SMF had no effect on muscle soreness (Reeser, et al., 2005) induced by isometric exercises. However, the interpretation of these results is controversial since this type of soreness heels itself within days. In the acute injury the efficacy of SMF is probably overshadowed by the body natural healing process, which might be different in the chronic conditions.

Several different intensities of SMF were used in clinical trials testing its effectiveness in treating pain. These clinical trials had also used different models of pathology. This might be a source of confusion because SMF may be effective in stimulating a healing process in one condition and not in another. The most prominent finding is that SMF has a reducing effect on pain. In support of that fact is the finding in our lab that static magnetic field has a depressive effect on the hippocampal evoked potential (Wieraszko, 2000). Although the static magnetic field-induced effects were demonstrated in the central nervous system, the outcome can be generalized and adopted for the peripheral nervous system since the process of transmission of information is similar in both systems. The sensation of pain is produced when stimulating the pain receptor with noxious stimuli. The pain messages are conveyed as action potentials travel from the site of generation to the central nervous system. The effect of static magnetic field on pain is probably mediated by depressing the generation and propagation of action potentials at the receptor level.

The SMF effect was tested on bone mineral density (BMD). Implanting a permanent magnet to a rat's femur bone showed significant increase in BMD (Yan, et al., 1998). The effect of SMF in that study was localized near the implanted magnet. These types of results indicate the possible clinical application of static magnetic field to stimulate bone formation in patients with osteoporosis.

When evaluating the influence of SMF on other sensory systems, it was revealed that the pairing of strong static magnetic field exposure with sweetened solution tasting caused taste aversion in rats (Nolte et al., 1998). Using T-mazes to investigate the aversive effect of magnetic field Weiss and colleagues (1997) have also found that rats avoided the region of strong static magnetic field. The authors suggested that this effect could be caused by the induction effect due to the rats' movement in the strong magnetic field region (Nolte et al., 1998).

Pulse Magnetic Field, PMF:

Alternating the static magnetic field between two states 'on' and 'off' with time intervals that can be manipulated by the experimenter is called pulsed magnetic field (PMF) (see methods and materials fig. 2.2 and 2.3). As illustrated by examples discussed below, PMF effects are quite different than those of SMF.

In cultured pineal gland cells from rats, PMF suppressed the production of melatonin, which indicated the direct effect of PMF on the pineal gland cells (Richardson, et al., 1992). Moreover, PMF suppressed the melatonin content in the pineal gland in rats, which was dependent on the photoperiod (Yaga, et al., 1993). PMF exposure disrupts the melatonin inhibitory effect on cAMP accumulation in breast cancer

MCF-7 cells and the disruption of that inhibitory effect was directly proportional to the field strength (Ishido, 2001).

In a study by Semm and colleagues, the influence of PMF was tested on the spontaneous firing rate in the trigeminal ganglion cells. Changes in vertical magnetic field intensity increased the spontaneous activity recorded from the trigeminal ganglion cells in the bobolink (Semm, et al., 1990). In this study, the intensity of the PMF was in the range of the earth's magnetic field. The responses had a positive correlation with the intensity of the field.

The exposure to PMF led to the stimulation of neurites re-growth in culture cells and the regeneration of the sciatic nerve in rats (Sisken et al., 1989, 1993, 1995; Kanje et al., 1993; Walker et al., 1994; Longo et al., 1999). In a similar protocol of exposure (Sisken et al., 1989, 1990, 1993, 1995; Kanje et al., 1993; Walker et al., 1994; Longo et al., 1999) PMF caused depression of both neurite outgrowth and differentiation of PC6 cells (Shah et al., 2001).

Exposing slices of chicken brain to PMF, increased calcium efflux (Blackman et al., 1985). In this study, Blackman and colleagues showed that the electromagnetic field interacted with the local earth's magnetic field. This interaction created a resonance-like relationship. The authors showed that the effect of the low frequency electromagnetic field on calcium efflux from the brain slices was tuned to a particular frequency that showed the window effect - the response to certain range of magnetic field frequency (Bawin and Adey 1976; Blackman et al., 1985, 1988). Similarly, in the case of chromaffin cells, which are often used as a model for studying processes in the nervous

system, exposure to PMF inhibited Ca^{2+} release from intracellular Ca^{2+} stores (Ikehara et al., 2002).

Furthermore, exposing hippocampal slices to amplified pulsed magnetic field evoked the potentiation of the population spikes recorded from CA1. Among many frequencies, pulsed magnetic field applied at 0.16 Hz was the most effective in increasing the hippocampal evoked potentials amplitude (Wieraszko et al., 2004). In these experiments, the amplification of the evoked hippocampal potential was accompanied by an increase in the synaptic glutamate release and increased the level of cAMP (Hogan and Wieraszko, 2004). These effects were most pronounced at a frequency of 0.16, again showing the window effect of PMF. Moreover, recording from a single and a network of pyramidal cells in hippocampal slices, showed that neural networks are more sensitive to magnetic fields than a single neuron (Francis et al., 2003).

The PMF effect was also tested on the protein level in rats. Short-term exposure of rats to pulsed magnetic field depressed the total protein levels (Kimberly et al., 2002). It simultaneously depressed the hematocrit value, lymphocyte number, and the concentration of carotenoid molecules. It elevated however, the number of granulosa cells (Kimberly et al., 2002).

The most remarkable finding in the pulsed magnetic field research is the promotion of bone regeneration. One should be aware that in the area of research pulsed magnetic field (PMF) and pulsed low frequency magnetic field (PLFMF) are used interchangeably (see a review by Shupak, 2003). However, in this dissertation, I used these two terms separately because their natures are completely different. PMF is the static magnetic field generated by direct current and then applied in pulsating form, but

the low frequency pulsed magnetic field referred to is the magnetic field that is generated by alternating current (ac), (to be discussed shortly), and then applied the same way.

The exposure to PMF for 3 to 4 weeks accelerated the bone formation and bone maturation in a rabbit's tibiae (Shimizu, et al., 1988). Although, in neurons, cAMP concentration increased after PMF exposure (Hogan and Wieraszko, 2004), fibroblast response to PMF was expressed as reduction in the level of cAMP (Farndale and Murray, 1985). PMF combined with alternating current (ac) pulsed magnetic field (low frequency pulsed magnetic field) and applied to a human osteoblast-like cell, was found to increase insulin-like growth factor II level and DNA synthesis. The authors of that study suggest that this might be the mechanism by which magnetic field stimulates bone regeneration (Ryaby and Fitzsimmons, 1994).

PMF has a beneficial effect on the endothelial cells, which may be significant in some pathologies. The endothelial cells make up the walls of capillaries and their function is crucial to maintain the health of body organs. Therefore, a noninvasive tool such as PMF can be beneficial in many pathological conditions. PMF effect on endothelial cells and microcirculation was tested in many studies. For example PMF stimulated growth rate and angiogenesis of the endothelial cells in vitro (Yann-Patton, et al., 1988). Application of PMF to a rabbit's arteriole significantly vasodilated the vessel and increased the system hemodynamics (Smith, et al., 2006). This effect of pulsed magnetic field could account for the wound and bone healing (Luben, 1991). Also, pulsed magnetic field was used in clinical trials to test its effect on peripheral vasculature; it was shown that pulsed magnetic field had improved the peripheral circulation in atherosclerotic patients (Yambe, et al., 2005). The potentially beneficial influence of

PMF on diseases that are caused by degeneration of the peripheral microcirculation (e.g. osteoarthritis) has not been evaluated yet.

Extremely low frequency pulsed magnetic field (ELF-PMF):

This form of magnetic field is the most utilized form in this area of research and in clinical applications. ELFPMF is usually generated by a source of alternating current with a frequency equal to or less than 100 Hz. ELFPMF was used to stimulate bone healing (Yasuda, 1953; Bassett et al., 1974, 1977, 1982), nerve regeneration (Romero-Sierra and Tanner, 1975) and tissue repair (Rowley et al., 1974; Cheng et al., 1993). The effects of pulsed ELFPMF at 65 Hz on the process of bone repair after a bone fracture, were investigated by Basset et al (1974). They found that the field accelerated the repair process. The same authors, using similar field characteristics, found that patients suffering from psuadoarthrosis had greatly benefited from the ELFPMF treatment, which induced the formation of new bone. ELFPMF (100 Hz) was found to stimulate bone formation in bony defects in the pre-maxilla of rats (Yamamoto, et al., 1992). Also, ELFPMF was tested in clinical trials for its effect on osteoporosis. Exposing 52 patients diagnosed with osteoporosis 30 minutes a day for few months a year, yielded satisfactory improvement in about 80 – 90 % of the participants (Jacchia, et al., 1985). The Food and Drug Administration (FDA)-has approved devices using pulsed ELFPMF to speed recalcitrant non-unions (the inability of bone ends after a fracture to unite) and spinal fusions (a surgical procedure to fuse vertebral bodies). The subject dealing with the effect of magnetic field on bone healing and on of other systems, was reviewed by Liboff (2004).

Similarly, ELFPMF at 75 Hz was found to regulate chondrocyte differentiation and expression of matrix proteins (Ciombor, et al., 2002). Exposing aged guinea pigs suffering from knee joint osteoarthritis to pulsed ELFMF for six hour a day, for 3 months preserved the morphology of the articular cartilage and slowed the lesion progression (Fini, et al., 2005) in the whole knee.

ELFPMF was evaluated in a few studies to test its effectiveness on neurite outgrowth. The effect of the field on neurite outgrowth was found to be intensity specific. Higher intensities seem to have an inhibitory effect but lower intensities tend to have a stimulating effect. Neuritis outgrowth was promoted under a field intensity of 0.23 mT, while it was inhibited under a field intensity of 1.32 mT (Fan, et al., 2004). Also, the sensitivity of neurite outgrowth to low frequency magnetic field was dependent on the condition of the cells' microenvironment (the extra-cellular medium). While magnetic fields characteristics induced neurite outgrowth when the culture conditions promoted cellular differentiation, the same fields were ineffective when the culture conditions were not strongly supportive of cell differentiation (McFarlane, et al., 2000). Also, the effect of low frequency magnetic field on neurite outgrowth was induced in the presence of forskolin and inhibited by melatonin (Takatsuki, et al., 2002).

ELFPMF has been used in many forms to treat chronic pain. In a clinical trial of 311 patients suffering of chronic pain, low frequency magnetic field exposure produced positive results in 147, slight results in 97, and no results in 67 patients (Paccagnella, et al., 1985). Low frequency magnetic field reduced pain in patients who underwent a mastectomy (Mustacchi and Nemez, 1985) and in patients suffering from chronic treatment-resistant low back pain (Zucco and Orlandi 1985). Cancer patients

treated with low frequency magnetic field experienced remarkable reduction in pain (Sauerwein, 1992). Extremely low frequency magnetic field exposure modulated the diurnal rhythm of pain in rats (Choi et al., 2003). The authors of that study suggest that magnetic field was probably associated with environmental light-dark cycle. Other experiments by Kavaliers and colleagues (1986) found that ELFPMF exposure reduced significantly the effect of stress-induced activation of endogenous opioid systems. In agreement with these results, exposing the human head to low frequency magnetic field at 80μT lowered pain threshold in humans, (Ghione, et al., 2005) and was correlated with increased alpha band amplitude measured after the exposure. Similarly the whole body exposure to low frequency magnetic field reduced the pain threshold in mice (Jeong, et al., 2006). These results seem to be contradictory, but that can be explained. When ELFPMF was applied on peripheral tissue (low back, knee and wound after surgery) it reduced the pain. One can then assume that ELFPMF was stimulating the healing process, thus reducing the amount of damaged tissues or activating the pain blocking mechanism in the tissue. On the other hand, stimulating the whole body or just the head will stimulate the brain. It seems that low frequency magnetic stimulation excites the neuronal tissue and increases its sensitivity to noxious stimuli.

The following are electrophysiological, chemical and behavioral experiments performed to investigate the epidemical effect of the ELFPMF exposure.

Testing the effect of ELFPMF on implantation in rats revealed that, although magnetic field exposure did not impair the process of implantation in the female rats, it caused significant changes in both the transport and development of embryos

(Huuskonen, et al., 2001). The exposure to this form of magnetic field is widespread in humans, so its disturbance of embryonic development could have deleterious outcomes.

Rats exposed to ELFPMF (2μT) for one or two weeks showed a significant reduction in the novel object recognition (NOR) task index, which indicated learning and memory impairment. Furthermore, the same animals in that study have shown significant increase in the corticosterone level, which was increased as a function of the exposure-time (Mostafa et al., 2002).

ELFPMF had an attenuating-effect on the conditioned taste aversion (CTA) in deer mice (Choleris et al., 2000). Moreover, in these experiments they found that ELFPMF modulated the effect of stress and anxiety in male deer mice only.

The exposure to ELFPMF caused a significant concern, as it is associated with increases the risk of cancer. A review of statistical data of the human population by Erren (2001) concluded that electromagnetic field generated by power lines are associated with the increase in breast cancer. This type of magnetic field was used in clinical trials to diagnose and treat seizures. For patients with intractable seizures, the exposure to low intensity magnetic field increased the interical (between seizures) firing rates (Fuller et al., 1995; 2003), illustrating the possible utilization of magnetic field as a diagnostic tool in detecting seizures. Successful attempts in the use of magnetic field in seizure therapy have been shown.

Magneto-encephalograph (MEG) was used to detect the intensity and frequency of emitted magnetic fields from brains of epileptic patients (Anninos and Tsagas, 1991). These researchers were then able to emit back the same intensity and frequency to the

epileptic foci and cause a cancellation effect. This procedure has reduced the seizure activity in many patients.

Repetitive trans-cranial magnetic stimulation (rTMS):

The idea of stimulating the brain through electrical or magnetic fields was conceived long before the development of TMS (Walsh and Pascual-Leone, 2003) and was developed into practical application as the method known currently as transcranial magnetic stimulation by Baker and coworkers (Barker et al., 1985, 1994).

The repetitive trans-cranial magnetic stimulation is a technique for generating very high voltage that can be discharged in a very short time (microseconds) into a coil that in turn generates a very strong magnetic field pulse (up to 3 Tesla) (Walsh and Pascual-Leone, 2003; George and Belmaker, 2000; Mills, 1999). When the stimulating coil is placed on the scalp, it can induce a current (eddy current), which crosses the cranium and reaches the cortex. The induced current, in turn, excites the neurons and causes them to fire and elicit a behavioral effect. Commercially available TMS devices can generate single and train pulses with frequency ranges from 1 Hz up to 30 Hz and intensity up to 3 Tesla. The coil can be applied directly to the head and stimulate the different regions of the cortex. The rTMS has been used in clinical trials to treat many psychiatric disorders (Post et al., 2001; Borsini et al., 1988; Cohen et al., 1999; Fleischmann et al., 1995). The rTMS application has shown improvement in patients with major depression (Pascual-Leone et al., 1996; Kirkcaldie et al., 1997). The results of these studies were obtained through the stimulation of dorsolateral prefrontal cortex (see Wassermann and Lisanby 2001). In healthy individuals, rTMS has shown a slight change in mood. In schizophrenia, although rTMS has been found not to be effective in

reducing psychotic symptoms (Klein et al., 1999), it was effective in reducing schizophrenic restlessness and auditory hallucinations. In addition, the stimulation of the left temporoparietal cortex reduced chronic auditory hallucinations (Pascual-Leone et al., 2002; Walsh and Pascual 2003).

In the case of obsessive-compulsive disorder, rTMS applied to the right lateral prefrontal cortex (Greenberg et al., 1997) reduced compulsive urges for 8 hours.

The second type of conditions that were greatly studied using rTMS were motor disorders like Parkinson's disease (PD), task-related dystonia and tic disorder. However, researchers had contradicting-results over the responses of the Parkinson's patients to rTMS (Pascual et al., 1994). While in some studies rTMS was beneficial, it worsened their condition in others (Ghabra et al., 1999; Topka et al., 1999). Their response depended on the frequency and the strength of the stimulation. In a chronic stimulation study, PD patients responded with a continuous improvement of their motor performance (Mally et al 1999., Shimamoto et al., 1999). Moreover, task-related dystonia patients showed improvement after rTMS. Patients with tic disorders have also shown reduction in the frequency of tics after the rTMS treatment (Siebner et al., 1999). One of the most exciting outcomes in these trials, is the reductive response of the intractable condition of epilepsy. In most epileptic patients treated with rTMS, the intensity and duration of the seizures have been reduced significantly (Michelucci et al., 1994, 1996; Tergau et al., 1999).

The mechanisms of rTMS were investigated in many animal models as well. While rTMS application modulated monoamine contents and turnover after a one-time application (Holsboer, 1995), its chronic application had no effect on the neurotransmitter

system. Moreover, the acute application of rTMS reduced dopamine in the frontal cortex and increased it in the hippocampus and striatum (Keck et al., 2000). The application of rTMS also increased the level of serotonin in the hippocampus (Ben-Schachar et al., 1999). In addition, rTMS increased the release of taurine, aspartate, and serine and decreased arginine, and vasopressin in the hypothalamic paraventricular nucleus (Keck et al., 2000). A one-time stimulus, as well as chronic rTMS, induced an increase in receptor binding (5-HT_{1A} and NMDA) in rats (Kole et al., 1999; Wassermann and Lisanby 2001), the result which is reminiscent to the action of antidepressive drugs. The application of rTMS was also found to increase the gene expression such as c-fos mRNA in the parietal cortex (Fleischmann et al., 1996). The rTMS was found to induce LTD (LTD is the long-term depression, which can be induced by low frequency stimulation) in vivo, in gerbils (Wang et al., 1996) and increased the neuronal reactivity in the rat dentate gyrus (Wang et al., 1996).

These findings leave no doubt that magnetic field exposure influences the nervous system. However, little is known at this time about the neural, biophysical or molecular mechanisms that might mediate these types of effects. This dissertation was aimed to characterize the effect and the mechanism of action of pulsed magnetic field.

The hippocampus

I was interested in studying the mechanism that mediates the effect of magnetic fields on the nervous system (Bawin and Adey 1976; Blackman et al., 1982, 1985; Semm, et al., 2003; Wieraszko, 2004) and how magnetic fields influence the processing of information in the nervous system. Therefore, I looked for a structure where these

processes are relatively well known. My choice was the hippocampus (Wieraszko, 2004; Wieraszko, et al., 2005).

The hippocampus is a part of the limbic system involved in memory and learning (Nadel 1991; Eichenbaum, 1992). Patients with bilateral damage to the hippocampus suffered from a severe loss in their ability to encode new information to a long-term memory. Since magnetic field has been found to effect memory formation (Mostafa et al., 2002; Lee et al., 2001) that makes the hippocampus a relevant model to study the effect of magnetic field on memory in mice. The hippocampus has the highest susceptibility for seizures, which can be induced by a magnetic field (Dobson et al., 2000; Fuller et al., 2003). In addition, the hippocampus was found to contain the place cells that are responsible for spatial orientation in animals (O'Keefe and Dostovsky's, 1971). Part of this dissertation was concerned with animal behavior (working memory), which depends on hippocampal activation. Therefore, using the hippocampus as a model would help to apply findings in vitro (influence of PMF on slices) to live animals (influence of PMF on animal behavior).

The hippocampus was also selected as the model for my project for several technical reasons. The hippocampus is a well-defined area of the brain. The fields and sub-fields of the hippocampus are easily visualized on a transverse hippocampal slice that makes it easy for electrode placement. From a practical point of view, the hippocampal slice has clear locations for different recordings such as for EPSP, fiber potential and population spike (fig.2.1). In addition, most of the electrophysiological characteristics of the hippocampal neuron have been intensively studied and are well known (Squire and Backercave, 1991; Eichenbaum, 1992; Johnston and Amaral, 1997).

Specific patterns of stimulation of hippocampal connections can transiently or permanently (Andersen et al., 1971) modify their efficiency. The mechanisms of these modifications are intensively studied and some will be described later. One of our goals was to investigate the mechanisms of the action of magnetic fields by evaluating their influence on different forms of synaptic plasticity, which is a change in the synaptic efficacy. These changes can be short or long term, and are believed to have behavioral consequences.

Hippocampal anatomy:

The hippocampal structures are the dentate gyrus, and the cornu ammonis (the hippocampus proper). The dentate gyrus (DG) is a trilaminar cortex. The main cell type is a granule cell that forms the dense cellular layer of the DG. The DG granule cells receive inputs from the Entorhinal cortex. There is also a polymorphic layer that contains different types of cells that connect within the DG and do not project anywhere else.

The hippocampus proper (cornu amminos) has three fields called CA3, CA2, and CA1. These fields contain several layers and one of them is a pyramidal cell layer. CA3 extends from the DG and it contains large pyramidal cells that receive the mossy fiber inputs from the DG (fig. 1.1). CA2 is a dense layer of pyramidal cells that receives inputs from the supramamillary region of the hypothalamus. CA1 contains many layers. The alveus is the layer that contains the axons from the subicular and hippocampal pyramidal cells that extend their connections to the fimbria of the fornix. The stratum oriens has basal dendrites of the hippocampal pyramidal cells and some interneurons. The stratum pyramidalis and the stratum lucidum contain mossy fibers. The stratum radiatum is a layer that contains Schaffer collaterals axons. These are associative connections, which

extend from CA3 and CA2 and go to CA1 field. The stratum lacunosum-moleculare contains the perforant pathway (fig. 1.1). The perforant pathway is a projection from the entorhinal cortex to the dentate gyrus (Berry et al, 1995).

The hippocampus connections:

Inputs

The hippocampus receives inputs from cortical and sub-cortical structures, like the amygdala, claustrum, septal nuclei, basal nucleus, supra mammillary nucleus, anterior thalamus, midline thalamus, ventral tegmental area, rahpe nucleus and locus coeruleus. Cortical inputs are from the perirhinal cortex, parahippocampal gyrus, cingulate cortex, piriform cortex, insular cortex, orbitofrontal cortex, and superior temporal gyrus (Berry et al, 1995).

Outputs

The hippocampus projects to cortical and subcortical structures. Cortical structure innervated by the hippocampus is the entorhinal cortex that receives projections from the subicular complex. The hippocampus projects to subcortical structures such as olfactory regions, clastrum, amygdala, septal nuclei, nucleus accumbens, caudate, putamen, hypothalamus, anterior thalamus and mammillary nuclei (Berry et al., 1995).

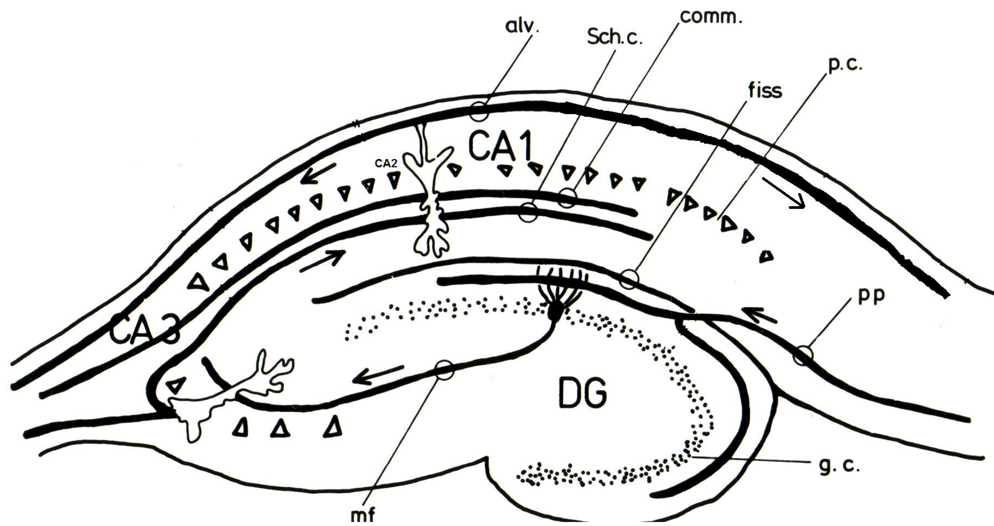


Fig. 1.1. A diagram of hippocampal connections as demonstrated on a cross-section perpendicular to the long axis of the hippocampus. It shows several fields of the hippocampus formations and the intrinsic structures and connections. Arrows indicate the direction of the propagation of action potentials. Abbreviations: DG, dentate gyrus; g.c, granule cell; pp, perforant path fibers; p.c, pyramidal cell; alv, alveus; Sch.c, Schaffer collaterals; comm., commissural fibers; fiss., fissure; m.f, mossy fibers. Modified after Wieraszko (personal communication).

Hippocampus and behavior:

O'Keefe and Dostrovsky (1971) were the first to demonstrate that the hippocampus contains many spike-complex cells that fire at a higher rate when the animal is at specific places within an environment. Most pyramidal hippocampal cells are silent in freely behaving animals. However, those silent cells showed high spontaneous activities when they received antidromic stimulation (Thompson 1989).

The hippocampus is linked to other areas of the brain. The interactions between the hippocampus and these areas are very important in many behavioral tasks. The interaction between the hippocampus and the ventral striatum is crucial in guiding the animal through a novel behavioral task that does not depend on previously acquired knowledge (Floresco, et al 1997). The exploratory goal-directed locomotion to find food in rodents is directed by the interaction between the hippocampus and ventral striatum pathways. The activities that depend on previously acquired knowledge for a particular task are taking place at another set of connections and interactions. For example, interactions between the hippocampus, ventral striatum and the prefrontal cortex direct the animal to specific guided steps to reach its goal (Floresco, et al 1997).

The electrophysiology of the connection between CA3 and CA1 fields:

The connection between the CA3 and CA1 fields in the hippocampus is a one-way connection exclusively originating from CA3 (CA2) sub-field to CA1 with no recurrent projections from CA1 to CA3. This axonal connection is called Schaffer Collateral.

The cellular potentials can be recorded extracellularly and intracellularly. The extra-cellular recording is a combined recording from many cells but the intracellular is from one cell.

Action potentials generated at the CA3 sub-field travel through the Schaffer Collateral to the synaptic area at CA1 sub-field stimulating synaptic release of the excitatory neurotransmitter glutamate. Glutamate in turn acts on two types of receptors, ionotropic and metabotropic. The ionotropic receptors are AMPA, kainate, and NMDA. They are named according to the ligand that is used to describe them. AMPA and kainate receptors mediate the fast excitatory post-synaptic potentials (EPSPs). NMDA receptor mediates the slow rise and slow decay of EPSPs (Johnston and Amaral 1997). These three types of receptors are jointly present in the hippocampus synapses with different variations. All ionotropic receptors are connected to non-selective channels allowing passage of monovalent cations Na^+, K^+, and extends to Ca^{+2} for some. All subtypes of NMDA receptor-channel complexes are permeable to Ca^{+2}. Another characteristic of the NMDA receptor-channel complex is that it is both chemically and voltage regulated. The channels are closed at the resting membrane potential because of Mg^{2+} blocks the channel. At depolarizing potentials, the magnesium block is removed and the channel opens. This characteristic of the NMDA receptor-channel complex is very important in coincidence detection and in LTP generation (Johnston and Amaral 1997). In addition, the hippocampus contains cholinergic receptors binding sites for acetylcholine. There are two types of ACh receptors: nicotinic (ionotropic) and muscarinic (metabotropic) in the hippocampus. The nicotinic receptor is present presynaptically and modulates the glutamate release. Muscarinic receptors are located both pre and post synaptically. While

the muscarinic receptors inhibit the release of glutamate presynaptically, they decrease the K^+ conductance postsynaptically.

Extracellular electrical recording could be performed in identifiable sites in the hippocampal subfields. When the recording electrode is placed at both the Schaffer collateral and the commissural fibers, it records the fiber volley, a compound action potential that is originally generated in the CA3 field. When it is placed on the synaptic layer (stratum lacunosm moleculare) it records the field excitatory post-synaptic potential (fEPSP). The recording from the laminar layer of CA1 (stratum pyramidale) would be called population spike, which represents the summed responses of neurons including feed-forward inhibitory inputs (fig. 1.2 recording electrode).

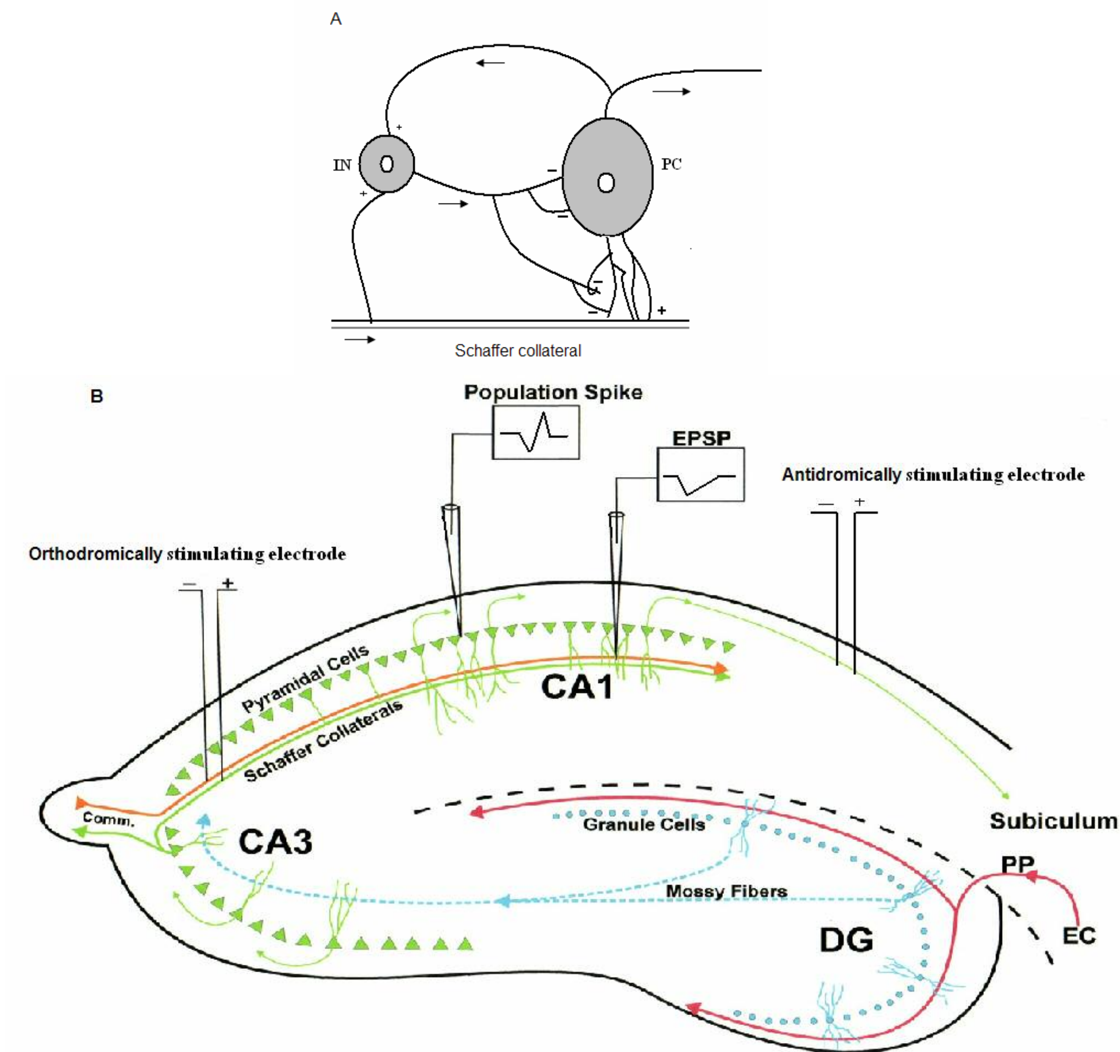


Fig. 1.2 The electrical stimulation of the Hippocampal pathways – The diagram shows a simple internal hippocampal network; IN and PC represent interneuron and pyramidal cell, respectively. The arrows in A show the direction of the action potential propagation. B – The diagram shows the hippocampal pathways and the location of stimulating and recording electrodes. The pathways mostly used in our experiments are the Schaffer collaterals – CA1 (orthodromically) and subiculum – alveus (antidromically). The diagram depicts the location of the recording electrodes, stratum radiatum for EPSP and stratum pyramidale for population spike (Modified from El-Sherif, 2003).

Hippocampal Synaptic plasticity:

Synaptic plasticity is defined as changes in the synaptic potentials amplitude, which are dependent on the history of the synaptic activity. It can be expressed in various forms in the hippocampus. There are short and long-term potentiation and depression.

Short-term plasticity:

There are four forms of short-term plasticity. First, paired pulse facilitation (PPF), which occurs when paired pulses separated by a specific interval (longer than 20 ms) are sequentially applied to the Schaffer collateral pathway. The response to the second pulse is greater in amplitude than the response to the first. PPF is a result of generation of the second elevated synaptic response on the elevated background of Ca+2 concentration induced by the first stimulus. There is a window of 25 – 55 ms where the PPF can be expressed.

The second type of short-term plasticity is paired pulse inhibition (PPI). Delivering paired pulses with short time intervals (less than 10 ms) would activate the recurrent inhibitory loop (in the hippocampus) that would coincidently inhibit the post synaptic pyramidal cell at the time of the second pulse arrival. That could be seen as a decrease in the second pulse response (fig. 1.2 A).

The third form of the short-term plasticity is the post-tetanic potentiation. After a brief train of stimulation, the cell expresses a transient increase in its responses to the same stimulus that has been applied before the potentiation. Post-tetanic potentiation has an exponential decay constant after the potentiation. However, it has two stages: one that is immediately after the stimulus end which has 5 – 10 sec decay time, and a later stage which is much slower. Both types of short-term plasticity are a result of the increase in

the probability of neurotransmitter release from the presynaptic site due to an increase in the level of the intracellular Ca^{2+} (Johnston and Amaral 1997).

The fourth type of short-term plasticity is the short-term depression due to the depletion of neurotransmitters after a particular type of repetitive activity. This form of depression can last from several hundreds of milliseconds to a few minutes (Johnston and Amaral 1997).

Long-term plasticity:

There are two types of long-term plasticity: long-term potentiation also known as LTP and long-term depression known as LTD. Although both have been discovered in the hippocampus, they occur almost in all brain areas.

Long term depression (LTP), was first described by Bliss and Lomo (1973), and it has a presumed role in learning and memory. High frequency (20 – 200 Hz) stimulation of the Schaffer Collateral pathway in the hippocampus will induce LTP at the synaptic connection between CA3 and CA1. LTP is an increase in the synaptic efficiency due to repeated high frequency trains, and this increase in response to control stimulation persists for a long period of time (30 minutes to several days (in vivo) after the stimulation).

LTD can be induced by low frequency (1-3 Hz) stimulation (Dudek and Bear 1992). It is a long-term depression of a synaptic response that persists from 30 minutes to more than an hour. Both LTP and LTD are dependent on the activation of the NMDA receptor channel complex. The NMDA receptor activation allows calcium to enter the cell to elevate the intracellular calcium concentration. The type of synaptic plasticity (either potentiation or depression) is dependent on the level of intracellular Ca^{2+}. High

but brief increase in the intracellular calcium induces LTP. However, moderate but persistent increase of the intracellular calcium induces LTD (Mulkey and Malenka 1992; Dudek and Bear 1992).

PMF-induced hippocampal plasticity

It was discovered that the exposure to pulsed magnetic field (in vitro) with frequency ranges from 0.5 to 0.16 Hz could induce a significant increase in the amplitude of the population spikes recorded from CA1 area in the hippocampus (Wieraszko, 2004). This type of induced increase in excitability was long lasting and was the subject of investigation in this dissertation.

Peripheral nerves

In this dissertation, the sciatic nerve was used to test the effect of magnetic field on compound action potential generation and propagation. These are several properties of the sciatic nerve, which made it a suitable model for researching. It has a massive size and length compared to other spinal and cranial nerves in mice and therefore, suitable for extra-cellular recording. It also allowed for multiple experiments to be performed each on individual nerve segment obtained from a single nerve. In addition, the sciatic nerve represents most of the peripheral nerves in axonal composition. The sciatic nerve originates from the lumbar area and runs down the lower extremities. The spinal roots – anterior and posterior – join and form the spinal nerves. The spinal nerves then unite to form the peripheral nerves such as the sciatic nerve. Therefore, the sciatic nerve is composed of both sensory and motor axons, (mixed nerve). Protective connective tissues layers cover all nerves. The connective tissue layer that wrapped around the whole nerve is called epineurium. The axons inside the whole nerve are separated by connective tissue

into fascicles; this layer of connective tissue is called perineurium. Another layer of connective tissue is called endoneurium wraps individual axon (myelinated and unmyelinated). The axons in general have several components. The axolemma is the axonal membrane and it is an extension of the neuronal cell membrane. The axolemma and the proximal Schwann cell layer make up a tight extracellular space; called the periaxonal space. There are many integral membrane proteins found in the axolemma of the axons of the peripheral nervous system. Among these proteins are ion channels, pump proteins, enzymes, and cell adhesion molecules. There are also other receptor proteins for GABAa, serotonin, and other known neurotransmitters. The distribution of the receptors in the peripheral nerves system differs from one nerve to another. The axolemma encloses a viscose fluid, the axoplasm. The axoplasm in turn contains the axoplasmic organelles like mitochondria, the axoplasmic-smooth endoplasmic reticulum, neurofilaments, dense lammelar bodies, neurotubules, and membrane cisterns. For more details see a review by (Berthhold and Rydmark, 1995).

Chapter 2 – Method and Materials

Animals

Mice (4 to 12 weeks of age), CD – 1 strain were used for the experiments. Animals were kept on a 12 hour / 12 hour light cycle. Food and water were provided ad libitum.

Preparation of hippocampal slices:

The hippocampal slices were prepared according to the procedure used in our laboratory (Wieraszko, 1983). The CSI Animal Care and Used committee approved this procedure. After decapitation, brain removal, placement into ice-cold Ringer's solution composed in mM of NaCl 124, KCl 3.1, KH_2PO_4 1.3, $MgSO_4$ 1.3, $CaCl_2$ 3.1, $NaHCO_3$ 25.5, and glucose 10, the hippocampi were sliced (350 - 400µM) with a manual tissue chopper. The slices were then transferred for at least one hour into an incubation chamber (33^oC) constantly oxygenated with a 5%/ 95% CO_2/ O_2 mixture. Following the incubation period, slices were transferred individually to interface – recording chamber maintained at 33^oC; constantly oxygenated with a 5% / 95% CO_2/ O_2.

Preparation of the sciatic nerve segments;

Following decapitation mice both hind limbs were skinned. The upper thigh muscles were then cut and either the femoral bone was broken or the hip joint was dislocated, carefully. The broken limb was pulled away carefully to expose the sciatic nerve from its origin in the vertebral column. With dissecting scissors the fascia and muscle tissue of the thigh were cut to expose the sciatic nerve. The two ends of the sciatic nerve were cut and the nerve pulled out and placed into ice-cold Ringer's solution. The nerve was then cut into 4 to 6 mm segments. The nerve segments were placed into an

incubation chamber maintained at 33°C; constantly oxygenated with a 5% / 95% CO_2/ O_2. The individual segment was transferred into a recording chamber. The epineurium (the outermost layer of connective tissue surrounding the entire peripheral nerve) remained intact. The recording electrode was placed inside the epineurium at the end of the cut and the stimulating electrode was placed out side the epineurium at a distance that was varied to obtain the robust compound action potential (fig. 2.1).

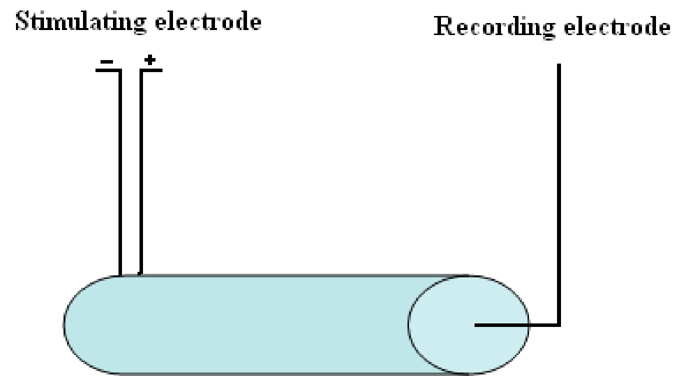


Fig. 2.1. The diagram shows placement of the electrodes on a sciatic nerve segment.

Electrophysiological recordings in the hippocampal slices

1. The population spike reflects a summation of the individual action potentials generated by activating pyramidal neurons (Andersen, 1971). The population spike was recorded in the hippocampus from the pyramidal cell layer (CA1) following the stimulation of Schaffer collateral pathway. The two ways to induce a population spike from the CA1 subfield are: First, orthodromic, which is the stimulation of the Schaffer collateral and then recording from the CA1 pyramidal cell layer. This involves synaptic transmission. Second, the antidromic stimulation, which is defined as the stimulation of the axonal area of the CA1 (Alveus) and recording from the cell bodies (subiculum pyramidale) (fig 1.2). It does not involve synaptic transmission. Stimulation of the Schaffer collateral pathway will excite both pyramidal cells of CA1 and interneurons through a feed forward inhibition pathway. In addition, the activation of the postsynaptic pyramidal neurons activates the feedback inhibitory loop. The population spike shows the resultant of that neural networks interaction (Wieraszko, personal communication). A computer program (LTP) measured the amplitude of the population spike, and it was calculated as the amplitude from the population spike peak to the intersection with a tangent line drawn between the pre-spike peak and the post-spike peak (LTP manual chapter 4).

2. The field post-synaptic excitatory potential (fEPSP) reflects the sum of the synaptic potential picked up by a recording electrode placed in the stratum rediatum (fig. 1.2). The slope of the fEPSP was calculated using the LTP software.

The strength of the stimulation was adjusted to record the evoked potentials of stable amplitude. Then the potentials were recorded for the next 10 – 15 minutes to

generate a baseline used as a control in each experiment. The slices that did not show that stable baseline were discarded.

LTP induction

Once the baseline was obtained by the low frequency test stimulus (every 30 sec or 0.03 Hz), the high frequency stimulation (HFS) was applied at 100 Hz for 1 sec, three times with 10 interval between trails. Then, the low frequency (control) test stimulus was resumed. The average of the sweeps amplitude calculated during the baseline period was compared to the average of the sweeps taken after HFS. The potentiation was considered LTP only if the potential remained elevated for at least 30 minutes.

Spontaneous activity recording

The spontaneous activity was recorded by connecting the recording electrode to a loud speaker that was in turn connected to a computer/or digital mini disc. The signal was analyzed using a Matlab signal processing toolbox (The MathWorks, Inc. USA). Each action potential generated by a single cell can be heard as a single tick of sound.

Paired-pulse stimulation

A paired pulse stimulation paradigm was used in both hippocampal and sciatic nerve preparations. It employed two consecutive pulses delivered at a frequency of 0.03 Hz; the inter-pulse interval (IPI) was adjusted accordingly with each experiment. To induce paired pulse inhibition (PPI) the IPI was adjusted to give the maximum inhibition. In the hippocampal slices, it was between 10 and 15 ms; in the sciatic nerve it was less than 10 ms (9 ms in most of the experiments). To induce the paired pulse facilitation (PPF) the IPI was adjusted to give the maximum facilitation. It varied between 15 to 55 msec. PPF and PPI were calculated as the difference in amplitude between the first

population spike (PS1) and the second population spike (PS2): in a paired-pulse paradigm, PS1 is the response for the first stimulus and PS2 is the response to the second stimulus. The reduction in the difference is interpreted as a decrease in inhibition and the enhancement of the deference is interpreted as an increase in inhibition.

In the case of the sciatic nerve, the paired pulse paradigm was used to induce short-term depression (STD); and to test the period of recovery the IPI was varied. The STD was calculated as the difference between the first (CAP1) and the second (CAP2) compound action potential.

PMF exposure

The pulsed magnetic field (PMF), was generated by DC-powered coils (Trabulsi et al., 1996; Wieraszko, 2000). The coils were wound around an acrylic frame, which surrounded the interface-recording chamber. The activation and deactivation of the coils was operated by a programmable timer, which was set at different frequencies to meet the goal of the specific experiment. A plastic tube (diameter 4 mm) with small holes was placed at the bottom of the acrylic frame. This plastic tube is connected to the building air supply to secure the flow of the air indicated by arrows E. The flow of air prevents the water chamber from overheating during electromagnetic field activation (Trabulsi et al., 1996; Wieraszko, 2000) (*fig. 2.4)*. Regardless of the frequency of the pulsed magnetic field, each pulse has four phases: Rising phase (period of coils charging), static magnetic field phase (period of no change; the power supply on), falling phase (period of coils discharging), and then an “off” phase (power supply off), (*figures 2.2 and 2.3*). The duration of the rising and falling phases was equal for all frequencies generated by our magnetic field setup (they were measured as shown in figures 2.2 and 2.3); however, the

number of these phases is substantially different for different frequencies. Unless specified otherwise, the frequency that was mainly used in this dissertation is 0.16 Hz. The phases and the shape of the magnetic field pulse were measured using an oscilloscope that was connected to a magneto sensor (fig.2.3). The frequency of 0.16 Hz was particularly used because of it previously showed the highest effect (Wieraszko et al., 2004; Hogan and Wieraszko 2004).

Frequency (*F*) is defined as the measurement of a repeated event per unit time (*T*). It is measured as the reciprocal time of the event's period $F=1/T$. Therefore, a frequency of 0.16 Hz was calculated as the reciprocal time of a complete cycle (3 sec "off" + 3 sec "on" = 6 sec), which is 1/6 or 0.16 Hz.

The coil's characteristics are: an 800 foot length wire, a diameter of 20 cm, number of 200 turns, height of 15 cm, and resistance of 6.108Ω per 1,000 feet. The calculated and measured generated magnetic field using that coil was around 15 mT.

Fig. 2.2. Thirty minutes of PMF recording. This graph shows - on line - magnetic field pulse recording for the period of thirty minutes that illustrates neither change in the PMF strength nor shape. A, B and C show on-line PMF recording of 4 minutes in the beginning, middle, and at the end of thirty minutes.

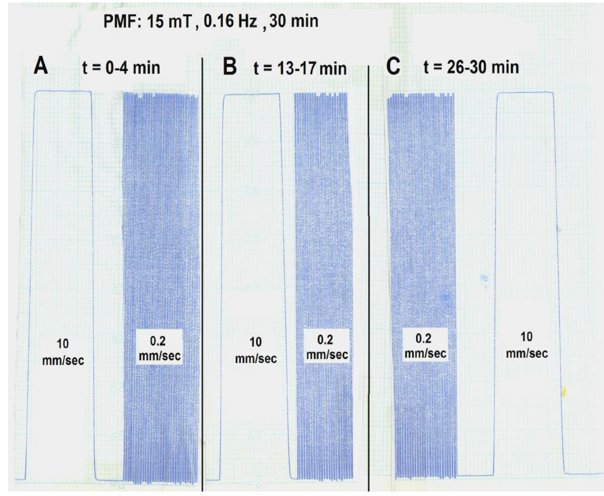
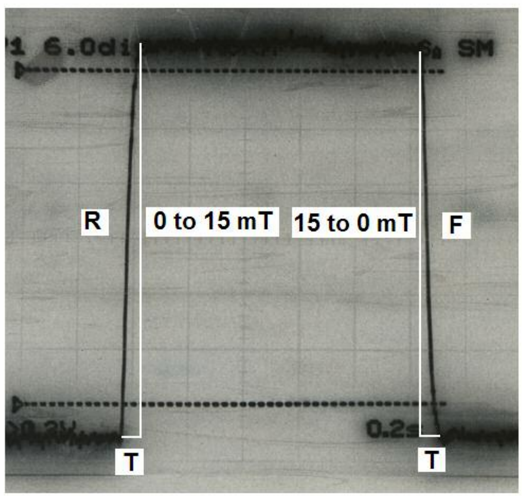
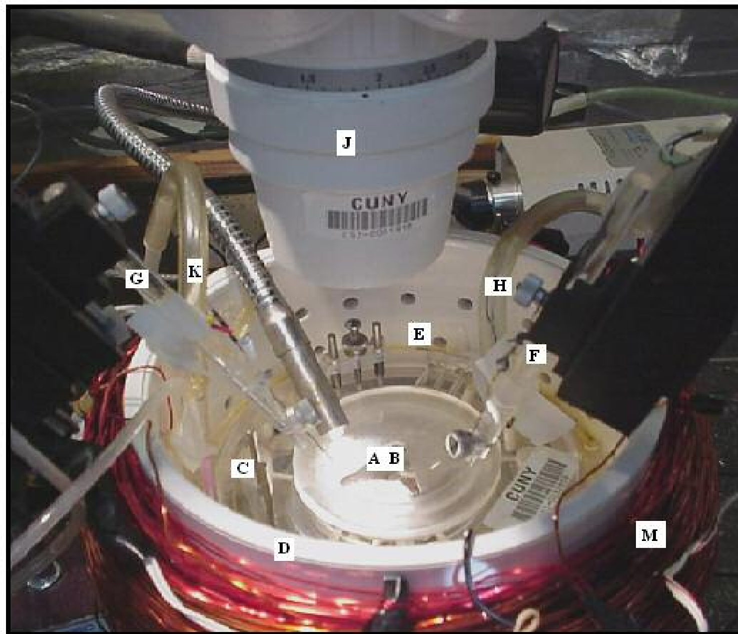


Fig. 2.3. A photograph of the PMF pulse taken from oscilloscope screen. It shows the rising phase (R), and falling phase (F). The white lines (T) are vertical line were drown to calculate the slope of the rising and falling phases of magnetic pulse (black trace).





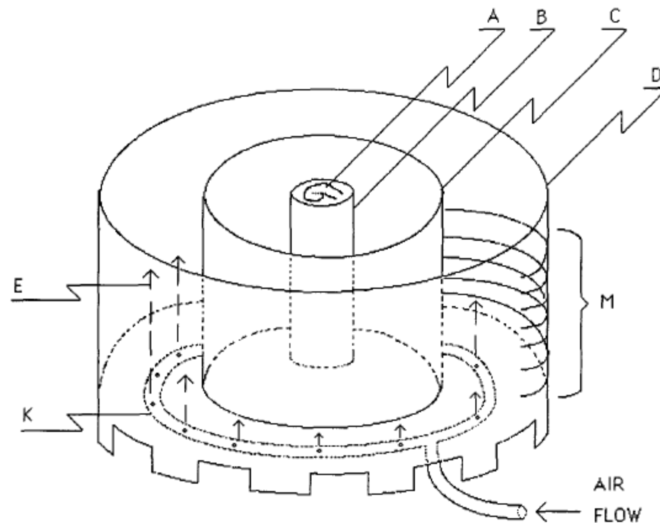


Fig. 2.4. A photograph and a diagram of the experimental test system. A photograph shows a superior view of the experimental test system, which includes the slice (A) and the slice chamber (B) that is filled with Ringer's solution and aired with 95%/5% O2/CO2. The water chamber (C) contains the temperature sensor (H) and the oxygenating airflow (comes through the water inside that chamber). The magnetic coil (M) encircled the acrylic frame (D). During the magnetic field application, airflow from the building system circulating through a holed tube (K) is turned on, creating an air flow (E) to dissipate any accumulated heat. The stimulating electrode (G), the recording electrode (F), and the dissection microscope (J) are shown as well.

Application of rTMS in vivo

The magnetic stimulation MagStim Rapid (MagStim, U.K.) used in our laboratory was equipped with 5 cm coil and could generate biphasic magnetic fields of up to 3.6 T. The pulse duration was 250 µsec and the magnetic field rising time was 60 µsec. The pulses were delivered either continuously, or in trains. At 50% power (approximately 1.8 T according to the manufacturer's specifications) 281 pulses can be delivered with a frequency of up to 30 Hz before the coil becomes overheated. Sixty four mice of the CD-1 strain (4-6 weeks of age) were used for the experiments. Since we had not observed any correlation between the sex of the animal and rTMS effects, the animals of both sexes were used in the current project. Half of the animals were used for behavioral studies and the second half for in vitro experiments. The mice used for behavioral studies and for in vitro experiments were divided into 4 groups (8 animals /group), and each group was exposed to a different paradigm of rTMS. While the control group did not receive any stimulation, each one of the experimental groups was exposed to rTMS of different frequencies (1, 8 or 15 Hz). The animals were held by hand and the coil was placed just above the head of the animal (fig. 2.5), as described by others (Belmaker et al., 2000; Ogiue-Ikeda et al., 2003). Each group received 450 pulses which were delivered in the following way: a) 15 Hz group: 6 trains lasting 5 sec delivered every 15 sec, 75 pulses in each train; b) 8Hz group: 6 trains lasting 9.5 sec repeated every 17 sec, 75 pulses in each train; c) 1 Hz group: 450 pulses delivered continuously for 7.5 min. The strength of the stimulation was 50% in all groups. Following exposure to rTMS one set of 4 groups of mice was tested in behavioral tests and the second set of animals was used to prepare

hippocampal slices. The control animals were held by hand for the same time period as the experimental group with the inactive magnetic coil placed just above the head.

The Behavioral test

In order to evaluate the influence of rTMS on memory retention we used a Novel Recognition Test (NOR), which has been suggested to involve hippocampus (Hammond et al., 2004). Each animal was individually placed in a box (40 cm x 30 cm x25) containing two objects located at a distance of 32 cm from each other. Each mouse was allowed to explore both objects 30 min per day for three days. Then one of the familiar objects was replaced with a new one and the time spent by the animals exploring both objects was recorded once for 5 min. The animals were divided into four groups. While half of the animals in each group were exposed to rTMS as described above, the second half served as non-stimulated controls. All rTMS stimulated animals were given the NOR test immediately, 1 hr and 3 days after rTMS exposure. The ability of the animals to distinguish between familiar (F) and non-familiar (N) objects was expressed as a recognition index calculated according to DeLima and collaborators (2005). The time spent in exploring either familiar or non-familiar objects, was expressed as a percentage of the total time spent exploring both objects in order to produce a relative percent preference for novelty score. A significant increase in the preference score of either object (novel or familiar) could be interpreted as a novelty response. However, insignificance of the preference score would be interpreted as memory impairment. Since the mice were not marked, the time spent on investigating familiar and non-familiar objects was averaged and taken for calculation for each of the group.

In vitro testing

Long-Term Potentiation (LTP) was induced by the application of high frequency stimulation (HFS: 100 pulses/sec for 1 sec repeated 3 times every 10 sec). The induction of LTP was considered to be successful, if the population spike was enhanced by at least 20% for no less than 30 min. The success rate of LTP induction was calculated by dividing the number of slices that showed LTP by the total number of slices in a given group. As an average, 2 -3 slices were tested from each animal. The slices from the control and rTMS exposed animals were always prepared and tested on the same day.

Statistical analysis

Paired t test was used to evaluate the difference between two groups and ANOVA analysis was used for the experiments consisting of more than two groups. Non-parametric tests such as Wilcoxon signed-ranks test was also used in the case of the population data that were categorical or were not normally distributed.

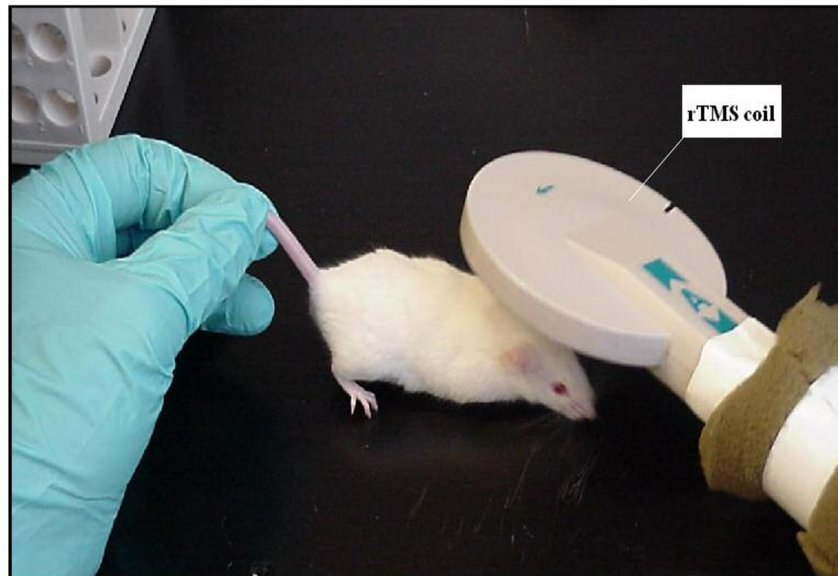


Fig. 2.5. Mouse being exposure to rTMS. The exposure lasted on average 7 – 15 minutes. Since the animals did not move much during the procedure, it was possible to focus the strongest magnetic field (the rim of the electrode) on the animal's head.

The Chemicals Used

The NMDA receptor competitive and selective antagonist, DL-amino-5-phosphonovaleric acid (APV) was purchased from (Sigma USA). Electrical synapse blocker carbenoxolone (cbx) (3β – hydroxyl – 11 –oxoolean – 12 –en – 30 – oic acid, disodium salt) was purchased from (Sigma USA). We have used non-NMDA receptors (AMPA and kainic acid) blocker 6-cyano-7-nitroquinoxaline-2,3-dione (CNQX), purchased from Tocris USA. Tetraethylammonium chloride (TEA), minimum of 98% and 4-Aminopyridine (4-AP) was used to block potassium channels. Both drugs were purchased from Sigma. To block GABAa receptors, SR 95531 hydrobromide, purchased from Tocris,USA, was used. To block glutamatergic synaptic transmission, Kynurenic acid (4-Hydroxyquinoline-2-carboxylic acid), Sigma, was used. Furthermore, Lidocaine hydrochloide (Sigma) tetrodotoxin (TTX) (Tocris) and veratridine (Sigma) were used to block sodium channels. Ascorbic acid (Sigma), (±)-α-Tocopherol (vitamin E) (Sigma) and Diphenylene iodonium chloride (Sigma) were used as free radical scavengers. A protein kinase A inhibitor (fragment 12-22, myristoylated trifluoracetate salt) (Sigma) and a protein kinase C inhibitor (P-1614) (Sigma) were used to inhibit both PKA and PKC kinases. All of these chemicals, except vitamin E, which was dissolved in 0.03% ethanol, were dissolved in a Ringer's solution.

Chapter 3 – Results

The goal of my dissertation was to characterize the effect of a magnetic field on the nervous system as well as to investigate the cellular mechanism mediating this effect. To achieve this goal, electrophysiological, pharmacological and behavioral methods were used

Comparing the mechanisms of LTP and the mechanisms of PMF-induced amplification of the population spike

The following set of experiments was designed to explore the nature of the amplification in population spike (PS) induced by the exposure to pulsed magnetic field (PMF) (Wieraszko 2004). Both high frequency stimulation (HFS) and PMF induce long-term enhancement in the excitability of the hippocampal neurons. To find out if the PMF-induced effect on excitability has similar or different mechanisms as HFS-induced LTP, we performed 15 experiments on different hippocampal slices. The slices were exposed to both treatments in turn: HFS and then PMF. A single experiment presented in *fig 3.1* shows the change in the excitability of the hippocampal neurons exposed to high frequency stimulation and pulsed magnetic fields. The HFS induced amplification of the population spike was further increased by the exposure to PMF. It is important to note here, that HFS was applied at least 30 minutes before the application of PMF. While a PMF-induced increase in excitability was observed in 100% of the slices, LTP only occurred in about 60 to 70 % of the slices. Thus, we inferred that PMF has induced the PS amplification even in the slices that did not express LTP. The PMF-induced amplification of population spike amplitude was accompanied by a pronounced increase

in the rate of spontaneous activity shown in *fig 3.1B*. However, no change in the rate was observed during the expression of LTP.

The PMF exposure induced detrimental seizure-like activity in 6 out of the total of 15 slices. The seizure- like activity usually started 15 minutes after the initiation of PMF exposure. Therefore, the electrophysiological recordings could not be completed and the results from these slices were not included for data analysis. Moreover, prior induction of LTP did not change the percentage of the slices demonstrating PMF-induced seizure activity. The seizure-like activity was expressed either as an increase in the firing rate, which is called epileptic field potential (EFP), or/and as multiple firing (increase in burst duration). Both forms of epileptic changes were observed in the slices exposed to PMF.

The average results of the 9 experiments (the remaining of the 15) are shown in *fig 3.2*. Although HFS induced a permanent, statistically significant increase in the amplitude of the population spike (18.5%, $p<0.05$), PMF exposure enhanced the population spike even further (326.4%, $p<0.0006$), implying that the HFS- and PMF-induced increases in the neuronal excitability were additive and the mechanisms involved in HFS-induced amplification (LTP) and PMF-induced enhancement of the population spike are different.

One of the most popular forms of LTP expressed in the hippocampus depends on the activation of the NMDA receptor. Since there was no overlap between the PMF-induced amplification and LTP, I assumed that the NMDA receptor was not involved in PMF-induced enhancement of neuronal excitability. To verify this assumption, the NMDA receptor was blocked with a competitive and selective antagonist, DL-amino-5-

phosphonovaleric acid (APV) (50µM). A representative experiment is shown in Fig 3.3A. The upper part of the diagram shows the shape of the potentials recorded 10-min prior (a) and 10-min after (b) application of HFS. As expected, LTP was not induced in the presence of APV. However, the subsequent exposure to PMF significantly amplified the population spike (*Fig 3.3A*, potential c). The average results of the 9 experiments presented in *Fig 3.3B* demonstrate that while the initiation of LTP was prevented, the PMF-induced amplification was similar to that observed when the NMDA receptor was not blocked (*Fig 3.1A*). These results support our previous assumption, that the NMDA receptor is not involved in the mechanism of PMF-induced amplification.

Interestingly enough, in the presence of the NMDA receptors, antagonist PMF-induced PS amplification and the epileptic field potential (EFP) were more readily induced (after 12 to 14 minutes) as compared to the control experiments (after 25 – 30 minutes).

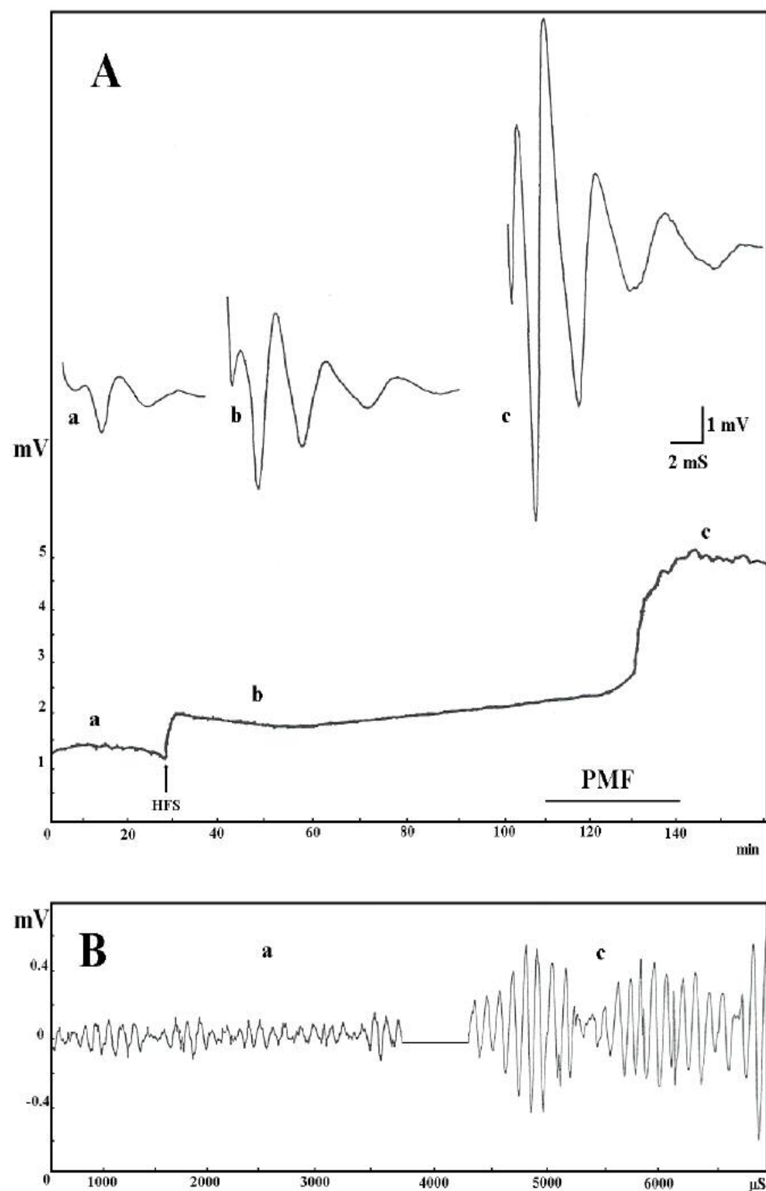


Fig.3.1. Changes in the excitability of hippocampal neurons exposed to high frequency stimulation (HFS) and pulsed magnetic fields (PMF). A - The amplification of the population spike by the application of HFS and exposure to PMF. The upper part of the figure shows the shape of the individual control potentials (a), the potentials recorded after application of HFS (b) and following exposure to PMF (c). Note that the HFS induced permanent amplification of the population spike was further enhanced by exposure to the PMF. The lower part of the figure depicts changes in the magnitude of the potential during the entire experiment. The horizontal bar under the graph illustrates the duration of the exposure to magnetic fields. B -the spontaneous activity of neurons in hippocampal slices exposed to PMF. The left and the right part of the graph show the spontaneous activity recorded before (a) and after (b) exposure to PMF, respectively.

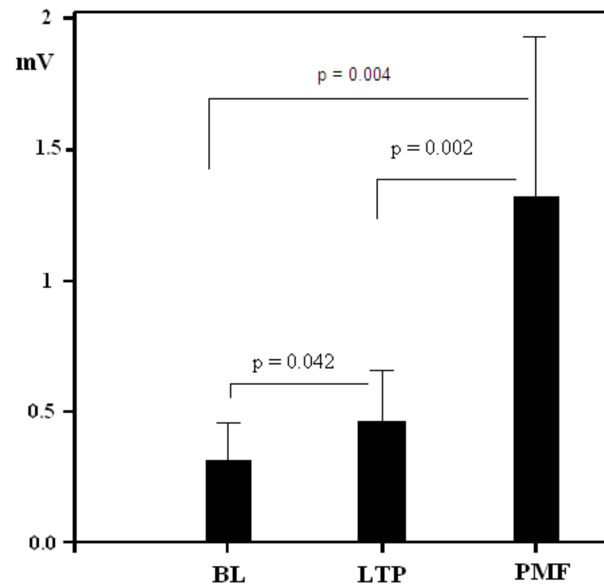


Fig. 3.2. The amplification of the population spikes by HFS and exposure to PMF. The averages of the population spikes were recorded during the baseline period (BL), following the induction of LTP (LTP) and after subsequent exposure to PMF (PMF) (n = 9). There are significant differences between control and LTP group (p = 0.042), between control and PMF group (p = 0.004) and between LTP and PMF group (p = 0.002). Paired t test was used.

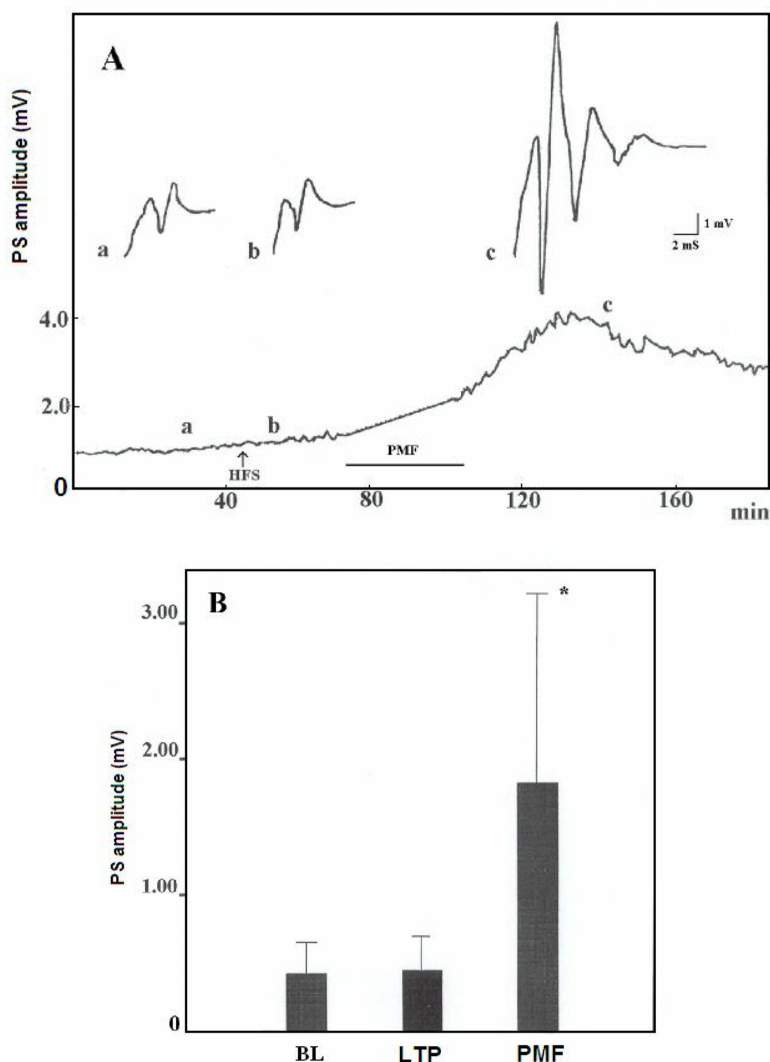


Fig 3.3. Exposure of hippocampal slices to PMF in the presence of APV, an antagonist of the NMDA receptor. A- An example of an individual experiment. The application of HFS does not induce LTP in the presence of NMDA receptor antagonists (compare potentials a and b). However, the subsequent exposure of the slices to PMF amplifies the population spike (potential c). HFS and PMF indicate the application of high frequency stimulation and the exposure to pulsed magnetic fields, respectively. The horizontal bar illustrates the duration of PMF exposure. Note that PMF has induced seizure activity, which is shown as an increase in the spike duration (measured as the duration from the peak of the first spike to the afterward DC line). B - Average results of 9 experiments demonstrating PMF-induced amplification of the population spike in the presence of an antagonist of the NMDA receptor. BL, LTP and PMF refer to measurements taken from the baseline period, after the application of HFS and after the PMF exposure, respectively. * denotes the difference between PMF and LTP ($p<0.05$).

<u>PMF and gap junction of the hippocampus:</u>

In my continuing efforts to reveal the mechanism of PMF-induced amplification, I turned my attention to electrical synapses. These synapses can increase synchronized discharges of hippocampal pyramidal neurons, contribute to generation of seizure activity (Carlen et al., 2000; Traub et al., 2002), and participate in the PMF-induced excitability. To test this idea I used carbenoxolone (cbx) (50µM), a blocker of electrical synapses (LeBeau et al., 2003). Cbx was added to the slice-containing chamber at the end of exposure to PMF. That then reduced PS amplitude significantly compared to PS amplitude after PMF exposure, but was still significantly higher than PS amplitude before PMF application. Furthermore, the reduction of the PS amplitude was accompanied by two changes: 1) a dramatic decrease in the spontaneous cell firing and 2) complete suppression of seizure activity. It is shown in figure 3.4 A, B and C.

It became apparent that the exposure to PMF activates electrical synapses, enhancing synchronized discharges of hippocampal neurons leading me to conclude that the PMF effect on the hippocampal slices is probably mediated through the two types of synapses (electrical and chemical).

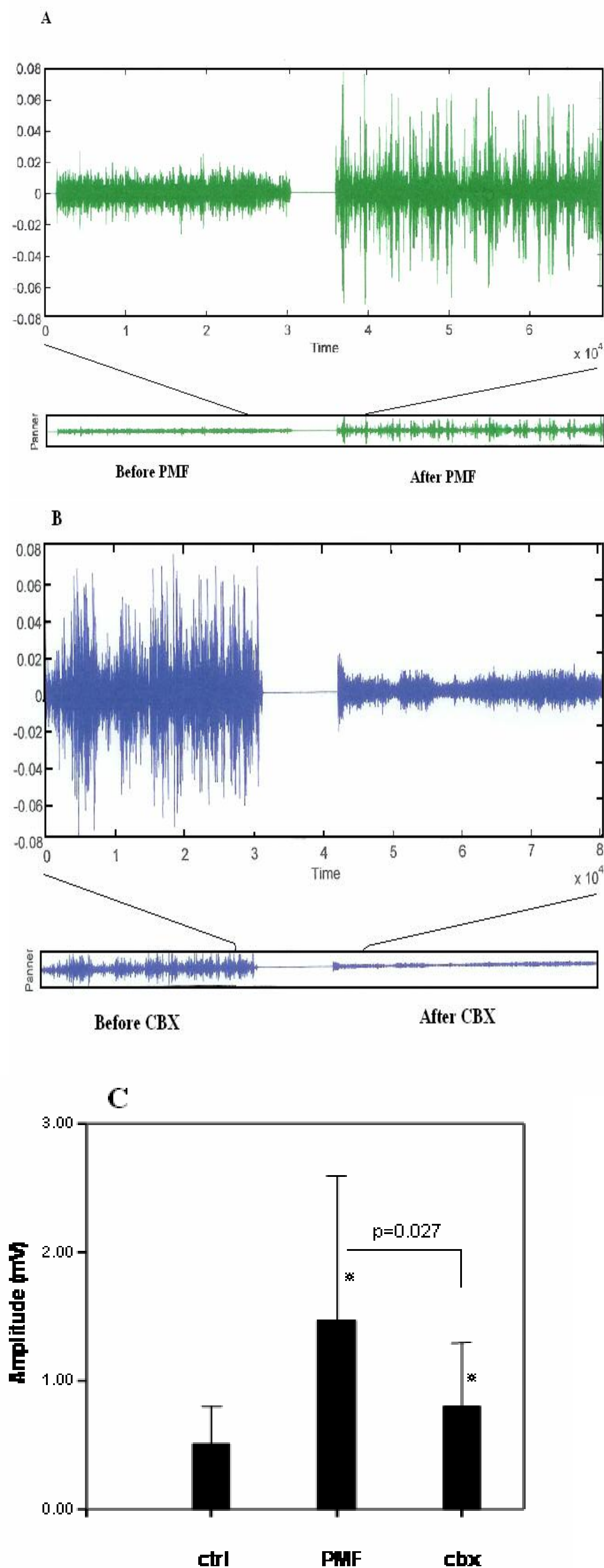


Fig.3.4. The role of the electrical synapse in the PMF-induced amplification of population spike and the PMF-induced spontaneous firing. A – the graph shows the intensity of the spontaneous firing before exposure to PMF(left), and the increase in this firing rate 30 minutes after the exposure to PMF(right). B – The graph shows the intensity of firing after the exposure to PMF (left), which was abolished by Cbx (carbenoxolone) (right). C - The average results of 6 experiments demonstrating a cbx-induced attenuation of the population spike, which has been previously amplified by the exposure to PMF. *denote statistical significant.

Effects of PMF on paired pulse facilitation (PPF)

The population spike reflects the number of active pyramidal neurons. Its increase may occur due to the changes in either presynaptic or postsynaptic mechanisms. In order to determine the synaptic location of the mechanism involved in PMF-induced enhancement of the population spike, we employed the PPF paradigm of stimulation. Two impulses, given in a rapid succession (25 - 55 ms interval), result in the greater amplification of the second population spike as compared to the first one. We can assume in general that changes in the PPF are related to modifications in the presynaptic mechanisms.

Although conventionally PPF and PPI be measured as either the ratio (PS1 divided by PS2) (Gottschalk et al., 1998; Saar et al., 1999) or as a percent of PS1 (the difference between PS2 and PS1 divided by PS1) (Kokaia et al., 1999), here, the PPF was calculated as the absolute difference between two population spikes (PS2 minus PS1). An increase in the second population spike relative to the first will produce higher PPF. The expression of PPF as the absolute difference rather than the relative difference has been chosen, since PMF increased both PS1 and PS2 (*figure 3.5*). That would change the reference value (PS1amplitude) between the baseline with that of PMF. Therefore, the relative difference may or may not show significant results when it comes to PPF and PPI and it may reduce the variability. However, a significant result from either the absolute or the relative difference may lead to a distinct interpretation of the data.

The experiment was initiated by collecting PS for 20 minutes during stable recording prior the exposure to PMF. The average of the PPF measurements that were calculated from the baseline period was compared to the averages taken from the

recordings after the exposure to PMF. The result of ten experiments showed significant increase in PPF after the PMF exposure (n=10, 150 %, p=0.000317, paired sample t test was used). Note, that although the magnitude of both population spikes increased following PMF exposure, the facilitation of the second one exceeded the facilitation of the first (See *figure 3.5*).

To determine if PMF-induced enhancement of PPF was related to the magnitude of the initial PPF, we calculated the change in PPF as the final PPF (the average PPF during the maximal effect of PMF) minus the initial PPF (the average PPF during the baseline period). A linear regression test (*figure 3.6*) revealed that there was no correlation between the change in PPF and the magnitude of the initial PPF (n=11, p>0.810, R=0.082). This may suggest that amplification of PPF was not due to PMF-induced increase in the number of firing neurons, but may be due to an increase in the excitability of the same population of neurons, which were active before PMF exposure.

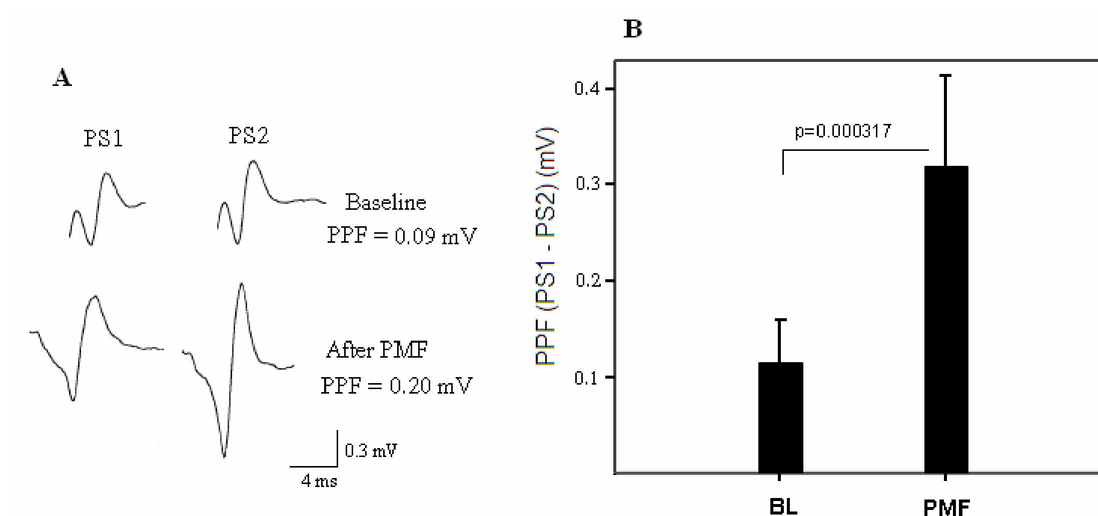


Fig 3.5.The influence of PMF on paired pulsed facilitation. A - Potentials marked PS1 and PS2 correspond to the first and second evoked potentials, respectively and were initiated by two consecutive pulses applied with a frequency of 0.03 Hz; inter pulse intervals (IPI) was adjusted accordingly to get the largest PPF (25 – 55 ms). The PPF was calculated as PS2 minus PS1. The upper and lower potentials represent potentials that were recorded during the baseline and after the PMF exposure, respectively. PPF was increased by about 120% after the exposure to PMF. Note, that both PS1 and PS2 increased after exposure. B - the two bars show the average changes in PPF after the exposure to PMF applied at the frequency of 0.16 Hz (n = 11). The baseline bar (BL) represents the average of PPF from the first 10 - 15 minutes before the application of PMF. The PMF bar represents the average of PPF during maximal effect of PMF amplification. Pulsed magnetic field has induced significant increase in PPF. The result of the paired t test is shown in the graph.

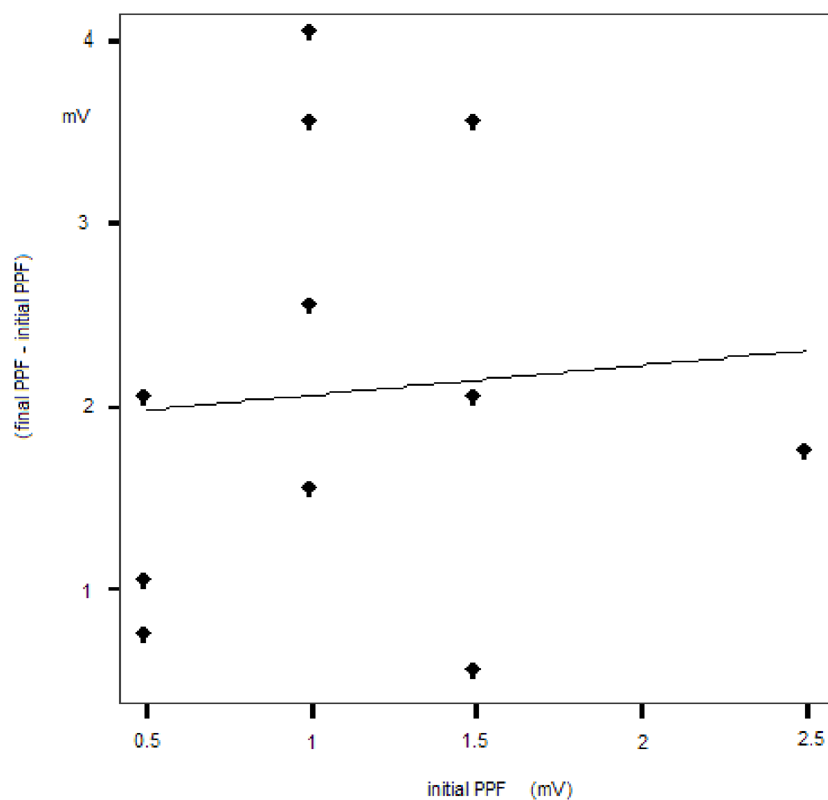


Fig. 3.6. The difference between the initial PPF (during the baseline) and the final PPF (after PMF exposure) was not dependent on the initial PPF. Plotting the difference on the Y-axis and the initial PPF on the X-axis showed no significant in a linear regression (n=11, p=0.810, R=.082).

Effects of PMF on paired pulse inhibition (PPI)

To investigate the effect of PMF on PPI, I used a two stimuli paradigm with inter pulse interval (IPI) less than 12 ms. This paradigm, which evaluates the efficacy of the inhibitory loop reduces the amplitude of the second PS or EPSP. The inhibitory loop can be axosomatic or axodentritic (fig. 2.1 A). Therefore, in two types of experiments both fEPSP and population spike were recorded. In these experiments, 8 and 11 slices were used in the EPSP and the population spikes experiments, respectively. Following 20 minutes of stable recording, PMF were applied for 30 minutes. The averages of PPI taken from the baseline were compared to the averages taken after the exposure to PMF. PMF increased the PPI of both fEPSP and population spike significantly ($n = 11$, $p<0.05$, paired t test). The PMF-induced increase in PPI occurred due to the relative increase in the difference between the two responses (PS1 – PS2) or (fEPSP1 – fEPSP2). As in the case of PPF, both PS1 and PS2 amplitudes increased after exposure to PMF. However, in opposition to PPF, the enhancement of a second response was much smaller, than the first we therefore concluded, that PMF exposure stimulates the activity of excitatory and inhibitory neurons. This is presented in Fig.3.7.B, as an increase in the difference between the amplitudes of both population spikes, and in the slope of both fEPSPs.

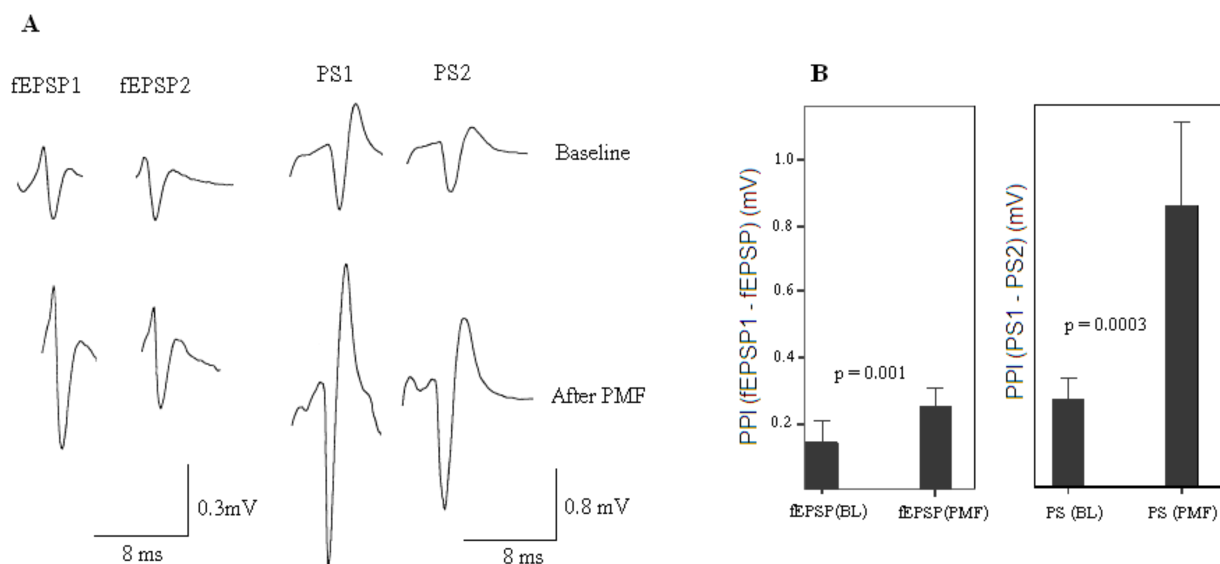


Fig.3.7. The Influence of PMF on PPI. As in the case of PPF, the amplitude of PPI was calculated as the difference between the first and second responses. A – The potentials marked fEPSP1 and PS1 correspond to the first field excitatory post synaptic potential and the first population spike, respectively, and fEPSP2 and PS2 correspond to the second field excitatory post synaptic potential and the second population spike, respectively. B – Bars represent the average changes in PPI in the baseline (BL) and after PMF exposure (PMF). Pulsed magnetic field has increased PPI in hippocampal slices significantly (n (fEPSP) = 8, n (PS) = 11), the p values are shown in the figure, which was obtained by the paired t test between groups.

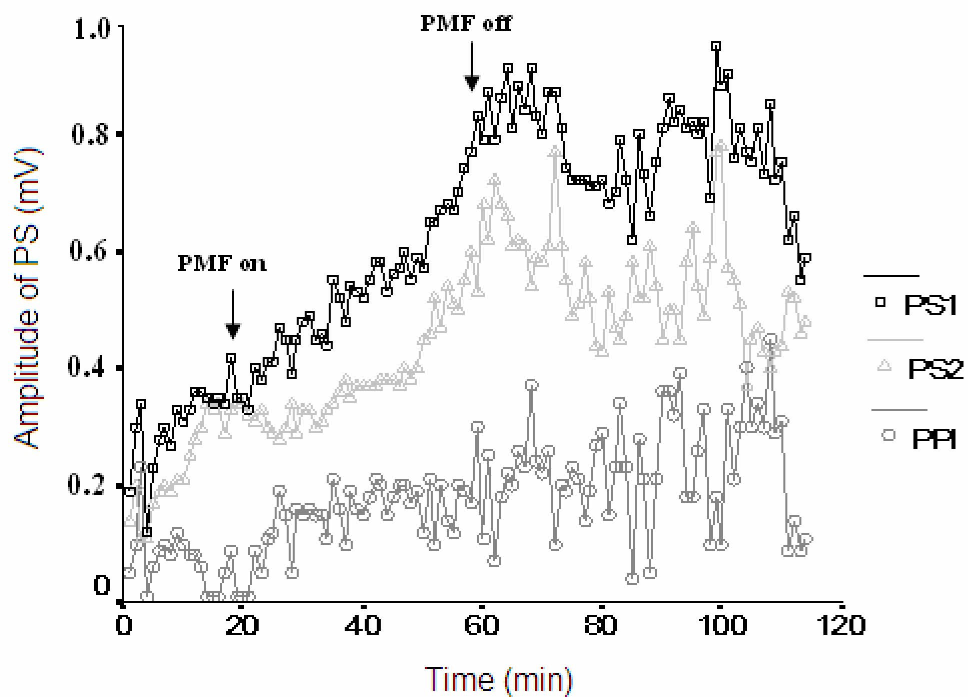


Fig.3.8. A representative experiment demonstrating the enhancement of PPI by PMF exposure. The first 20 minutes shows the PPI before the exposure to PMF. The first and the second arrows indicate the initiation and the termination of the PMF exposure, respectively. PS1 and PS2 indicate the first and the second population spikes, respectively. The PPI (open circle) indicates the calculated paired pulse inhibition (PS1 – PS2). Note that PMF has simultaneously increased PS1, PS2, and PPI.

PPI was a result of a simultaneous, but unequal increase in both spikes, as it is shown in *figure 3.8.* This conclusion is supported by the linear regression in *figure 3.9*. In this graph, 115 paired spikes evoked potentials from one experiment were compared. PMF caused positive correlation between PS1 and PS2. This positive correlation does not express a decrease in inhibition, but indicates that there was an increase in the amplitude of both evoked potentials. Simultaneously, the absolute difference between the paired evoked potentials was increased (increased inhibition).

Control experiments were performed in order to determine the correlation between enhancement of the first and second response without participation of PMF. To elevate the amplitude of the first response the strength of the electrical stimulation was increased. Although this paradigm led to an increase in the first spike, it reduced the amplitude of the second one (opposite to the effect observed after PMF exposure). See *figure 3.11.* Using SR95331 hydrobromide, to block GABAa receptors, reduced the amplitude of PPI partially (Fig. 3.11B). Moreover, in the controlled condition, where there was no change in the strength of electrical stimulation strength or addition of drugs, the correlation between the first and the second population spikes was negative *figure 3.10*.

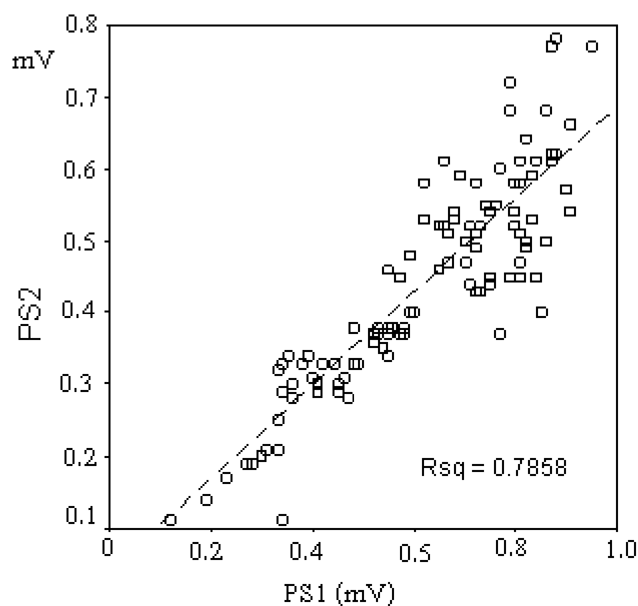


Fig.3.9. This figure demonstrates a representative experiment of 115 spikes recorded during and after the exposure to PMF. The first population spike was (PS1) plotted on the X-axis and the second population spike (PS2) was plotted on the y-axis. A linear regression line shows a strong correlation between the two spikes (r = 0.886, p = 0.0003).

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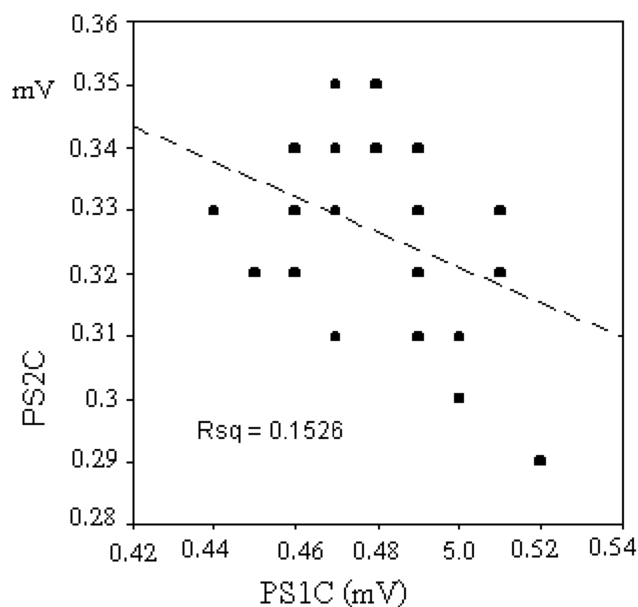


Fig.3.10 The inverse correlation between PS1 and PS2 in controls are as shown here. The figure demonstrates an inverse correlation between the amplitude of the 1st (PS1C) and the 2nd (PS2C) population spikes (9ms ISI). Linear regression, n = 33 spikes, r = 0.391, p = 0.030.

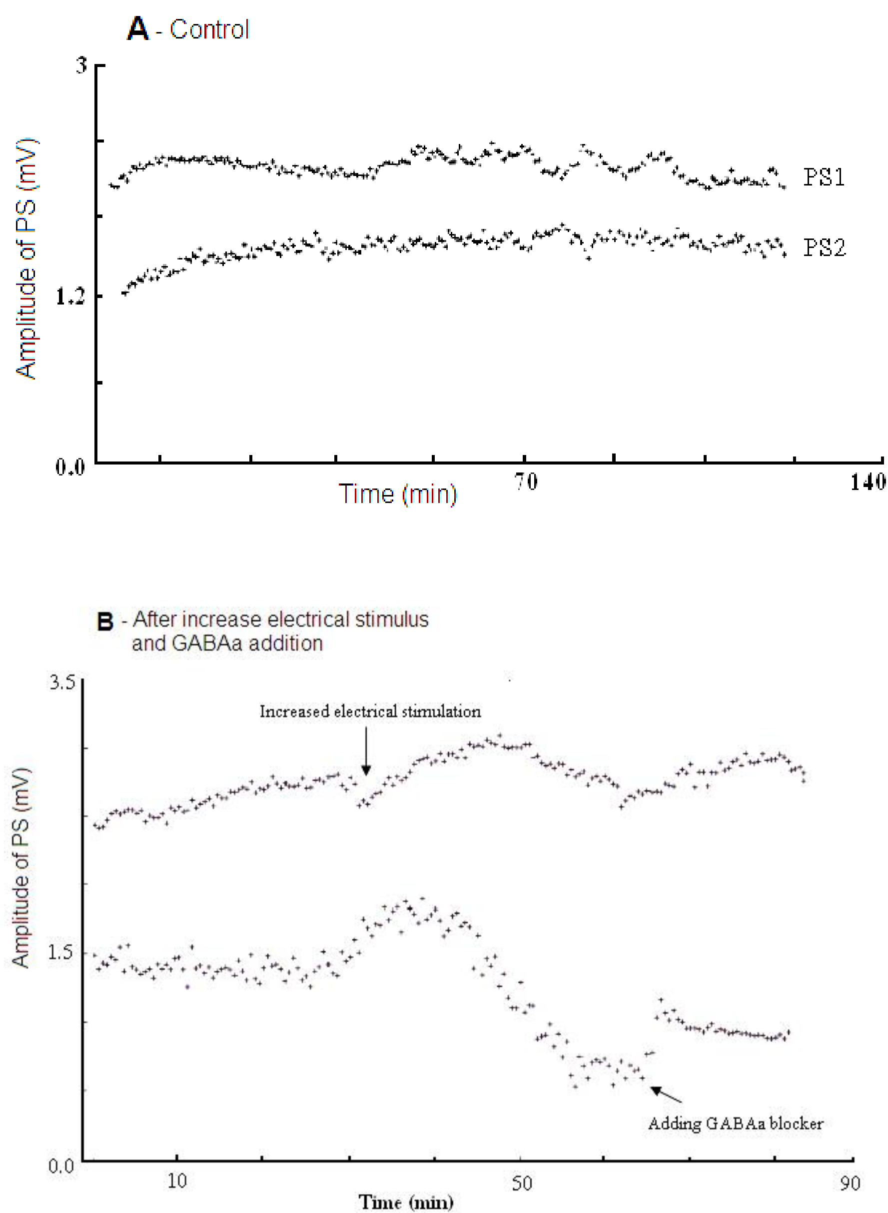


Fig.3.11. The influence of factors other than PMF on PPF. A – Control experiment showing the stability of both PS1 and PS2 over long recording time. B – The influence of increased electrical stimulation and subsequent addition of GABAa receptor blocker (SR 95531 hydrobromide), (400uM) on PPF.

The effect of PMF on antidromic evoked population spikes in the hippocampal slices

To inquire into the mechanisms of PMF, we considered an experimental paradigm that would express the action potential without concomitant involvement from synaptic transmission. Stimulating the hippocampal pyramidal cell layer CA1 with antidromic stimulus (stimulating electrode on the subiculum, recording electrode on the pyramidal cell layer CA1) (Fig.2.1) would induce pre-synaptic action potentials only. The addition of kynurenic acid to the slice containing chamber and the elimination of calcium would eliminate any contribution of glutamatergic synaptic transmission to either the initiation or size of recording potentials. To maintain a calcium free environment, the Ringer's solution of the slice incubation chamber was replaced by fresh calcium free Ringer's solution every 15 minutes.

I performed 15 experiments on 15 different slices taken from 5 animals. The potentials were recorded for 20 minutes to create a stable baseline, and then a PMF of 0.16 Hz was turned on for 30 minutes. Averages from the baseline and at the maximal effect of PMF (20 potentials) were compared. The PMF has not only increased significantly the size of population spikes (n=15, 183.3%, p=0.0004, *figure 3.12 C*) but also induced multiple firings and seizure like activity (*figure 3.12 B).*

To verify the assumption that the PMF-induced effect does not depend on the initial excitability of the slice, expressed as the amplitude of the population spikes, I analyzed the same previous 15 experiments using scatter plot. As it is shown in *figure 3.14*, there is no correlation between the amplitude of the baseline (averages) and the amplitude of the change (the PS amplitude after PMF exposure minus the PS amplitude of baseline) after the exposure to PMF. That means that the effect of PMF was

independent of the amplitude of the initial population spike. In *figure 3.14*, the change and the absolute values after PMF exposure were plotted on the x-axis and the absolute values of the baseline on the y- axis. The graph shows that the change after the PMF exposure shifted toward the left and away from the PMF-induced amplification values, indicating that the PMF effect does not depend on the initial amplitude of the evoked population spikes. Moreover, the PMF effect can be quantified by using a histogram such as the one in *figure 3.15,* that shows a mean value for the change caused by the PMF, (0.390 ± 0.205 mV).

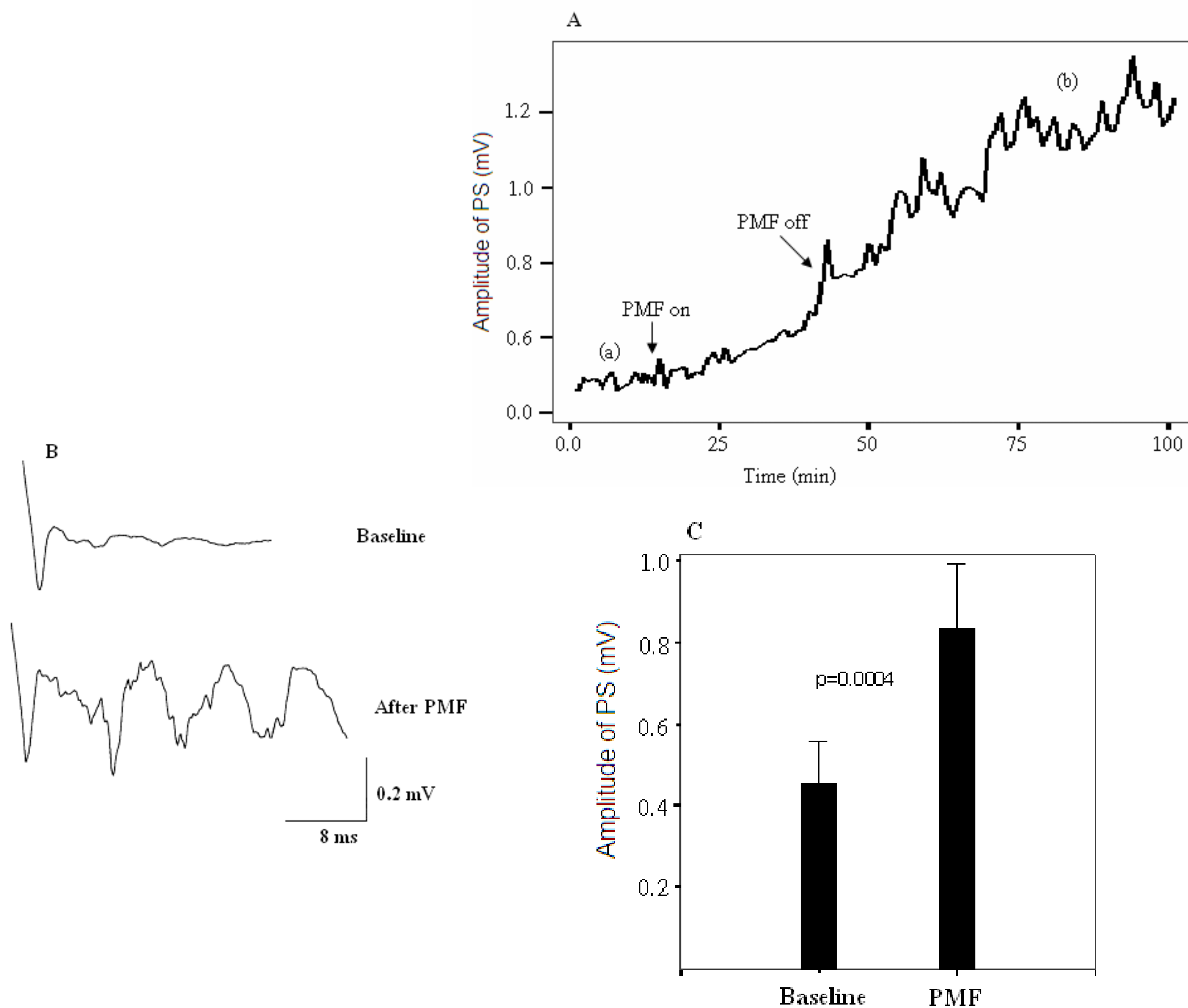


Fig.3.12. The effect of PMF on the antidromically evoked population spike. The slices were incubated in a calcium free medium and with 2mM Kynurenic acid. A – is a representative experiment showing the amplitude of the population spikes before (a) and after (b) the application of PMF. B – A waveform that shows the potential amplitude before and after PMF exposure; note the increase in the burst duration. C – Average results of baseline and at maximal PMF effect (n = 15, p = 0.0004). Paired t test was used.

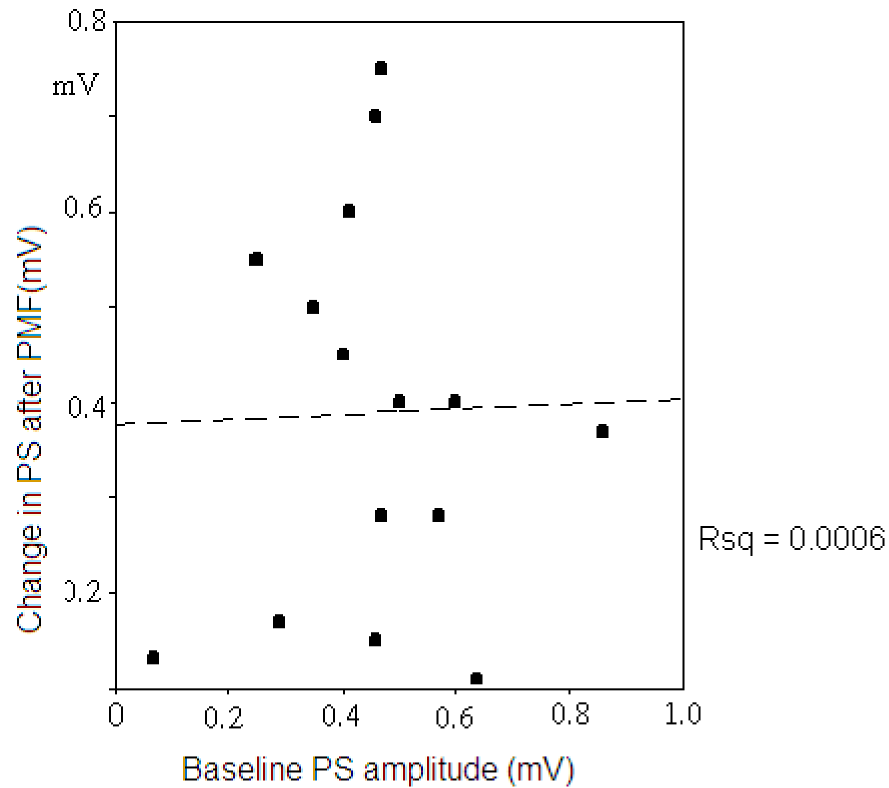


Fig.3.13. The change in population spike (PS) size does not depend on the baseline of the PS amplitude. The change is calculated by subtracting the baseline averages from the averages recorded at the maximal effect of PMF. The graph illustrates that the baseline PS and the change are not correlated (n=15, r=0.004, p=0.932). Note that most of the values are clustered between 0.4 – 0.6 mV, demonstrating that same or close initial PS size can have different amounts of change.

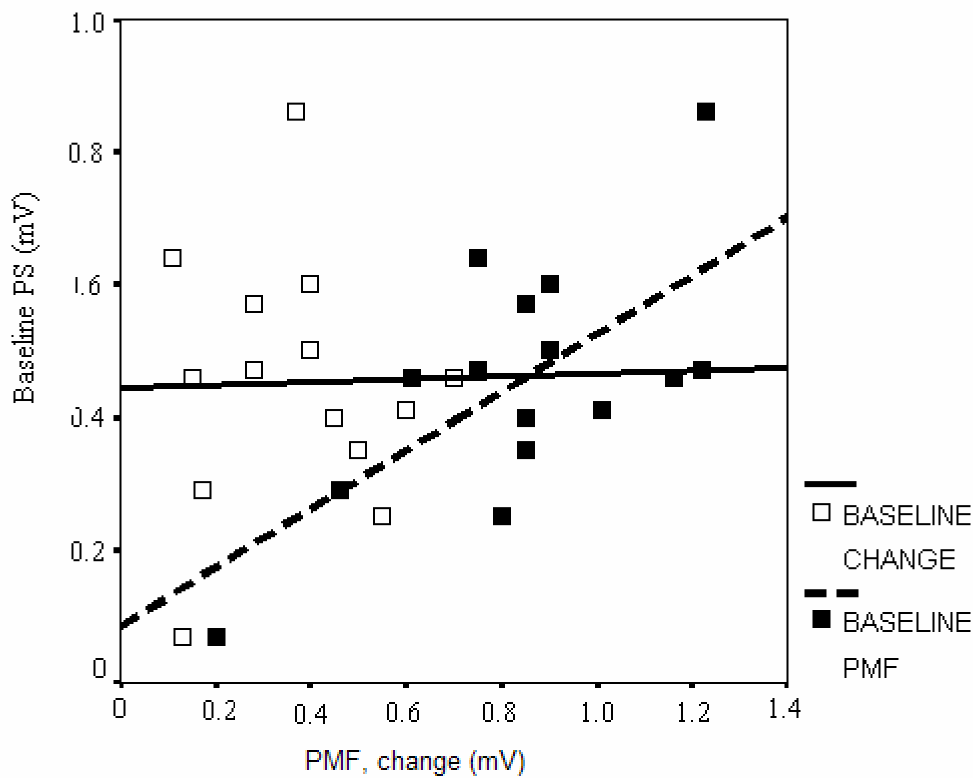


Fig.3.14. This graph demonstrates the correlation between the baseline and the values illustrating the influence of PMF on the population spike. While the change value represents the difference in the amplitude of PS before and after PMF application, (empty square) the PMF value (solid square) expresses the maximal amplitude of the PS after PMF exposure. Both the change and the PMF values were plotted (X-axis) against the PS amplitude of the baseline (Y-axis). Note that the change values after PMF exposure are completely shifted to the left from the PMF values and it is not correlated with the baseline PS amplitude (horizontal line). The PMF value is positively correlated to the baseline amplitude (the dashed line) ($r = 0.438$, $p = 0.007$). This data analyses may suggest that there is no increase in the number of the cells, which fire action potentials, but an increase in the excitability of the same population of neurons. That may hold true when using maximal stimuli, however. At sub-maximal stimuli the increase in both recruitment and excitability may take place.

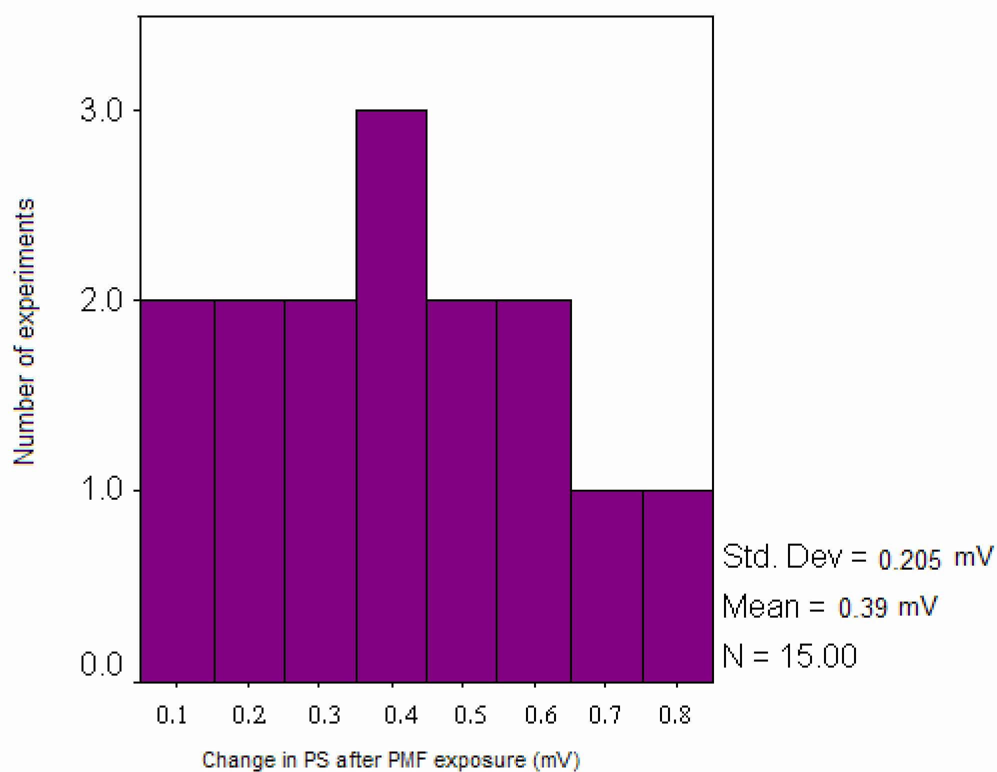


Fig.3.15. The magnitude of PMF-induced effect is predictable. This histogram illustrates the mean value of the change caused by the exposure to PMF (mean=0.39 mV, Std. Dev =0.205 mV). The change was calculated by subtracting the PS amplitude at the maximal effect after the PMF exposure, from the PS amplitude of the baseline of each experiment.

The role of gap junction and potassium channels

There are electrical gap junctions between pyramidal cells in the hippocampus. I assume that while the synaptic transmission was blocked in the experimental design mentioned above, the electrical gap junctions mediated the PMF effect. Therefore, I used the same experimental conditions (2mM kynurenic acid and calcium free medium) and then blocked the electrical gap junction using carbenoxolone (cbx) (50µM). The PMF has significantly increased the antidromically evoked population spikes (n=5, 158.5%, p=0.008*). See figure 3.16.*

Next, I tested the hypothesis that PMF may magnify the evoked population spike by inhibiting the function of potassium channels. Therefore, the potassium channels were blocked by using 1 mM 4-aminopyrimidine (4-AP) shown *in figure 3.17,* 4-AP increased the evoked population spike amplitude significantly (n=9, 145.8%, p=0.01), most likely due to cell hyper-polarization. After the increase due to 4-AP had reached its maximum amplitude and stabilized for at least 20 minutes, the PMF was turned on for 30 minutes. The PMF increased the evoked population spike significantly (208.6%, p = 0.03) of 4-AP, and (312.5%, p = 0.01) of baseline, as well as causing multiple firings (*figure 3.18).*

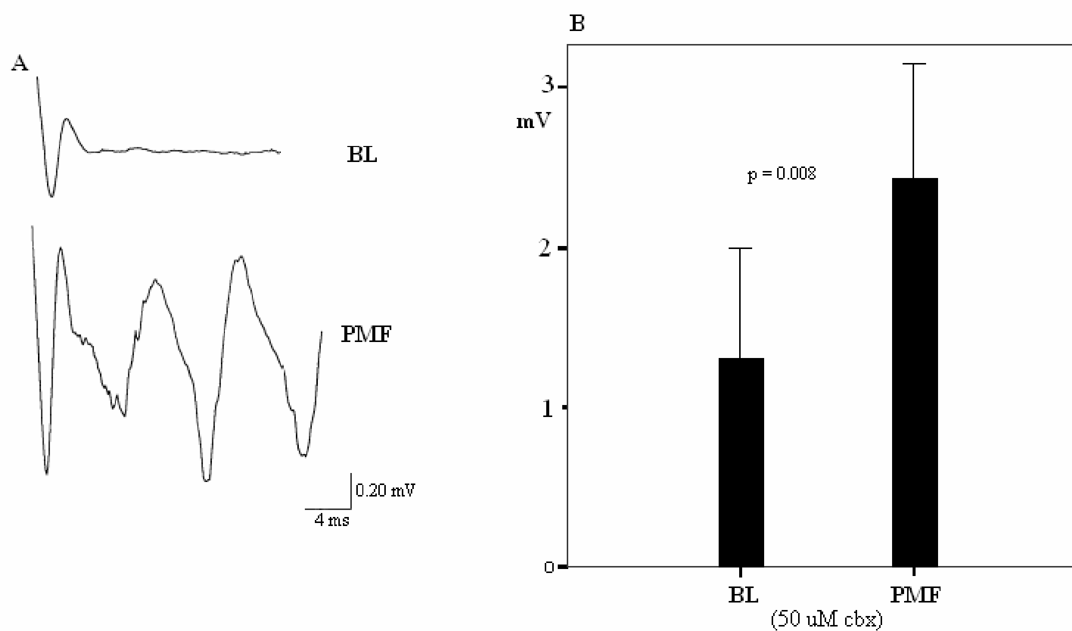


Fig.3.16. The role of gap junction on the PMF-induced effect. The slices were incubated in calcium free Ringer solution and 2mM Kynurenic acid, and cbx was added into the recording chamber. In A, two potentials from the baseline (BL) and after the exposure to PMF (PMF) are shown. These waveforms illustrate two things, one is that PMF increased the amplitude of PS (recorded antidromically), the second is that PMF increased the duration of the PS (repetitive firing). In B, PMF significantly increased population spikes in this preparation (n=5, 158.5%, p = 0.008). This result indicates that gap junction not contribute to PMF-induced increase excitability. However, cbx has reduced the PMF-induced repetitive firing in three of these slices.

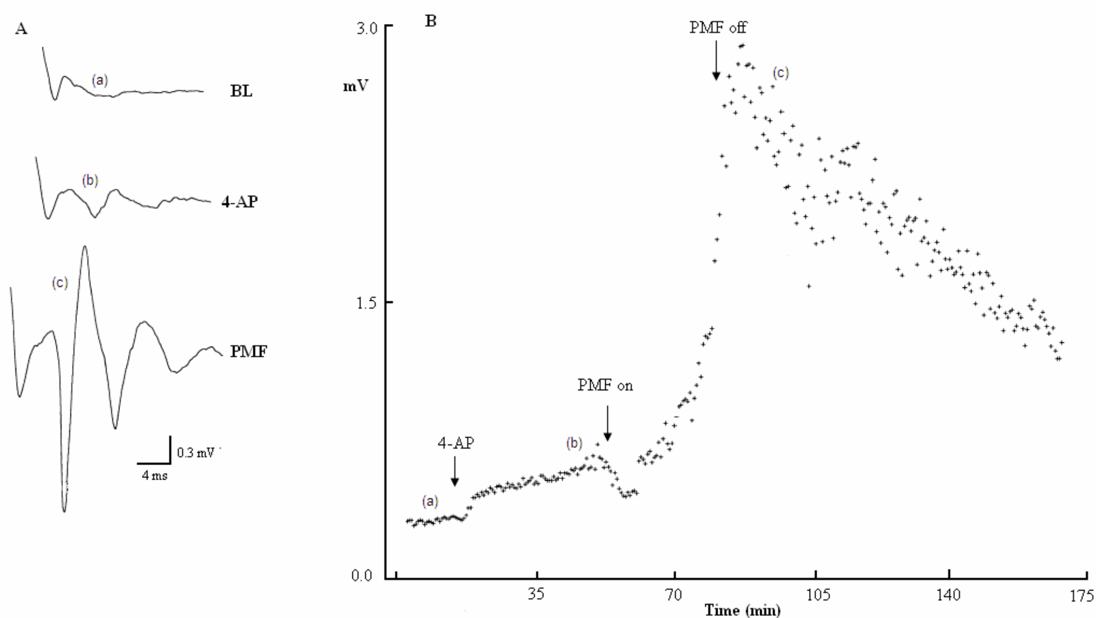


Fig.3.17. A representative experiment shows the effect of 4-AP on antidromically evoked potentials and the result of a subsequent PMF exposure. A – Baseline (a), at the maximal effect after the addition 4-AP (b), and at the maximal effect after PMF application (c). B – The whole experiment is shown here. Arrows indicate the time of treatment (addition of 4-AP and PMF exposure).

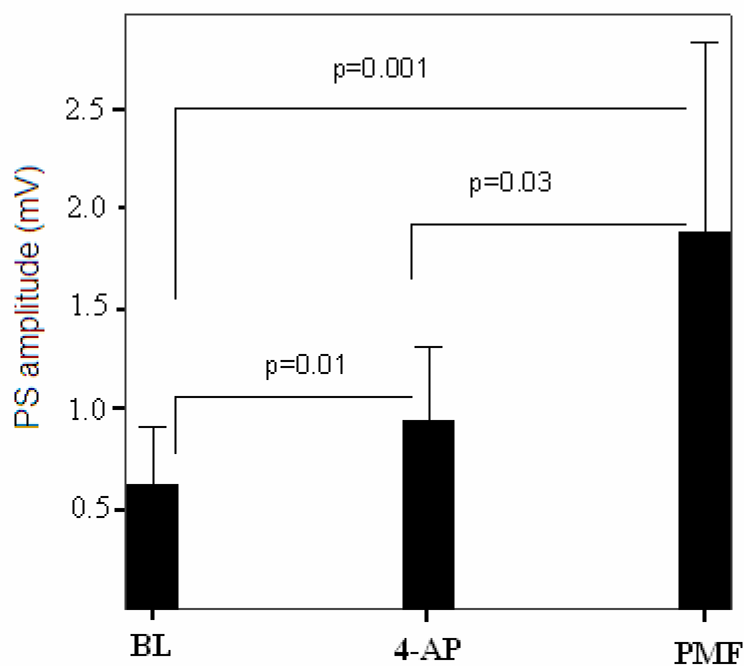


Fig.3.18. The graph illustrates the influence of PMF on antidromic potentials recorded from the hippocampal slices pre-incubated in calcium free Ringer's solution in the presence of kynurenic acid and subsequent addition of 4-AP. The increase induced by PMF exposure was significant (n = 9, 208.6% of 4-AP and 312.5% of baseline). The paired samples t-test result is shown on the graph.

Effects of PMF on compound action potential:

It can be surmised from the preceding experiments that the PMF effect probably influences the mechanism of action potential through changes in its initiation and propagation. Therefore, I decided to investigate the effect of PMF exposure on the sciatic nerve, which represents simpler preparations without any synaptic connections. This simplicity can help narrow the interpretation of the results of the effect of PMF on the component of action potential generation and propagation. The interpretation of the PMF-induced effect could then be circumscribed to the processes responsible for generating, modulating and propagating action potentials. The sciatic nerve contains sodium, potassium, and calcium channels and at least some of these channels (sodium) are known to be modifiable by phosphorylation with protein kinases (PKA and PKC). The other advantage of using the sciatic nerve is its durability, expressed as continuous recording, for many hours with a high level of stability.

Effects of the PMF on the sciatic nerve excitability

To investigate the effect of PMF on the compound action potential recorded from the sciatic nerve, 10 experiments were performed. The averages of the CAP amplitude recorded in the first 20 minutes (we called this period the baseline) as in the case of the hippocampus, were compared to the averages of the CAP amplitude recorded 30 minutes after the exposure to PMF. The PMF increased the mean amplitude of the CAP significantly (n=10, 187.1%, p = 0.005,) (figure *3.20*). The graph in *figure 3.19* shows a complete experiment.

To verify that this increase did not occur spontaneously, we followed the CAP in a controlled condition with no exposure to PMF for 2 hours. As indicated in *figure 3.21 B*

the amplitude of the CAP remained unchanged throughout the experiment ($p=0.396$, $n=10$, *see figure 3.21A* for a representative experiment).

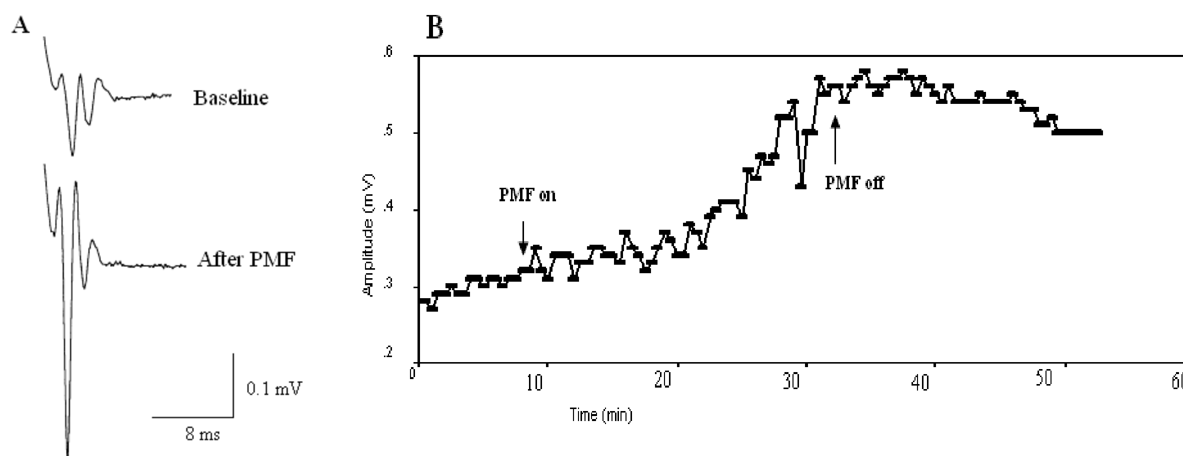


Fig.3.19. The graph above shows a representative experiment of the effect of PMF on CAP. A – The upper and lower potentials represent the CAP before and after PMF exposure, respectively. B – An example of a typical experiment; PMF on / PMF off indicates initiation and termination of the magnetic field exposure, respectively.

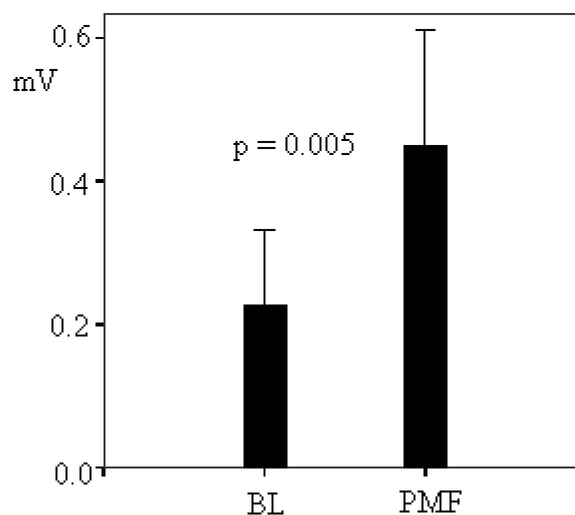


Fig.3.20. The influence of PMF on CAP recorded from the sciatic nerve. The bars show the averages of 10 experiments, performed on ten different segments of the sciatic nerve. The sciatic nerves were used in these experiments were taken from eight mice. BL refers to the baseline-recording period. The PMF bar represents the recordings at the maximal effect of the PMF. PMF significantly increased the compound action potential (n=10, 187.1%, p = 0.005). Wilcoxon signed ranks test, for 2 related samples was used.

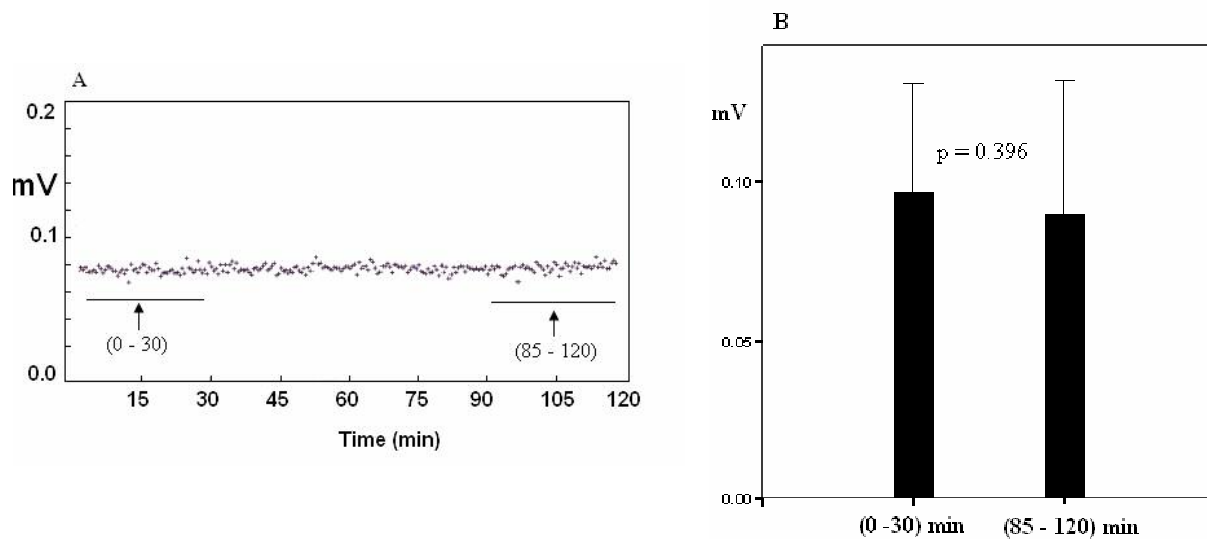


Fig. 3.21. The CAP recorded from control sciatic nerve, not exposed to PMF. A – A representative experiment; the horizontal lines under the graph indicate the time of collecting the potentials taken to calculate the averages illustrated in figure B. B – Bars represent averages that were taken at the two mentioned periods of recording time. There was no change in the potentials followed for 2 hours period (p=0.396, n=10). Paired t test was used between the two groups.

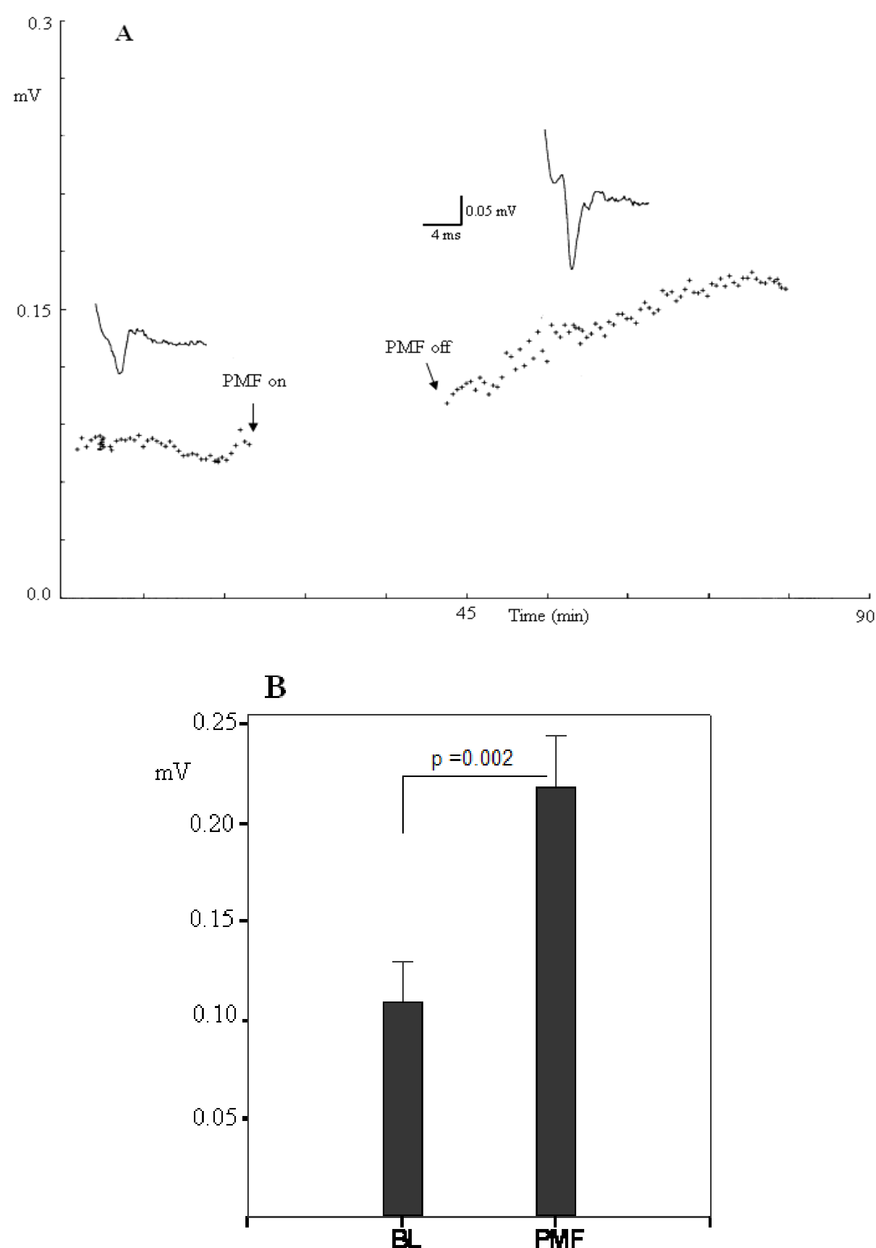


Fig. 3.22. PMF effect was independent of the concurrent electrical test stimulus. The recording was followed for 20 minutes and then the electrical stimulation was stopped during the 30 minutes exposure to PMF. After the PMF was turned off electrical stimulation was resumed and potentials were followed for another 30 minutes. A – An example of a typical experiment: the upper part of the graph represent potentials recorded before and after PMF exposure and the lower part of the graph shows the potential amplitude that was followed throughout the experiment except the period of PMF exposure. B – Bars represent the averages that were taken from the baseline period (BL) and at the maximal effect after the PMF exposure (PMF). PMF induced significant increase of the CAP amplitude (n=11, p=0.002, 185.7%, Wilcoxon signed ranks test).

<u>Effects of PMF on the short-term depression (STD) in CAP</u>

When the axon is stimulated twice with a short inter-pulse interval (IPI) (less than 12 ms), the second action potential (in response to the second stimulus) is lesser in amplitude than the first action potential (in response to the first stimulus). This phenomenon is similar in its expression to the previously discussed PPI, observed in hippocampus slices and results from internal hippocampal conditions. However, the sciatic nerve just contains the processes of neurons located at the spinal cord, and the potentials recorded from it, are axonal action potentials only. Therefore, to distinguish between these two phenomena, we will use the term "short-term depression' (STD) to describe the processes occurring in the nerve during paired-pulse stimulation. The two stimuli paradigm was used to investigate the effect of pulsed magnetic field on the short-term depression generated in the sciatic nerve. Short-term depression is believed to be a reflection of alteration in the initiation, reliability, and/or waveform (amplitude) changes of action potentials. As another form of stimulation, I used 10 stimuli to see if increasing the repetition of generation of action potentials was related to the PMF induced effects. In all experiments where the two and the ten stimuli paradigms were used, I used 9ms inter-stimulus intervals. The PMF induced effect on STD was investigated at 20, 50, and 100 milliseconds IPI.

STD was calculated by subtracting the amplitude of the first CAP from the second CAP (CAP1-CAP2). In the two stimuli paradigm at 9 ms interval the PMF increased the STD significantly (n=8, 329.4%, p=0.012, paired t test, see *figure 3.23B*). In figure 3.23A a representative experiment depicts the shape of the potentials in the paired stimulus paradigm (CAP1 and CAP2) at the baseline period and after the PMF

exposure. The dotted lines represent the amplitude of CAP1 and CAP2 throughout the whole experiment. The arrows indicate the time at which the depicted potentials were recorded. Note that while there was practically no difference between CAP1 and CAP2 before the PMF exposure, the difference between these two potentials increased significantly after the application of the PMF. It is interesting to note that the PMF not only increased STD, but it also increased the amplitude of the second CAP (*figure 3.23A).*

The stability of the sciatic nerve potentials in the controlled condition (no PMF), during the two stimuli paradigm was investigated (Figure 3.24A). Potentials evoked by the two stimuli paradigm and changes in amplitude during the entire experiment were depicted as the upper and lower part of the figure, respectively. A series of arrows indicate the parts where the represented potentials were recorded. The bars in figure 3.24B represent the average STD that was taken at 2 different periods: 0 to 30 minutes and at 90 to 120 minutes. Under the controlled condition, the STD was stable ($p = 0.465$, $n = 6$, paired t-test).

PMF increased STD in the 10 pulses train paradigm when averages calculated before (baseline) and after PMF exposure, were compared ($p = 0.018$, 304.5%, $n = 8$). Since the highest depression occurred in the tenth potential, only the amplitude of the first and the tenth CAP were taken for calculation. Figure 3.25A shows the potentials of one train of ten pulses with a 9ms inter stimulus interval during the baseline (left) and after the PMF exposure (right). Note that PMF increased the amplitudes of all potentials, as well as, the difference between CAP1 and CAP10 (STD10). Figure 3.25B shows the average change after the PMF exposure.

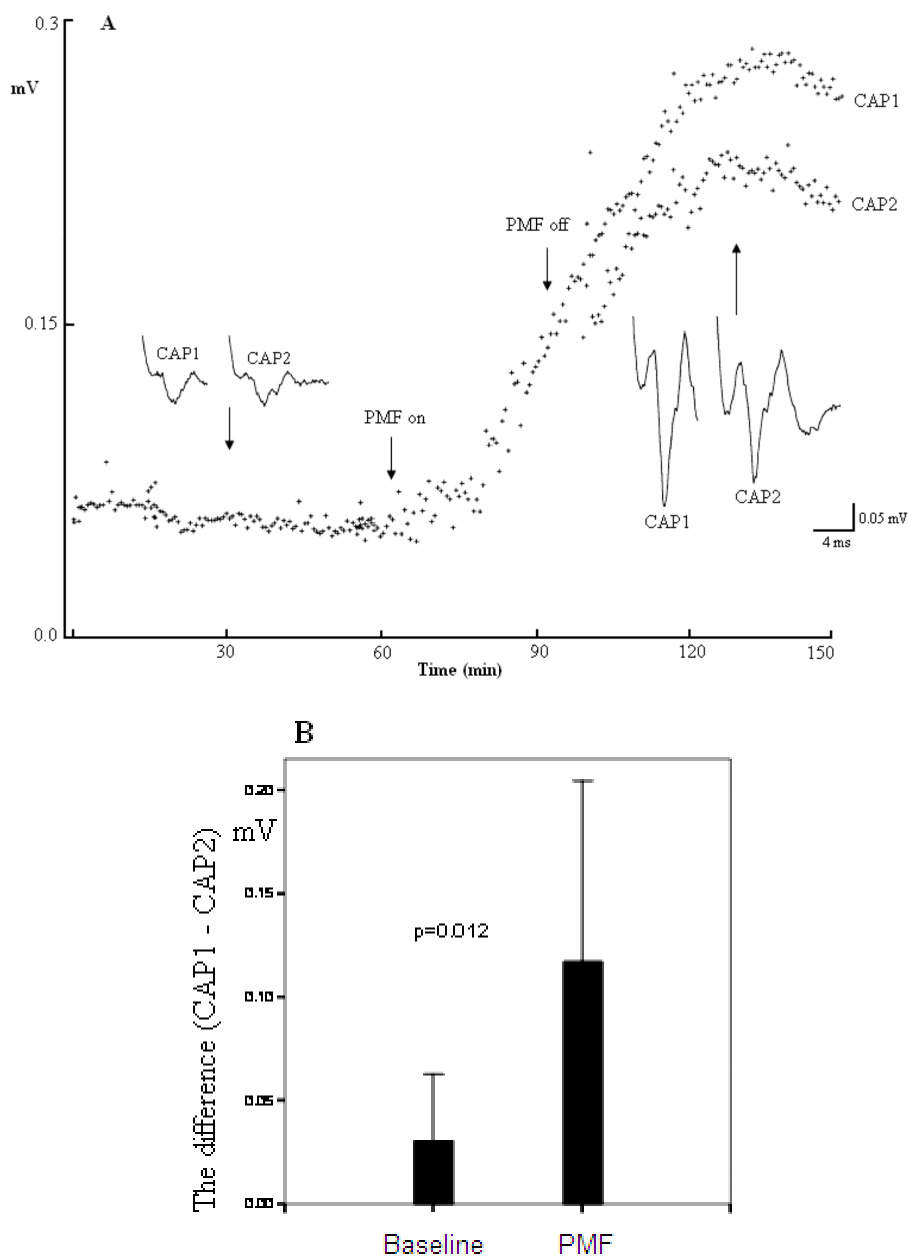


Fig.3.23. PMF increased the difference between CAP1 and CAP2 recorded from the sciatic nerve. A – a representative experiment showing the PMF induced effect on the short-term depression at 9ms ISI. CAP1 and CAP2 indicate the first and the second compound action potential, respectively. PMF-on and PMF-off indicate the time of the start and the end of the PMF exposure. The arrow indicates when the potentials were recorded. B – The bars illustrate the influence of PMF on the compound action potential. The first bar (baseline) represents the average difference between the first and the second compound action potential (CAP1 minus CAP2) before the exposure to PMF and the second bar (PMF) represents the difference between the two compound action potentials at the maximal effect of PMF exposure. PMF increased the difference between CAP1 and CAP2 significantly ($p=0.012$, 329.4%, $n=8$). Wilcoxon signed ranks test was used for 2 related samples.

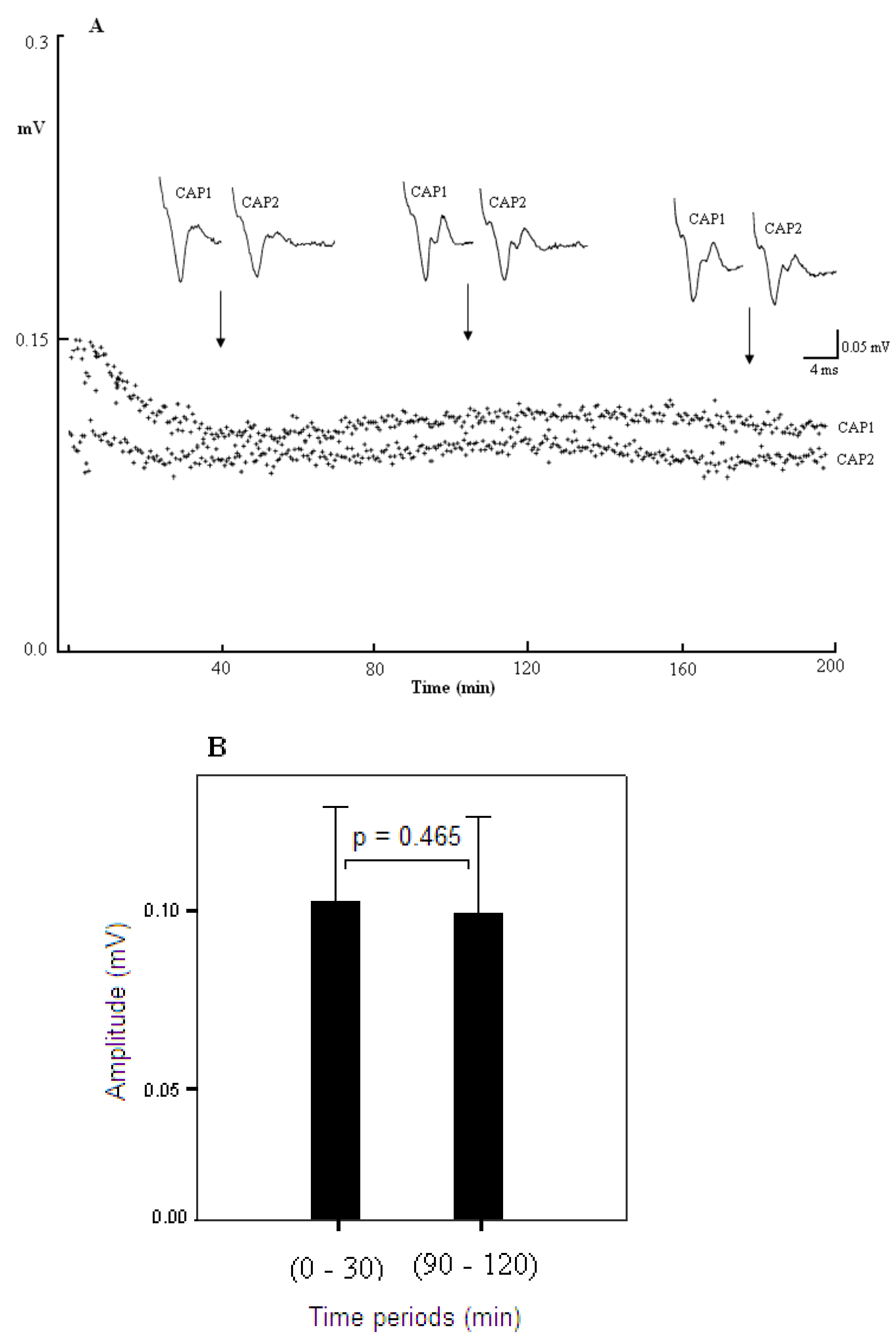


Fig.3.24. STD in control condition. A – A representative experiment shows the stability of the STD in control condition. CAP1 and CAP2 are the first and the second potentials, respectively. The paired pulse responses (CAP1 and CAP2) are plotted over time. The nerve was stimulated and sweeps were recorded every 30 seconds. In these experiments, the inter stimuli interval was 9 milliseconds. B – The bars represent the average of STD calculated at indicated time periods: (0 – 30) and (90 – 120). STD was stable throughout the experiments ($p = 0.465$, $n = 6$, paired t-test)

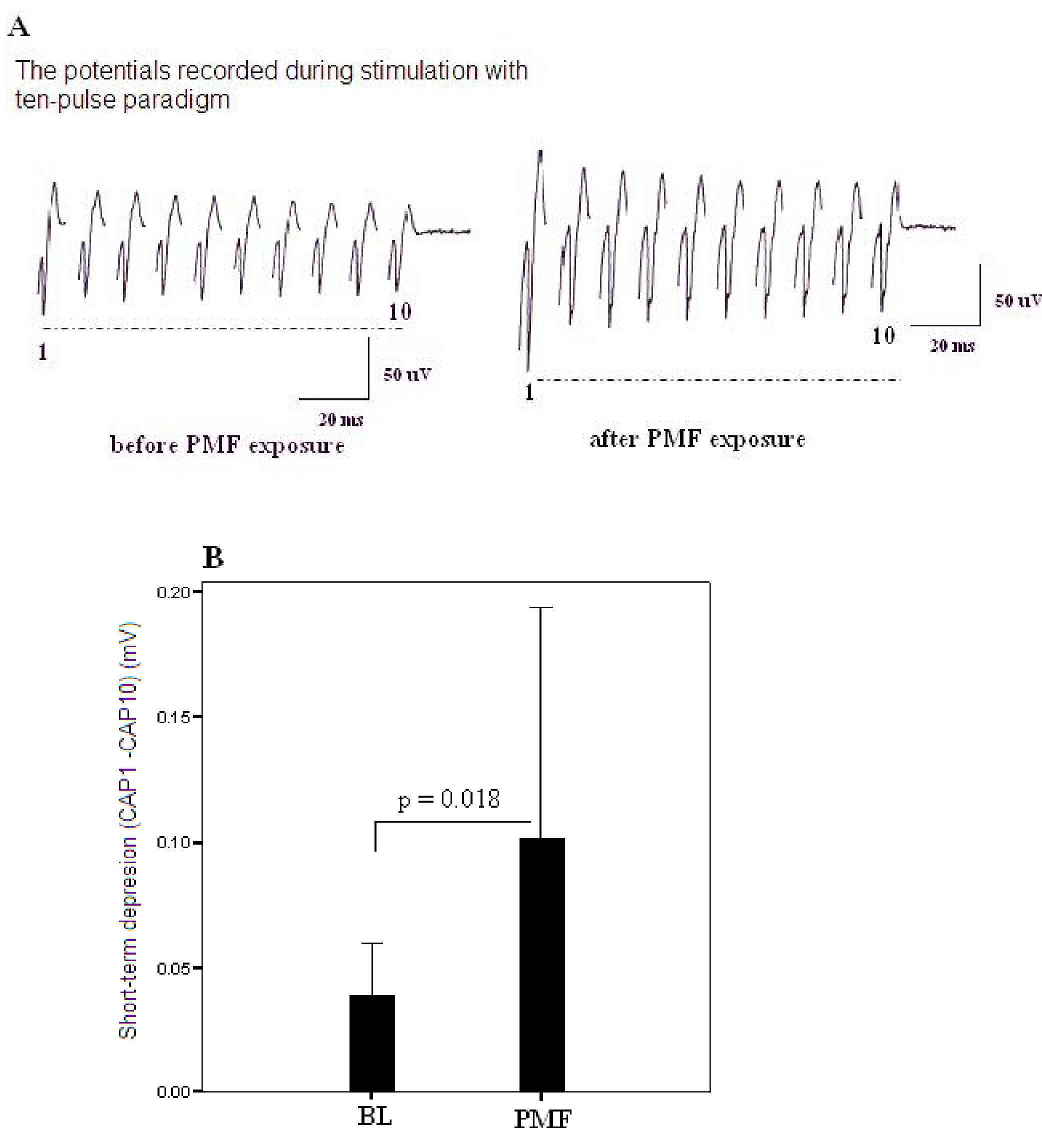


Fig.3.25. PMF increased the difference between the 1st and the 10th CAP. To investigate the effect of PMF on the short-term depression, we adapted a ten-pulse train paradigm and the potentials were recorded at the beginning of the experiments (baseline (BL)), and after the exposure to PMF. A – A representative of ten potentials induced by the ten-train paradigm are shown. Note the difference between the first and the tenth spike before and after the PMF exposure. B – Bars represent the potentials in short-term depression before (BL) and after the PMF exposure (PMF). The difference between the 1st and the 10th CAP was calculated as the first minus the tenth response. PMF increased the difference significantly (p=0.018, n=8). Wilcoxon signed ranks test was used.

Effect of the PMF on STD at different IPI

We previously tested the effect of the PMF exposure on STD that was induced at one IPI (9 ms). To better characterize the influence of the PMF on STD, we evaluated the action of the PMF at longer inter-pulse intervals.

Although the PMF induced a significant increase in the STD in the two stimuli paradigm with IPI of 9 ms ($p < 0.05$, $n = 9$), its effect was not statistically significant at IPI of 20, 50, and 100 milliseconds ($p > 0.05$). Figure 3.26 shows the influence of the PMF exposure on CAP at different ISI. In this figure, the black bars represent the average STD magnitude after the PMF exposure and the gray bars represent the average STD magnitude at the baseline period.

As show in figure 3.27, the averages of the compound action potential amplitudes calculated from the baseline (gray) and after the PMF exposure (black) were compared. The first two bars marked CAP1 show the average change in the first CAP induced by the PMF. The remaining bars show the average change of the second CAP at different IPI. The PMF significantly increased the average amplitude of CAP1 (black), compared to the CAP1 from the baseline (gray) ($p < 0.05$, $n = 9$).) Similarly, the comparison of CAP2 at all IPI (9, 20, 50 and 100 ms) after the PMF exposure (black bars) to baseline (gray bars) showed that the PMF increased CAP amplitude significantly ($p < 0.05$, $n = 9$). Note that figures 3.26 and 3.27 were derived from the same set of experiments; and represent STD and absolute CAP amplitude, respectively.

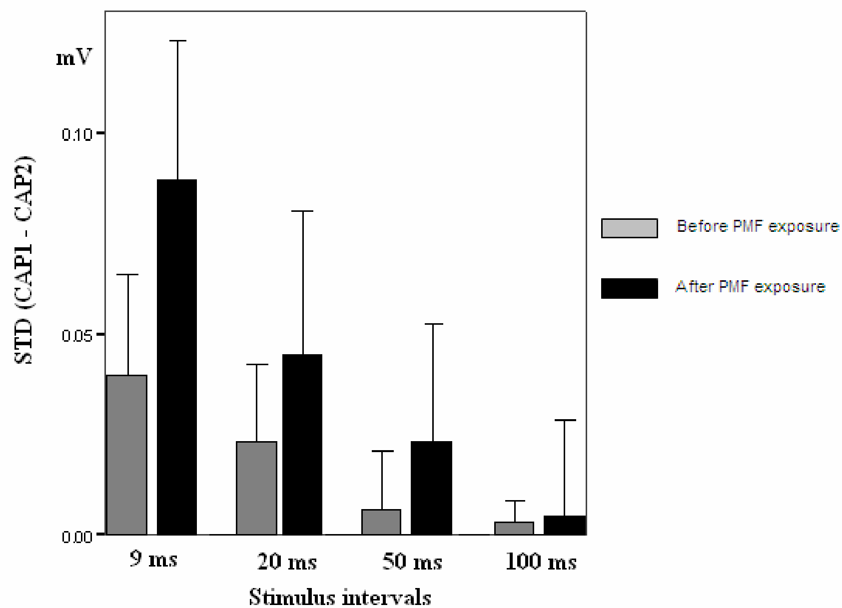


Fig.3.26. The influence of PMF on STD evaluated on the mouse sciatic nerve preparation. CAP1 and CAP2 correspond to the first and the second evoked compound action potential in a two stimuli paradigm, respectively. The difference was statistically significant for 9 ms ISI only ($p = 0.012$, $n=9$, paired t-test).

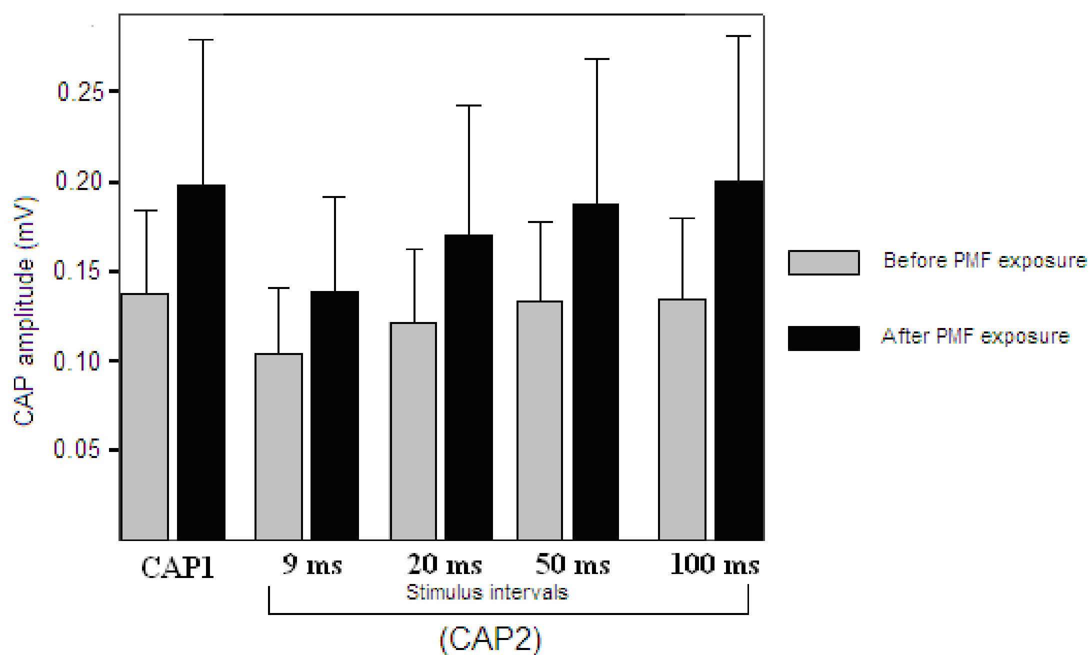


Fig.3.27.The exposure to PMF increased the amplitude of the first as well as the second CAP at different stimulus intervals. The gray and black bars represent the average amplitude before and after PMF exposure, respectively. The first two bars show the results of CAP1 before and after the exposure; the increase was statistically significant ($p<0.05$, $n = 9$). At all intervals (9, 20, 50, and 100 milliseconds) PMF increased CAP amplitude significantly ($p<0.05$, $n = 9$, paired t-test).

PMF effects on STD at different IPI in the presence of Tetraethylammonium chloride (TEA)

In order to investigate the mechanism that mediated the PMF-induced effect on the short-term depression, TEA (500µM) was used to block potassium channels. The blocking of potassium channels abolished the PMF induced increase in STD ($p = 0.307$, $n = 9$, paired t-test) *see figure 3.28.* TEA not only abolished STD, but it modified the action of the PMF, changing this inhibition into induced short-term facilitation in 20 ms and 50 ms intervals. In figure 3.28 the negative values of STD indicate facilitation (CAP2 amplitude greater than CAP1). Although, TEA blocked the increase in STD, it had no effect on the PMF-induced increase of CAP amplitude (fig. 3.29). In four of the experiments, CAP2 was nearly twice the CAP1 amplitude, especially at 20 and 50 ms IPI. This is shown as negative STD on the graph (*figure 3.28B*).

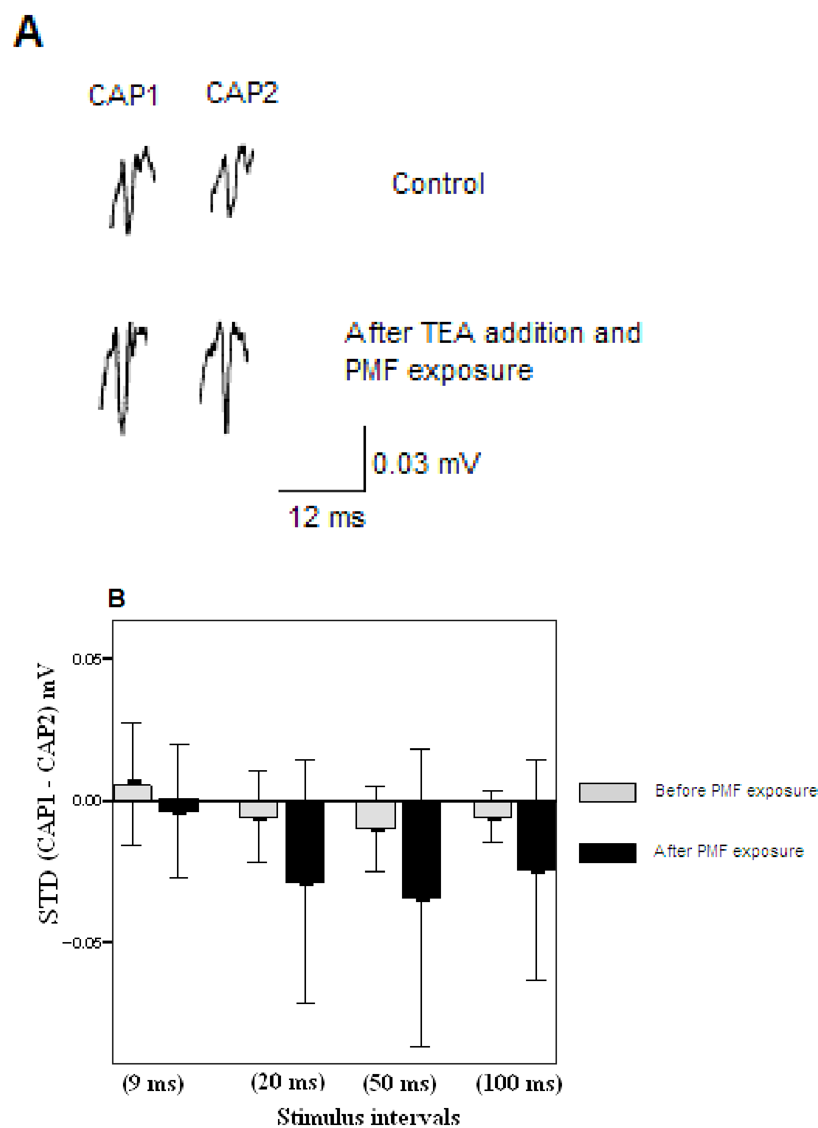


Fig.3.28. The effect of TEA (500µM) on PMF-induced short term depression. A – Representative potentials show the relation between CAP1 and CAP2 in a controlled condition (neither TEA nor PMF applied) and after PMF exposure. B – The average STD before PMF exposure and after the addition of TEA (gray bar); the average STD after the exposure to PMF in the presence of TEA (black bars). TEA abolished STD completely ($p = 0.307$, $n = 9$). ANOVA one-way was used. Negative STD indicates that CAP2 had higher amplitude than CAP1 indicating facilitation.

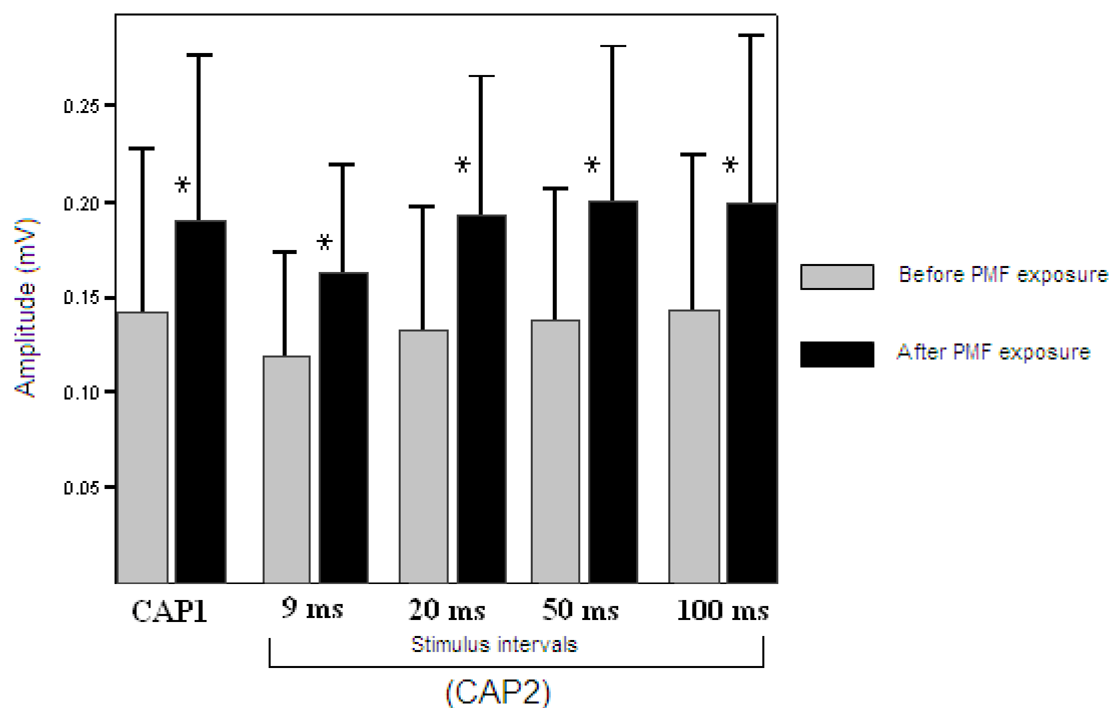


Fig.3.29. Influence of TEA on PMF-induced effect. TEA was used to investigate the increase in the difference between the first and the second CAP (500uM). Gray bars represent the measurements before PMF exposure (control) and black bars represent the measurement after it. In the presence of TEA, PMF significantly increased the CAP amplitude, ($p<0.05$, $n=9$, Paired samples t-test). At all stimulus intervals PMF has significantly increased CAP2 ($p<0.05$, $n = 9$, paired t-test) as compared to CAP2 prior to PMF exposure. Asterisk means statistically significant.

Effect of PMF on the CAP2 IPI recovery-time (IPIRT)

The results of the previous experiments (the effect of PMF on STD with and without TEA) were analyzed to evaluate the shortest time needed for the CAP2 to have the amplitude equal to CAP1. We named this time Inter-Pulse Recovery Time (IPIRT). Figure 3.30 graphs the recorded data for CAP1 and CAP2 amplitudes (at different intervals) before and after PMF exposure without (triangles) and with (squares) TEA. BL1 and BL2 refer to the data collected at the baseline with and/or without TEA addition, respectively. While PMF1 and PMF2 refer to the data collected at the maximal effect of the PMF with and/or without TEA addition, respectively. Note that there was no significant difference when CAP1 amplitudes of BL1 and BL2 were compared. Similarly, there was no significant difference between the amplitudes of PMF1 and PMF2. There was also no significant difference between CAP2 amplitudes at 100 ms IPI between BL1 and BL2, and between PMF1 and PMF2. There was a significant difference between CAP2 amplitudes of BL1 and BL2, and PMF1 and PMF2 at IPI 9 ms 20 ms, however. The difference between PMF1 and PMF2 only became significant at IPI of 50 ms, indicating that a shorter IPIRT was induced by the PMF exposure. Table 1 shows another presentation of the same data allowing me to calculate the IPIRT, which was approximately 50 ms between BL1 and PMF1 and it significantly shorter (by 75 ms) between BL2 and PMF2.

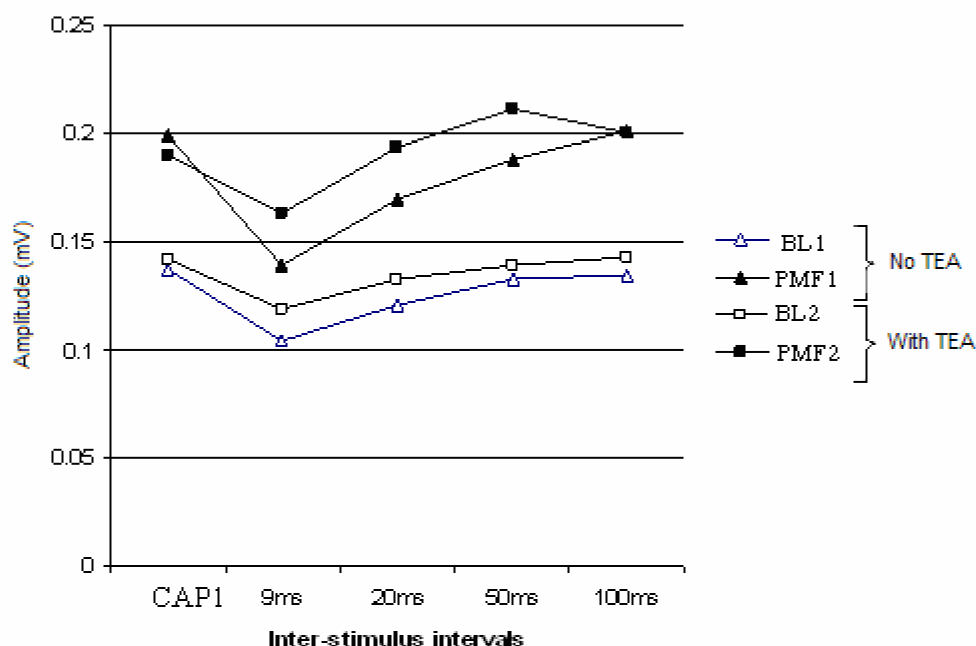


Fig.3.30. Shown here is the recovery time of the compound action potential. We define the recovery time as the time of the interval that is needed for CAP2 to reach amplitude equal to CAP1. The squares and the triangles are the averages of nine experiments. The open and solid squares (BL2 and PMF2) show the data collected before and after the exposure to PMF, respectively, in the presence of TEA (500µM). The open and solid (BL1 and PMF1) triangles show data collected before and after PMF exposure, respectively, without the presence of TEA. Note that in the experiments without TEA, PMF led to shorter recovery time (about 50 ms for the calculation; see table 1) and higher STD (depicted as a deeper curve). After the addition of TEA the recovery time was dramatically shorter (75 ms), and there was no change in STD, which was reversed at 50 ms inter-stimulus interval. Also note that TEA did not alter the PMF induced effect on CAP1 amplitude. This could be determined by comparing PMF1 (1st square) with PMF2 (1st triangle).

Table 1.3 The Effect of PMF on the CAP recovery time.

	TEA	CAP1 (mV)	CAP2 (mV) at: 9 ms	20 ms	50 ms	100 ms
BL1	_	0.1378	0.1044	0.1211	0.1333	0.1344
PMF1	_	0.1989	0.1389	0.1700	0.1878	0.2011
BL2	+	0.1422	0.1189	0.1333	0.1389	0.1433
PMF2	+	0.1900	0.1633	0.1933	0.2110	0.2000

- BL1: the baseline data that was collected before both the addition of TEA and PMF exposure.
- PMF1: the data that was collected after PMF exposure with no TEA.
- BL2 and PMF2 are data collected in the presence of TEA.
- The numbers in the table are averages of CAP amplitude from 9 experiments.
- The recovery time was approximated by comparison of the amplitude of CAP1 and CAP2 at different time intervals. The shortest time interval, at which the difference in the amplitudes of CAP1 and CAP2 was the smallest, was considered as a recovery time.
- Note the recovery occurred shortly before the interval of 20 ms in PMF2.

Effects of PMF on axonal threshold

The previous results indicate that the PMF decreases the IPIRT (BL2 was at IPIRT of 100 ms, but PMF2 was at ISIRT of 20 ms), as a result of the reduction of the nerve threshold.

To test the effect of the PMF on the axonal threshold, eight stimulus strength values were chosen (in Volts) 0.1, 0.2, 0.4, 0.9, 1.5, 2.5, 3.5, and 4.5. In most of the experiments, 0.1 V was the lowest strength used to evoke the lowest compound action potential, and for maximal strength one Volt was used. Any stimulus strengths above one Volt were considered supera maximal. During the baseline period of each experiment, one recording was taken at the strength of each stimulus (specified above) and repeated after the PMF exposure. Figure 3.31 presents potentials that were recorded in the same experiment before and after the PMF exposure. Note that the same electrical stimulus strength evoked higher CAP amplitude after the PMF exposure. Figure 3.32A shows that the PMF reduced the threshold significantly ($n = 9$, $p<0.05$) in all stimulus strengths except at 0.1 V, where the reduction of the threshold was small and statistically insignificant ($p>0.05$).

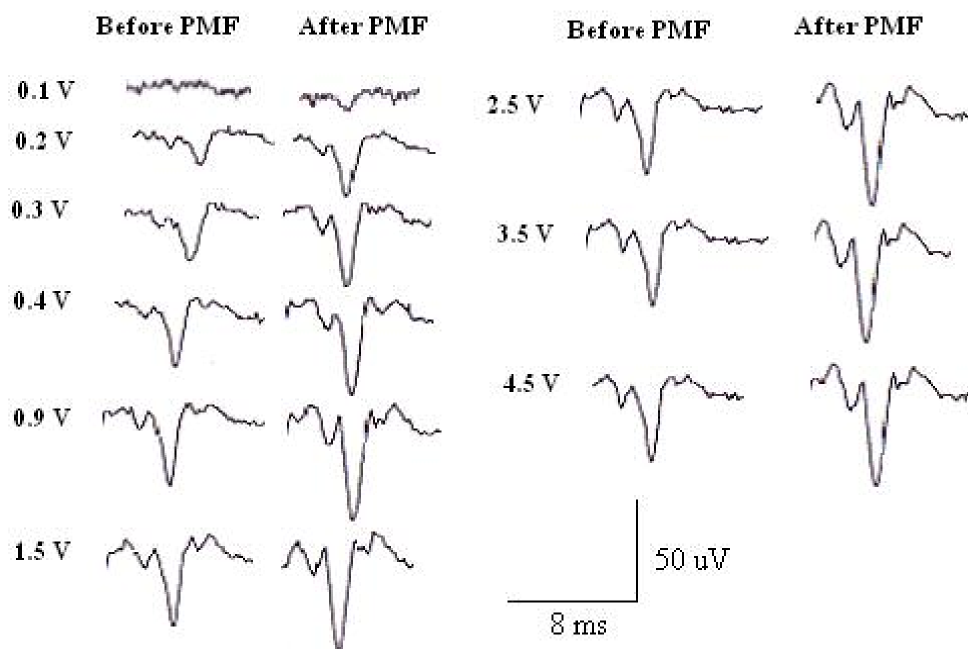


Fig. 3.31. Results of the influence of PMF on the sciatic nerve threshold are shown. These are waveforms from a typical experiment recorded before and after the PMF exposure.

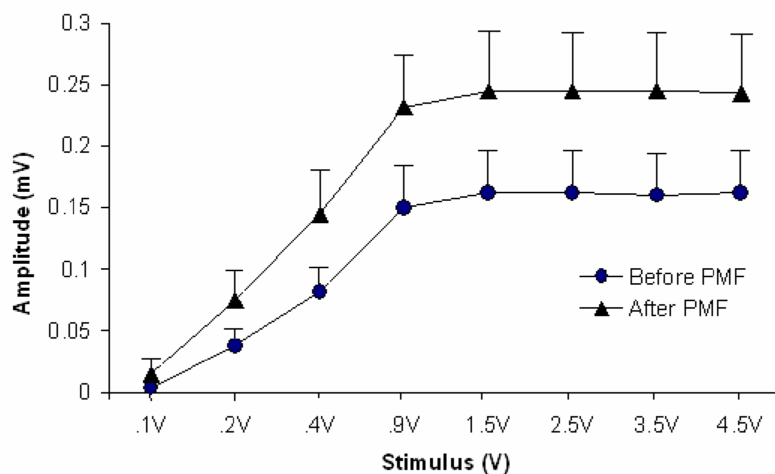


Fig.3.32. Effects of PMF on the sciatic nerve threshold. The stimulus strength ranging from 0.1 V to 4.5 V was applied to the sciatic nerve preparation before (●—●) and after (▲—▲) the exposure to PMF. Eight measurements (one measurement at each value of stimulus strength) were taken from each experiment before PMF exposure (solid circles) and another eight measurements were taken after the PMF exposure (solid triangles). The PMF shifted the threshold curve to the left, which suggests reduction in the axons threshold. That left shift was statistically significant in all stimulus strength values ($p<0.05$, n=8) except at 0.1V ($p>0.05$, n=8, paired Samples t test). Each point on the graph represents the average of eight experiments.

PMF and axonal gap junction

The axon-to-axon interaction occurs in two ways 1) ephaptic: impulse in adjacent axon influences another axon through extra cellular potential (Binczak et al., 2001) and, 2) electrical coupling, which is mediated by axoaxonal gap junction (Debanne, 2004).

Experiments using the gap junction blocker carbenoxolone (cbx), were conducted to verify the assumption that electrical gap junction might mediate the PMF induced effect. The PMF increased significantly the amplitude of CAP even in the presence of 50μM carbenoxolone ($n = 8$, $p = 0.003$ paired t-test). Figure 3.33A shows potentials from the baseline and after the exposure to the PMF, as the upper and lower potentials, respectively. Bars in figure 3.33 B represent the average change induced by PMF exposure.

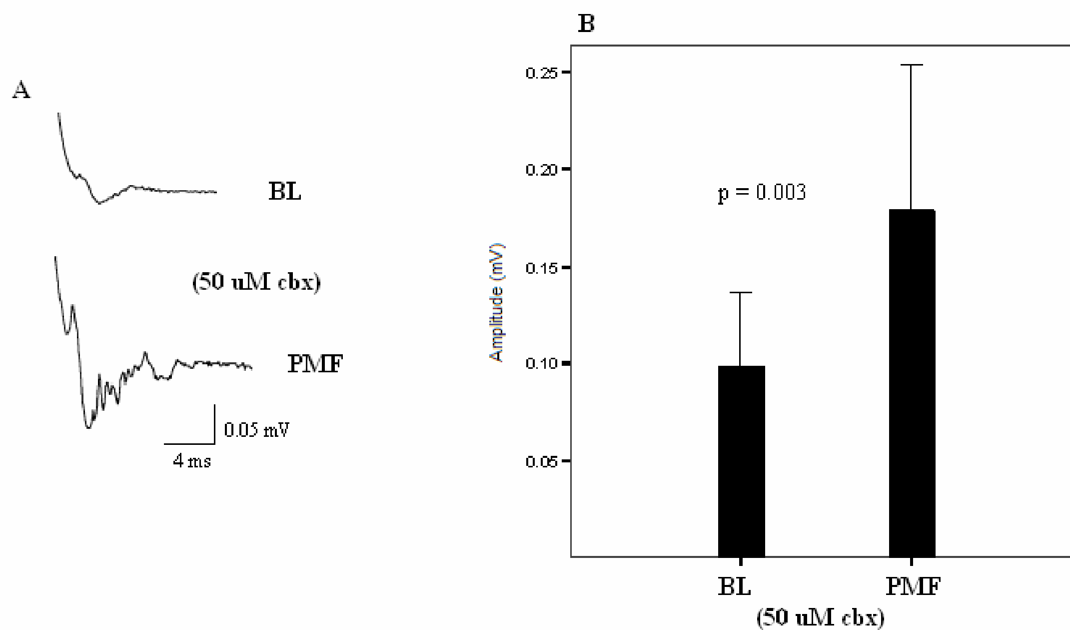


Fig.3.33. Results of the effect of PMF on the compound action potential (CAP) of the sciatic nerve in the presence of cbx (50uM). A – Two representative potentials from the baseline (BL) and after the PMF exposure (PMF) are shown. B – Bars represent the average of potentials recorded during the baseline period (BL) and at the maximal effect of PMF exposure (PMF). PMF has increased the CAP significantly (p=0.003, n=8, paired t-test).

The PMF and PKC and PKA inhibitors

Throughout literature it has been established that, protein kinase A (PKA) and protein kinase C (PKC) phosphorylate tetrodotoxin-sensitive (TTX-S) and tetrodotoxin-resistant (TTX-R) sodium channels. This phosphorylation reduces the macroscopic Na^+ conductance of TTX-S sodium channels attenuating neuronal excitability (Bevan and Story, 2002).

If the PMF somehow modulates the neuronal excitability through PKA and PKC activities, blocking these enzymes should influence the PMF induced effect. To test this assumption, eight experiments were performed while PKC (P-1614) and PKA (fragment 12-22, myristoylated trifluoracetate salt) inhibitors were added to the incubating and to the recording chambers (10μM). The nerve segments were incubated with the inhibitors for at least 90 minutes before the start of the PMF exposure. The averages before PMF exposure were compared to the averages taken at the maximal effect of PMF. In the presence of PKA and PKC inhibitors, the PMF increased the CAP significantly ($n = 8$, 228.6%, $P = 0.04$, paired t-test) (fig.3.35). A representative experiment is shown in Figure 3.34.

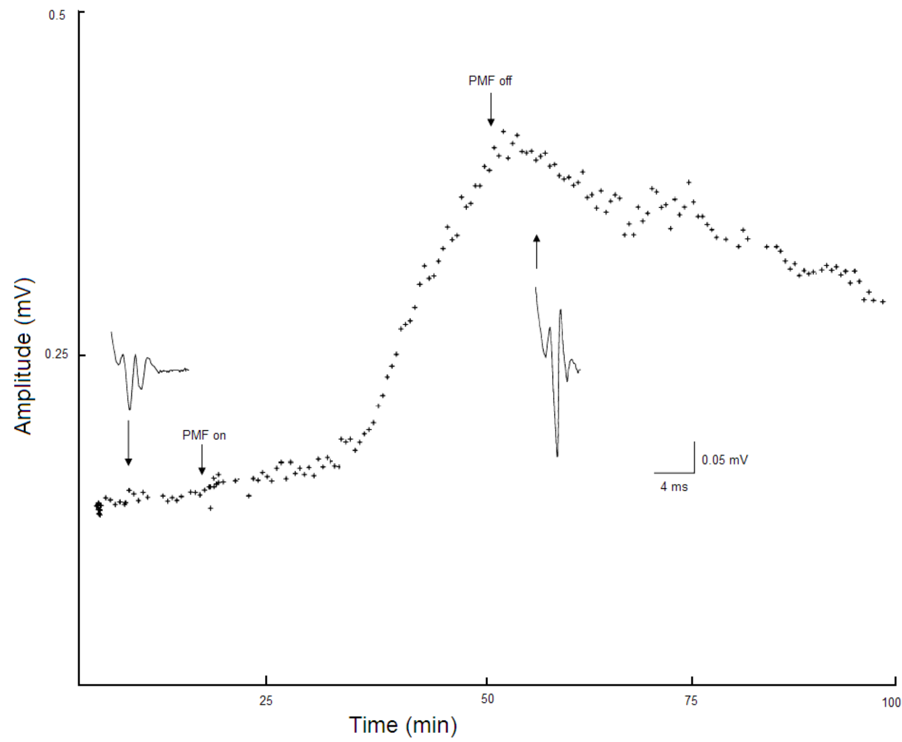


Fig.3.34. A representative experiment illustrating the effect of PMF exposure on sciatic nerve potential in the presence of PKA and PKC inhibitors. The sciatic nerve segments were incubated with the inhibitors for at least 90 minutes before starting the experiment.

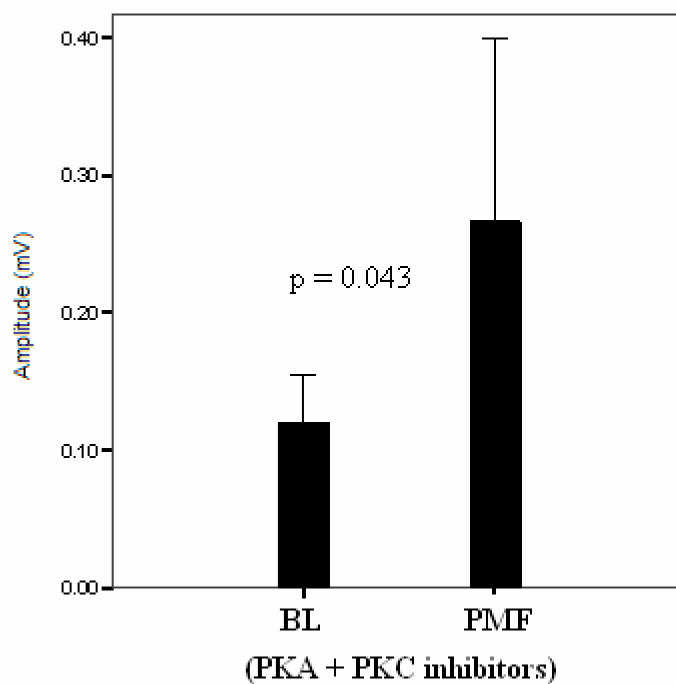


Fig. 3.35. The effect of PMF on the CAP of the sciatic nerve in the presence of PKA (fragment 12-22, myristoylated trifluoracetate salt) and PKC (P-1614) inhibitors. In these experiments the inhibitors were added (both at the concentration of10uM) at least 90 minutes before starting the PMF exposure. Bars represent the average results (p=0.043, n=5, paired t-test).

Effect of the PMF on CAP in the presence of free radical scavengers:

To test the hypothesis that the PMF might influence the nervous system through a free radical mechanism, I chose known strong free radical scavengers like vitamin E, C, and diphenylene iodonium (Dpi) to evaluate their effect on the PMF induced amplification of action potentials. Although, the nerve segments were incubated with the three scavengers simultaneously, the PMF increased the CAP amplitude significantly (n = 8, 174.2%, p = 0.0001, paired t test) see *figure 3.36 A and B.* Vitamin E was dissolved in 0.03% ethanol.

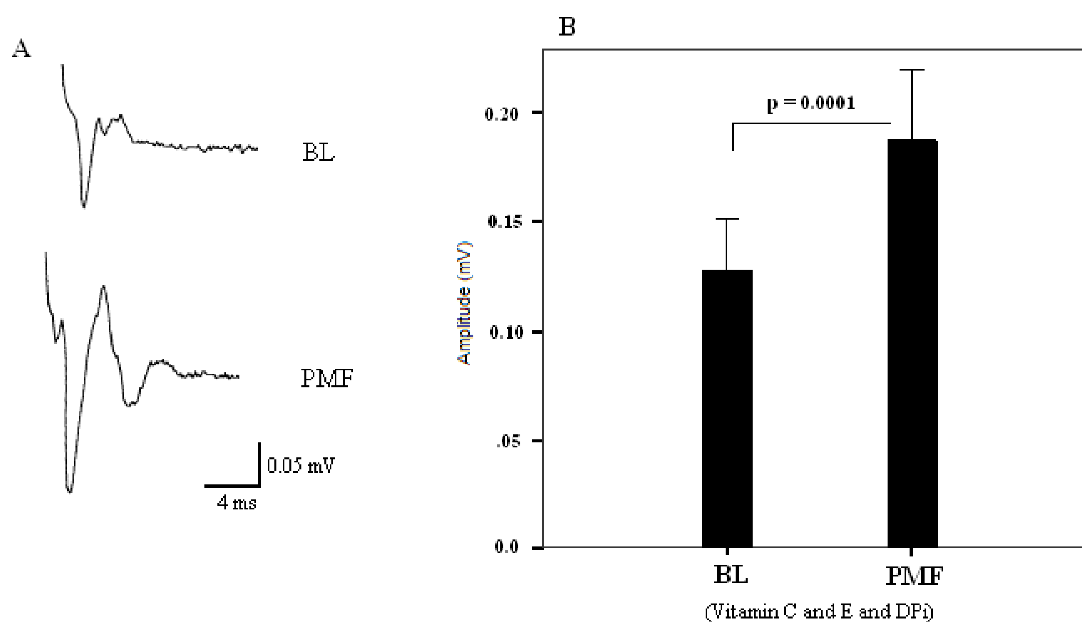


Fig.3.36. The effect of PMF on the sciatic nerve CAP in the presence of vitamin E, C, and Dpi. The compounds were dissolved in distilled water, except vitamin E, which was dissolved in 0.03% ethanol. A – The baseline potential (BL) and potential after PMF exposure (PMF) recorded in the same experiment. B – The average results of eight experiments demonstrating that PMF induced a significant CAP increase (n = 8, 174.2%, p = 0.0001, paired t test).

The effect of PMF on sodium channels

The experiments previously described, suggest that Na^+ and K^+ channels are involved in the mediation of the PMF-induced effect. I studied the effect of blocking potassium channels on the PMF induced amplification of CAP, and determined that the PMF led to a shorter recovery period in the presence of TEA, indicating there was a specific influence of the PMF on sodium channels. This assumption was further verified when I conducted experiments employing inhibitors of sodium channel.

1. The effect of PMF on CAP in the presence of 10nM TTX:

After stable baseline recordings were established, TTX (10nM) was added to the recording chamber. TTX significantly reduced the CAP amplitude ($p = 0.009$, 68.2%, $n = 6$). When the amplitude of the potential stabilized at its minimum value after the addition of TTX (15 - 20 minutes), the PMF was applied for 30 minutes. The depressive effect was not only eliminated within first 10 minutes of the PMF amplification, but was reversed in the later stages of the PMF application ($p=0.027$, 80% ± 55%, $n=6$). Figure 3.37A shows a representative experiment showing the CAP potentials and the change in their amplitudes depicted as the upper and lower parts of the graph, respectively. The bars in figure 3.37 B represent the averages of potentials recorded from three periods during the experiment: 1) baseline (before the addition of TTX and the PMF exposure) marked as (BL); 2) at the maximal of the depressive effect of TTX (TTX) and 3) at the maximal amplification after the PMF exposure (PMF).

2. The effect of the PMF on CAP in the presence of lidocaine:

Lidocaine is a potent local anesthetic whose action is mediated by the blocking sodium channel. After stabilization of the potential, lidocaine (150 μM) was added to the

recording chamber, it significantly reduced the amplitude of the compound action potential ($p = 0.0005$, $n = 8$). When the amplitude of the potentials stabilized at its minimum value after the addition of lidocaine (15 -20 minutes), the PMF was turned on for thirty minutes. Surprisingly, the PMF reversed the depressive effect of lidocaine within 7-10 minutes. The reversal effect of the PMF was significant ($p = 0.0005$, $n = 8$). Washing away the lidocaine amplified the potential even further ($p = 0.003$, $n = 8$). Figure 3.38A depicts a typical experiment that shows the potentials shape and amplitudes at different periods of the experiment. Bars in figure 3.38 B represent the average potential for different periods of the experiments.

In control experiments (no PMF) the potential depressed by lidocaine was followed for over an hour to determine if the CAP would spontaneously recover its baseline value. After the addition of the lidocaine, the average potential was observed at the first 10 minutes mark, at around the 40 and at around the 60 minutes mark for comparison to the average potential from the baseline (BL)., No spontaneous recovery was observed, however. Lidocaine depressed the potentials permanently during the course of experiment ($n = 6$, $p <0.05$). The depression was reversible upon perfusion with lidocaine free Ringer's solution ($p = 0.158$). Figure 3.39A shows a representative control experiment. Bars in figure 3.39 B represent the average potential for the marked periods during the experiment.

3. The effect of PMF on the sciatic nerve CAP in the presence of veratridine (VrD):

VrD binds to site 2 on the sodium channel and modifies its inactivation gate, inducing a persistent activation of the sodium channel (Hille, 2001). To verify the

assumption that the PMF increases the inward current by changing the gating process of the sodium channel, we used a preparation of sciatic nerve segments incubated with VrD (100µM) for 20 minutes before exposure to PMF. In the one pulse paradigm, the VrD did not change the size of CAP ($n = 10$, $p = 0.619$) but reversed the action of the PMF from facilitation (no VrD) to depression (VrD) ($p = 0.002$). These results indicate that the PMF potentiated the effect of VrD probably by facilitating its binding ability. In addition, in paired pulse paradigm, the simultaneous application of the PMF and the VrD eliminated the CAP2. Figure 3.40 shows traces of the potential of two stimuli paradigm and the amplitude of the potential in a representative experiment depicted at the upper and lower parts of the figure, respectively. The bars in figure 3.40 B represent the average of CAP1 amplitude at different stages of the experiment. BL, VrD and PMF are the average potentials from the baseline, after the VrD addition and after the PMF, respectively.

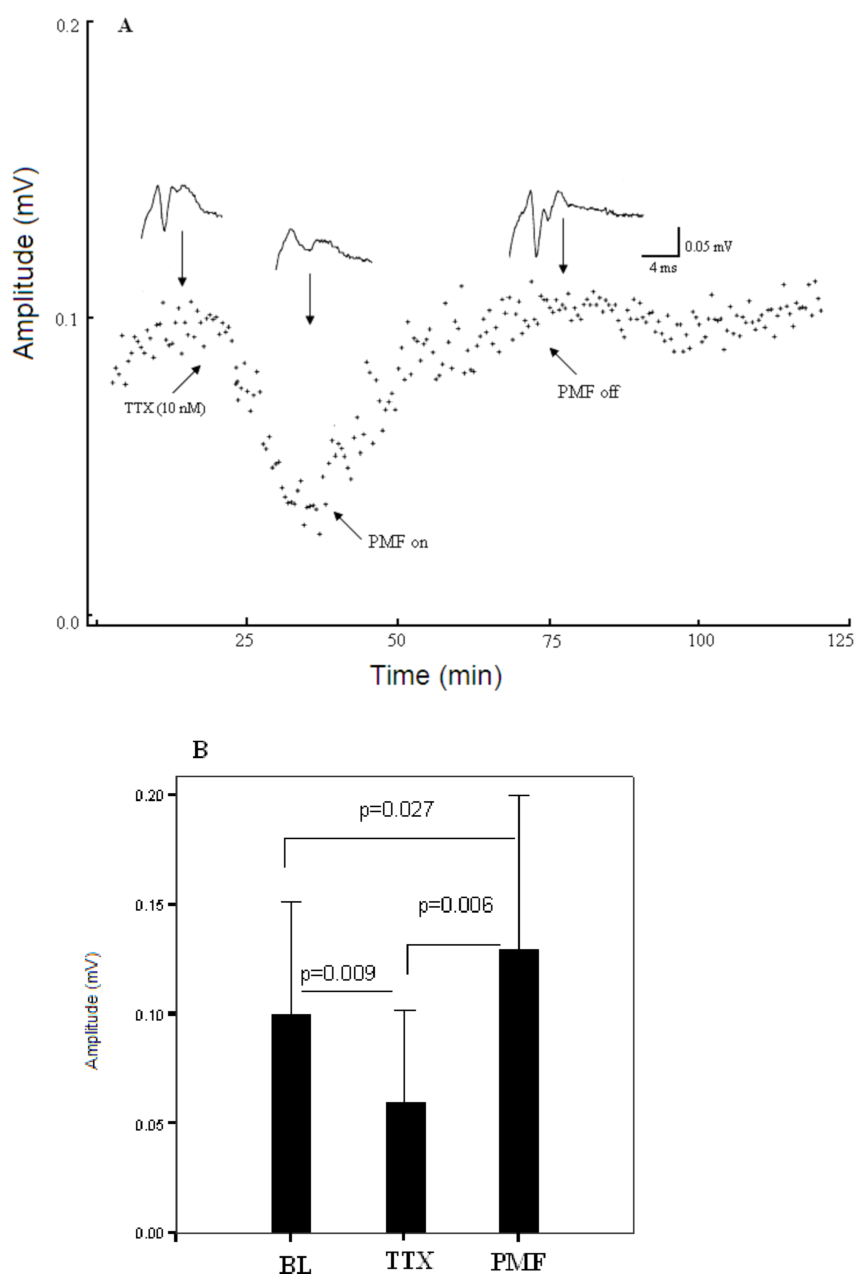
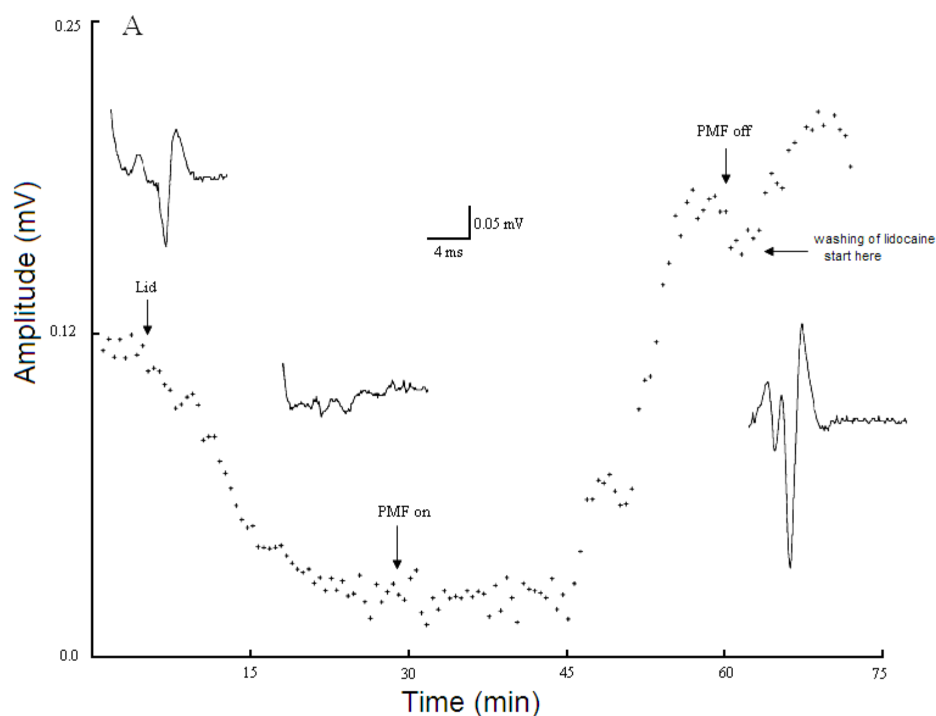


Fig.3.37. Results of the influence of PMF on TTX-induced depression of CAP. A – A representative experiment of TTX (10nM) and PMF is shown. Following 15 minutes of baseline, TTX was added to the recording chamber. TTX reduced the CAP significantly (p=0.009, 68.2%) of baseline. After the recording in the presence of TTX showed stability, PMF was turned on for 30 minutes. It reversed the TTX-induced depression and in later stage it significantly amplified the potential over the baseline. B - Bars represent the average potential from the baseline (BL), after TTX addition (TTX) and after PMF exposure (PMF). PMF significantly reversed the effect of TTX and increased the CAP significantly (p=0.006, 80%, n=6, paired samples t-test).



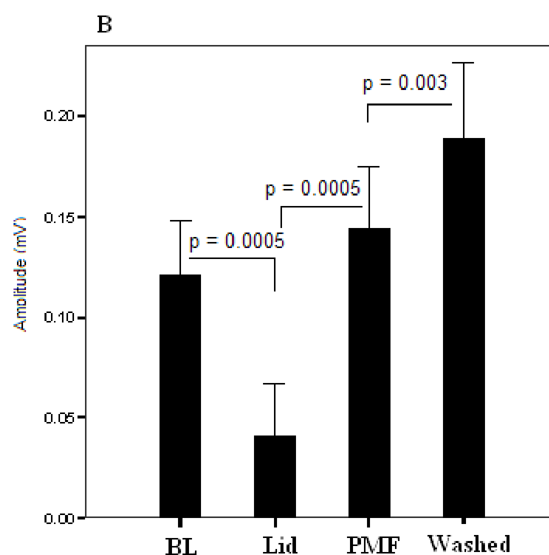


Fig.3.38. The influence of PMF on Lidocaine-induced depression of CAP. A – A representative experiment demonstrating the depression of CAP by lidocaine (150µM) and the reversal of lidocaine's action by PMF exposure. B – The magnitude of CAP in baseline (BL), after the addition of lidocaine (Lid), after PMF exposure (PMF), and after lidocaine was washed away (Washed). Lidocaine-induced attenuation of CAP ($p=0.0005$, $n=8$), was reversed by PMF exposure ($p = 0.0005$, $n = 8$, paired t-test). The increase that was induced by PMF exposure was significantly higher than the mean of the CAP of the baseline ($p=0.04$). Washing of Lidocaine away induced additional increase in CAP amplitude, which exceeded the value of the potentials recorded after PMF exposure ($p=0.003$).

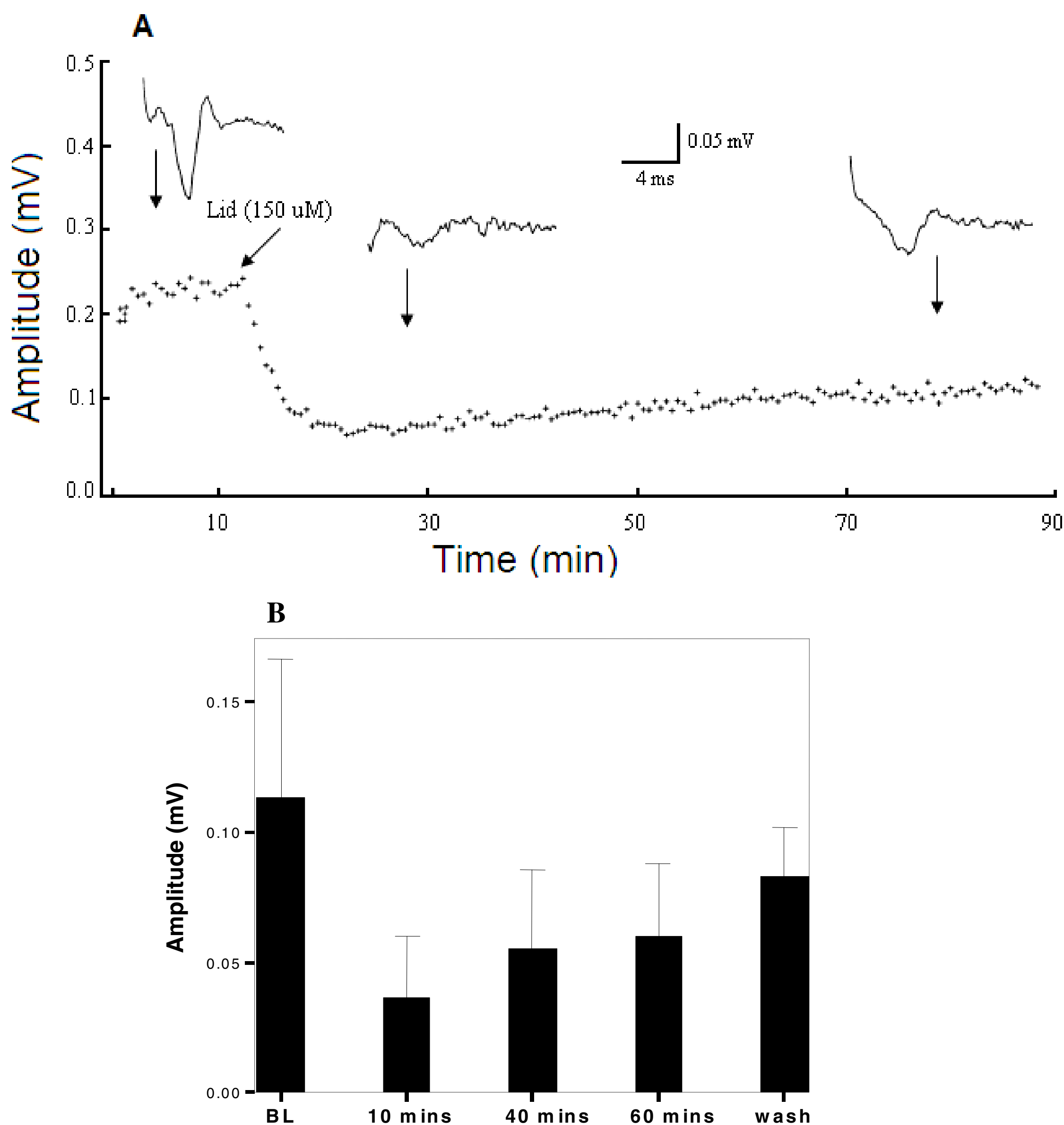


Fig.3.39. The lidocaine-induced depression on CAP in a controlled condition (no PMF exposure). A – A representative experiment showing permanent depression of the potential by lidocaine. B – Bars represent the average of potentials recorded at the baseline (BL), 10, 40, 60 minutes and after washing. Lidocaine reduced the CAP significantly in 2 to 10 minutes after the addition ($p = 0.003$, $n = 6$). Although CAP was slowly recovering, it did not reach its value recorded at the baseline even 60 minutes later. Washing the lidocaine away accelerated the recovery process ($p = 0.158$, $n = 6$, paired samples t test).

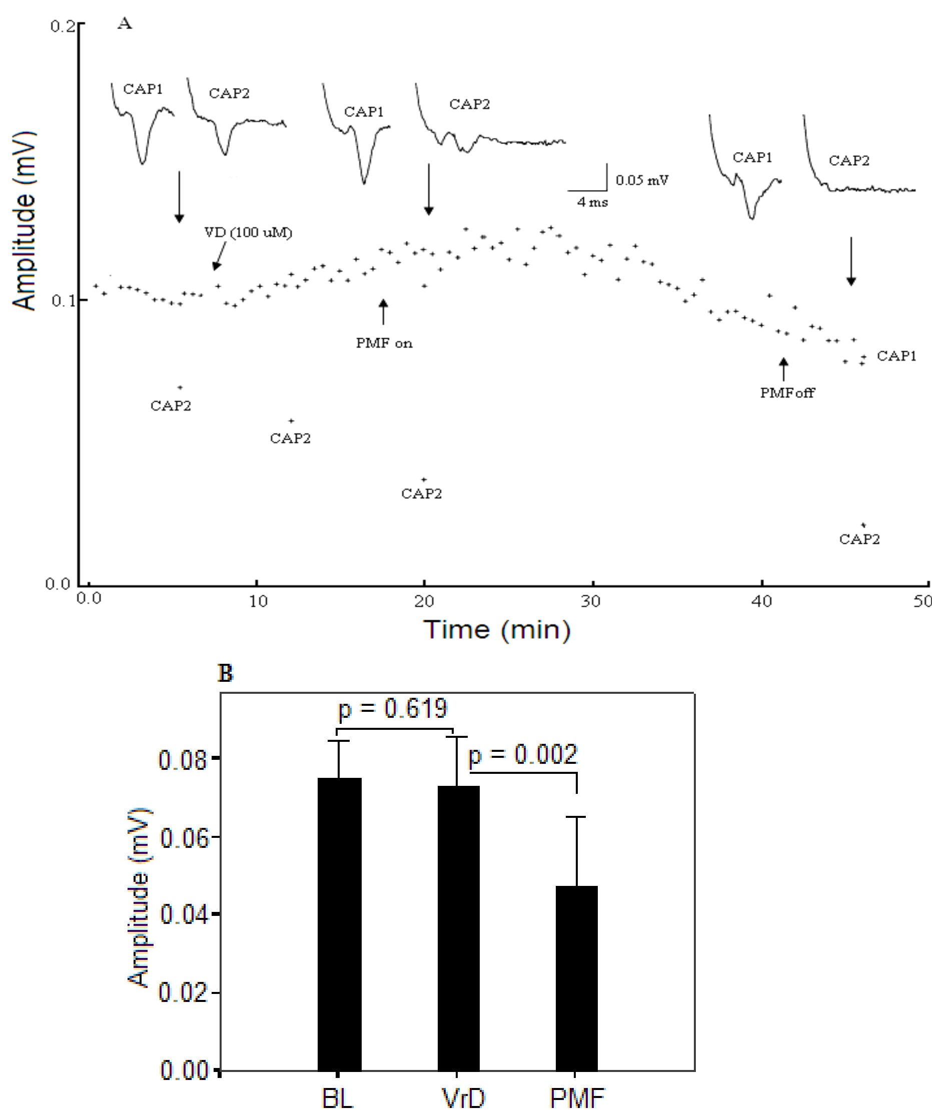


Fig.3.40. Veratridine (VrD) blocked the effect of PMF-induced CAP amplification. After recording of 20 minutes of baseline CAP, veratridine (100uM) was added into the recording chamber. The CAP was followed for another 20 minutes in the presence of VrD, before PMF was turned on. PMF was then turned on for 30 minutes. A – A representative experiment shows the effect of VrD on CAP1 and CAP2 and the effect of the subsequent application of PMF. Arrows show when the potentials evoked by paired pulse were taken. Note that paired pulse stimulation was turned on at the indicated time to measure the amplitude of CAP2. Otherwise it was a single pulse. In two stimuli paradigm, CAP2 needed at least 5-10 seconds of inter stimulus delay to recover completely after the application of VrD and PMF exposure enhanced that effect. B – BL (baseline CAP) is the average of 10 experiments recorded from 10 different segments of the sciatic nerve; VrD and PMF are the averages (CAP1) that were taken after the addition of VrD (15 min) and after PMF exposure (immediately), respectively. VrD did not cause any significant changes in the first CAP amplitude within the first 20 minutes after the application ($p = 0.619$, $n=10$). At the end of the PMF exposure period, PMF enhanced the effect of VrD and reduced the CAP1 significantly ($p = 0.002$).

Effects of elevated $[K^+]_o$ on the PMF induced effects:

The elevation of $[K^+]_o$ slows down the recovery of Na^+ channels from inactivation and attenuates the action potential amplitude (Meek and Mennerick, 2004). I suggest that the PMF-induced effect is partly mediated by increasing the leakage rate of potassium (efflux) that in turn hyperpolarizes the cell membrane. This increase in the leakage rate would increase the level of $[K^+]_o$, but not enough to cause changes in the sodium channels dynamics and be buffered away quickly by glial cells. A moderate increase of $[K^+]_o$ should add to the PMF-induced elevation of $[K^+]_o$. This would reduce the action current, alter Na^+ channel dynamics (activation and inactivation) and subsequently the PMF-induced amplification of CAP. The experiments in *figure 3.41A and B* suggest that the PMF-induced effect is partially mediated by increasing the outward leakage of potassium, leading to the hyper-polarization of the cell membrane, and to the increase in the action current. The increase in the action current may partially account for the amplitude changes due to the PMF exposure. Moreover, as it is shown in *figure 3.41B*, the PMF-induced effect was blocked by the elevated $[K^+]_o$ ($n = 10$, $p = 0.146$, paired t test). The elevated extracellular potassium reduced the CAP amplitude significantly within the first 10 – 15 minutes of the application ($p = 0.004$). The action of the elevated extracellular potassium was spontaneously reversed after 10 – 15 minutes. However, the PMF did not lead to a statistically significant increase of CAP amplitude of the baseline.

In the following set of experiments, the extra-cellular potassium was elevated up to 10 mM. This extra-cellular concentration of potassium was a sub-threshold and did not cause CAP depression. On the contrary, 10 mM of extra-cellular potassium led to a similar increase of CAP1 and CAP2. The difference between CAP1 and CAP2 remained

practically unchanged, see *figure 3.41 and 3.42.* Surprisingly, the PMF exposure reduced the CAP2 markedly ($p = 0.001$) and did not significantly reduce the CAP1. Note that 10 mM $[K^+]_o$ plus PMF behaved exactly like VrD plus PMF.

The representative experiment in Figure 3.41 shows the additive effect of elevated extracellular potassium and the PMF on STD. In this experiment two stimuli impulse with 9 ms inter-pulse intervals caused a short-term depression that was reduced by elevating the extra-cellular potassium. Subsequent exposure to the PMF markedly depressed the CAP2. Collectively, these experiments suggested that the PMF increased the leakage rate of potassium, which in turn increased the bulk of the extra-cellular potassium.

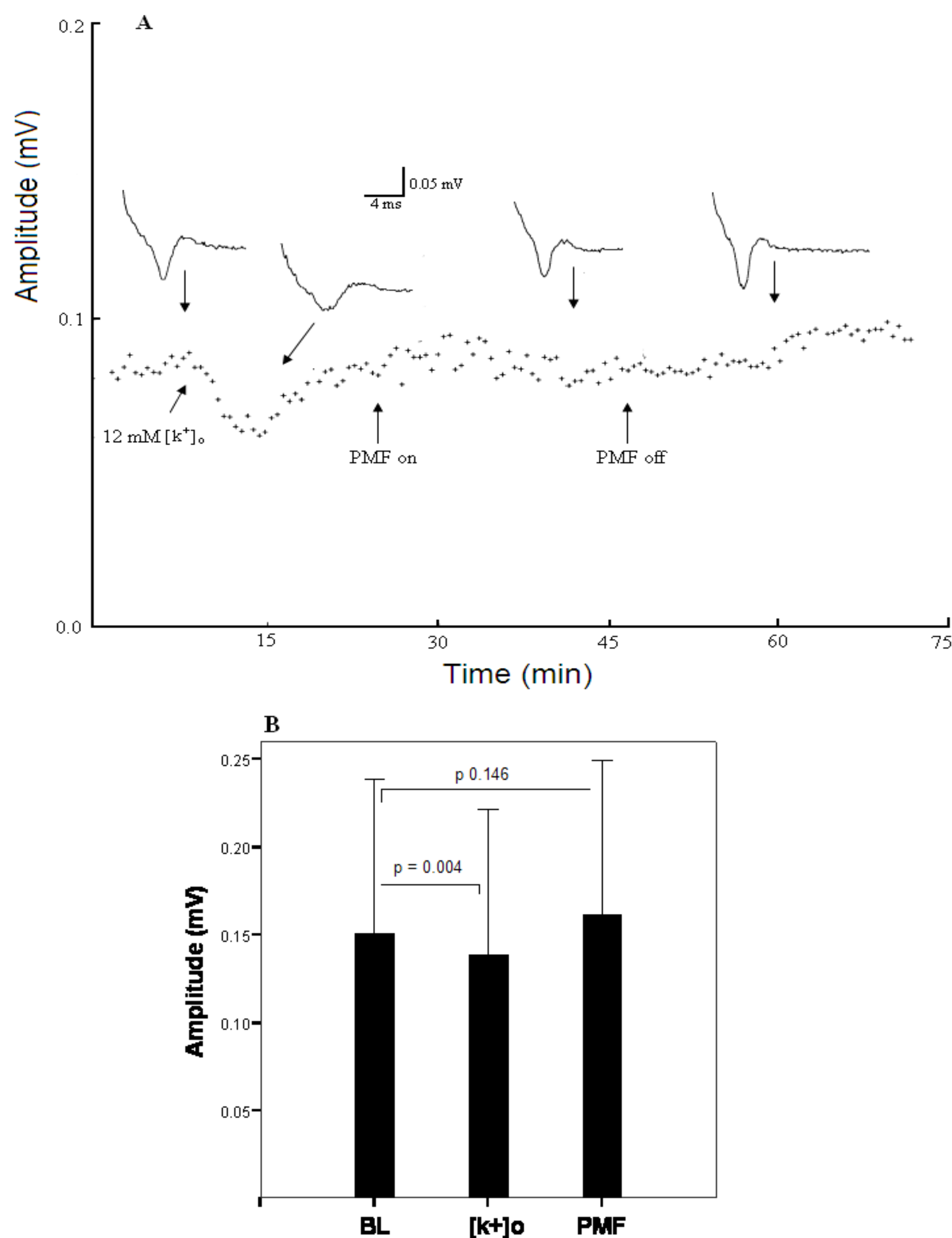


Fig.3.41. The effect of elevated extra cellular potassium $[k^+]_o$ on the PMF induced effect. Elevated $[k^+]_o$ (12 mM) reduced CAP1 significantly (n = 10, p = 0.004), and it then recovered spontaneously. After the recovery from the elevated $[k^+]_o$ the potential has stabilized . PMF was then turned on for thirty minutes. In A, a representative experiment that shows the effect of elevated extracellular potassium on the compound action potential of the sciatic nerve and on the PMF-induced effect. In B, bars represent the averages that were taken at the baseline period (BL), after the elevated $[k^+]_o$ and after the PMF exposure (PMF). PMF did not increase amplitude of CAP (p = 0.146). These results suggest a synergistic interactions between the effects of both PMF and $[k^+]_o$.

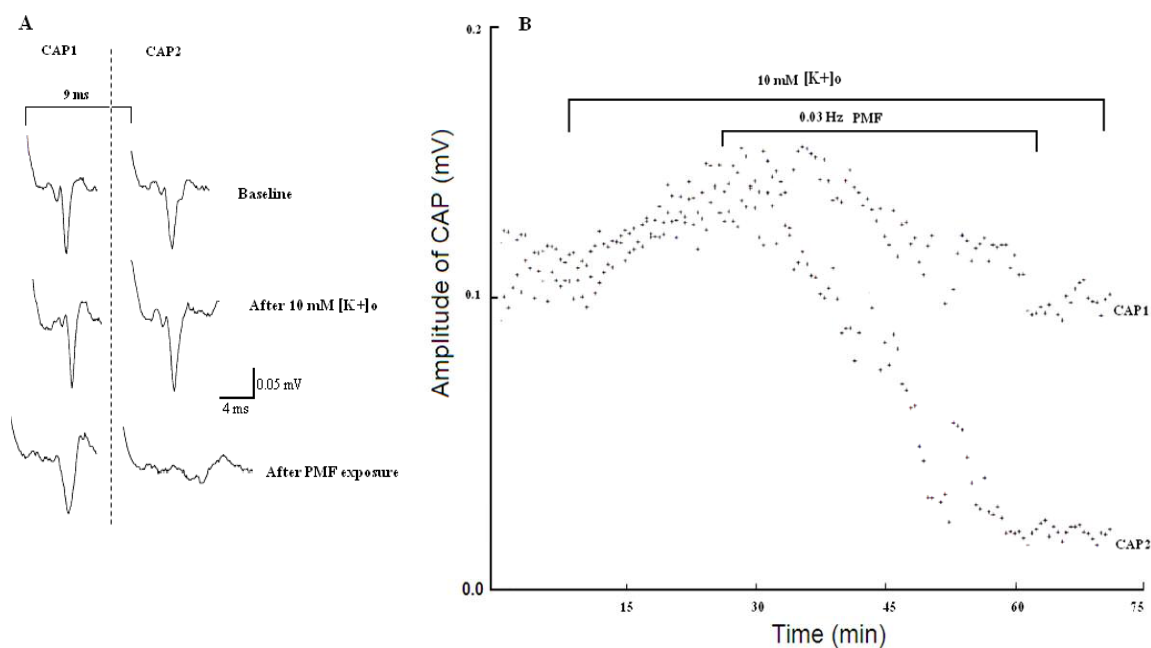


Fig.3.42. The influence of elevated $[k^+]_o$ (10 mM) and PMF on the sciatic nerve CAP. A – Representative potentials from the same experiment show changes of both CAP1 and CAP2 after each treatment (baseline, after elevating $[k^+]_o$ and after the PMF exposure). B – A representative experiment is shown. The horizontal lines indicate the duration of each treatment. Note that increasing the extracellular potassium to 10 mM led to amplification in CAP1, and slight reduction in STD. Subsequent application of PMF led to gradual decrease in both CAP1 and CAP2. PMF noticeably increased the difference between CAP1 and CAP2. The increase in STD was mainly because the decrease in CAP2 exceeded the decrease in CAP1.

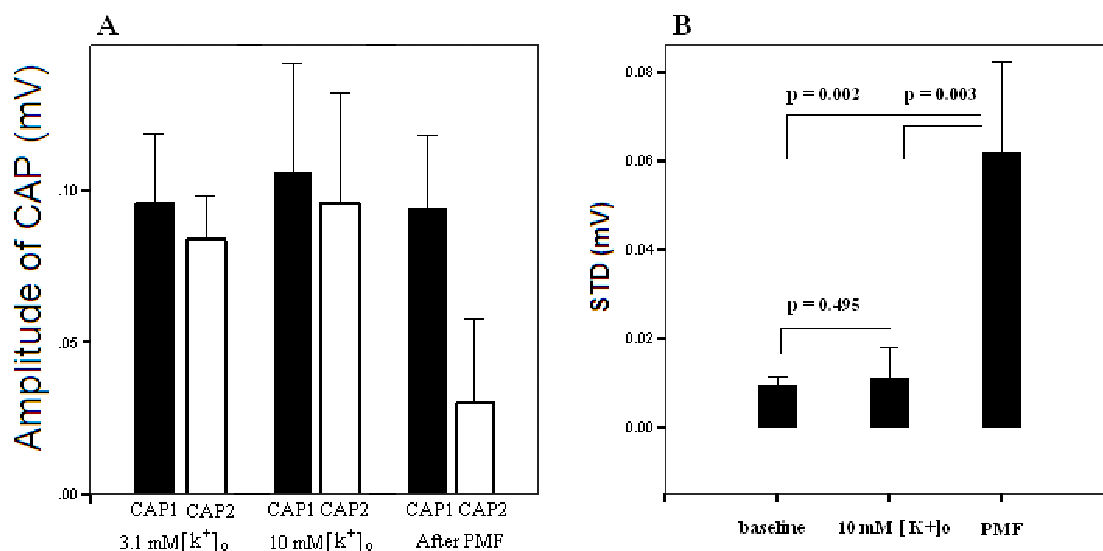


Fig.3.43. Results of the combined effect of elevated extracellular potassium (10mM) and PMF exposure on the sciatic nerve CAP. A – the absolute values of both CAP1 (black bars) and CAP2 (empty bars) were measured before and after the elevation of the extracellular potassium from 3.1mM up to 10 mM, and after PMF exposure (potassium concentration remained at 10 mM during the PMF exposure). The difference between CAP1 and CAP2 in the three measurements was statistically significant, before potassium elevation ($n = 5$, $p = 0.033$), after potassium elevation ($p = 0.016$), and after PMF exposure ($p = 0.001$). Comparing the average potentials of CAP1s of the three treatment (black bars in A) yield no statistical significant ($F = 0.403$, $p = 0.677$), which indicates the blockage of PMF-induced effect. One-way ANOVA was used between groups. B – STD is shown in the baseline (before elevation of potassium), after increasing the extracellular potassium to 10 mM, and after PMF exposure. Note the remarkable increase in the difference after the exposure to PMF.

The pulsed magnetic field frequency and its effect on CAP:

The magnetic field impulse consists of four phases: rising, static, falling, and off phase. These phases depend on three things, 1) the characteristics of the magnetic coil that determines the slope and the magnitude of both rising and falling phases and 2) the applied frequency that determines the duration of both the off and the static phases and 3) the intensity of the supplied electrical current that determines the intensity of magnetic field . For example, at frequency 0.16 Hz the duration of the static and the off phases is 3 seconds. The rising and the falling phases are fixed in all frequencies because the characteristics of the coil and the intensity of the magnetic field remains unchanged. I therefore, performed the following experiments to discern the effect of PMF at different frequencies on the sciatic nerve. I chose five types of frequencies, 0.5 Hz, 0.16 Hz, 0.07 Hz, 0.03 Hz, and 0 Hz (static magnetic field) (30 minutes exposure time) so that I could compare their effect on the centeral nervous system (previous research in the lab) and on the peripheral nervous system (sciatic nerve).

The percentage of the baseline was calculated as the average taken before the treatment minus the average taken after the treatment, divided by the average taken before the treatment (average before - average after / average before). Increasing the frequency led to higher magnification of CAP ($n = 7$, $F = 7.566$, $p = 0.0002$) (figure 3.44). ANOVA one way was used between groups. The results of one-sample t test are shown on the graph. The static magnetic field temporarily reduced the CAP amplitude during the application ($n = 16$, $p = 0.0009$) then the CAP returned to its original amplitude ($p = 0.481$) prior to the field application (figure 3.45).

According to these results the effect of the magnetic field depends on the architecture of the field: the number of rising and falling phases and the duration of the static phase. Figure 3.44 shows an example of a typical experiment that represents the amplitude of the potential. Furthermore, figure 3.45 shows the potential shape at different stages of the experiment.

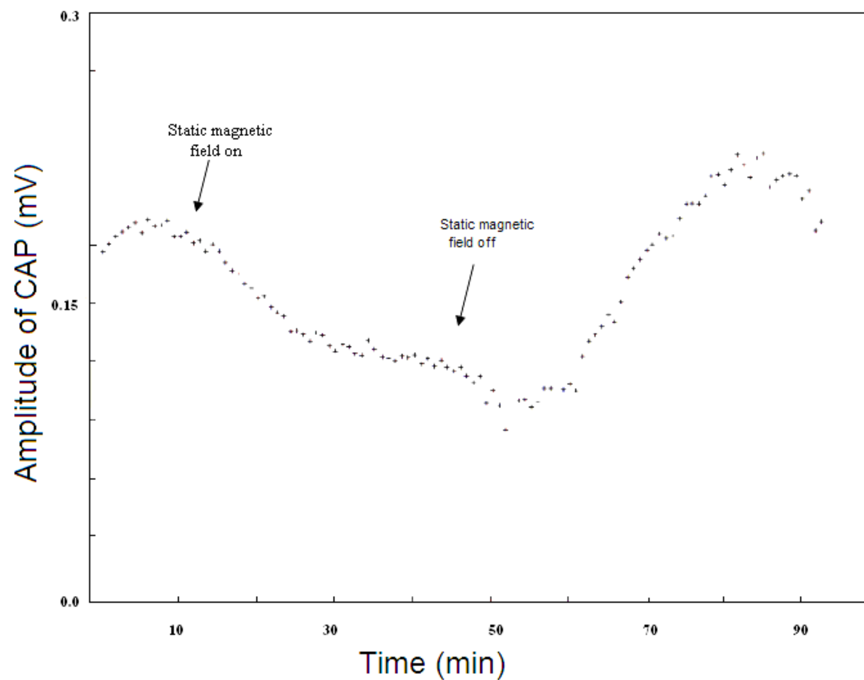


Fig 3.44. A representative experiment showing the influence of static magnetic field on CAP recorded from the sciatic nerve. Static magnetic field induced biphasic effect: depression phase during the application of field and recovery/amplification phase after the termination of the field.

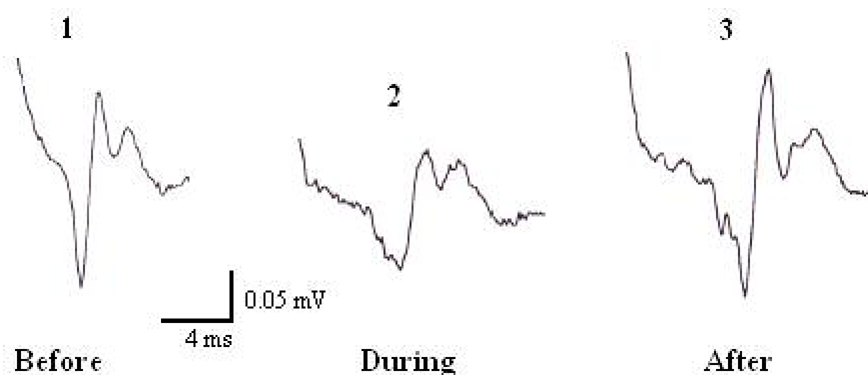


Fig.3.45. Three waveforms show the shape and the amplitude of CAP before (1), during (2), and after (3) the static magnetic field exposure.

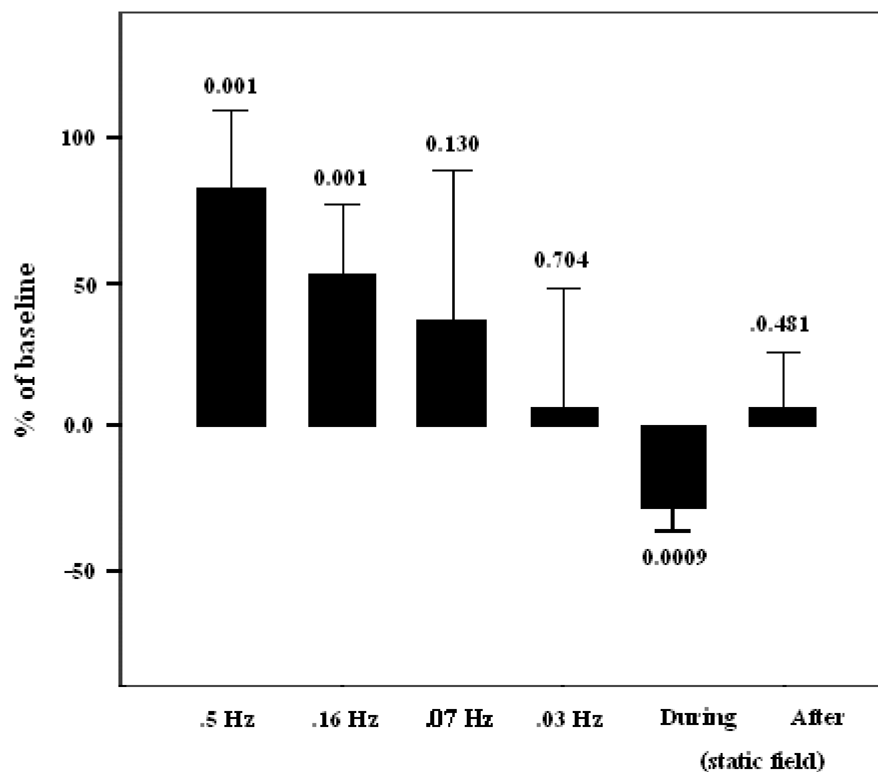


Fig.3.46. The influence of different frequencies of PMF on CAP recorded from the sciatic nerve. The bars represent the percentage of control calculated as the difference between the average before and after the PMF exposure divided by the average before PMF exposure (percentage = the average before – the average after ⁄ the average before). The numbers above the bars represent the p value, which was calculated using one sample t test. The difference between groups was statistically significant One way ANOVA was used ($F = 7.566$, $p = 0.0002$).

Effect of the PMF on memory and learning in mice:

My next step was aimed at testing the effect of the PMF in vivo. The novel object recognition task (NOR) was used to test the animals' ability to recognize a novel from a familiar object. The task is based on the natural tendency of the rodents to spend more time exploring the novel object rather than the familiar object. The experiments were performed on 12 exposed and 12 control animals. I habituated the animals in an open field in the presence of two objects for 20 minutes every day for 3 days. On the day of the test, each animal was exposed to the PMF for 30 minutes, and then tested. After replacing one of the familiar objects with a novel one, I recorded the time each animal spent exploring each object. The time allotted for exploration was 5 minutes. I then conducted the NOR test after one hour and again after three days. The time spent exploring the familiar and the novel objects was divided by the total time that each animal spent exploring the two objects. This yielded the difference between the times they each spent exploring each object in the form of a percent. This difference was then statistically compared using a paired t test. I found that less time was spent in exploring the familiar object as opposed to the novel object by the control in both time-delays of the test (after one hour, $p = 0.001$; after 3 days, $p = 0.0001$). The experimental group showed no significant difference in the results within the two time delays of the test, (after one hour, $p=0.958$; after 3days, $p=0.481$), see figure 3.47. These results indicate a significant impairment of memory in exposed animals.

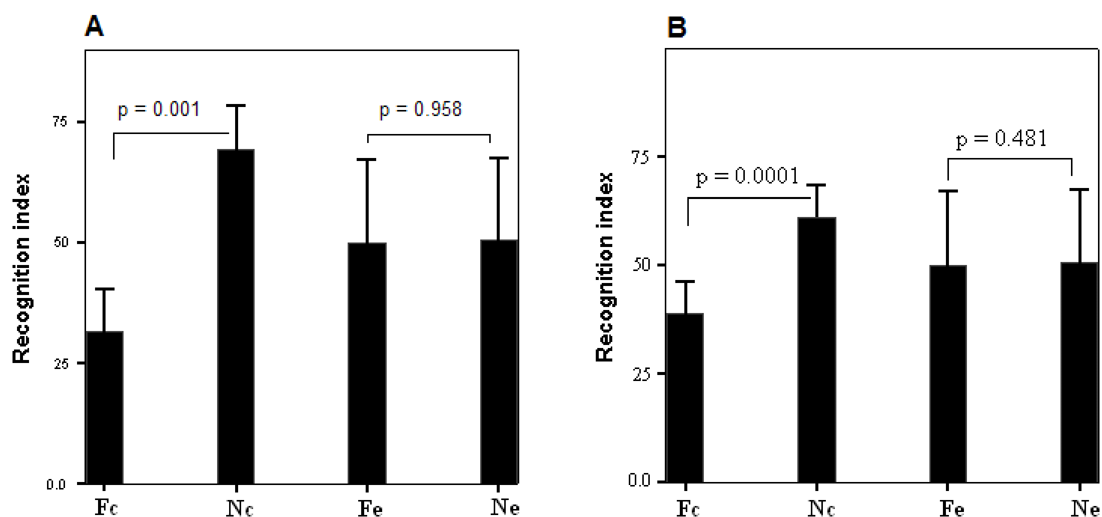


Fig.3.47. effects of PMF on memory and learning in mice. The above graphs are the results of testing 24 animals (12 control and 12 experimental animals) either after one hour (A) or three days (B) of PMF exposure in case of experimental animals (e) or sham exposure in case of control animals (c). The results were expressed as recognition index, which was calculated as the time each animal spent exploring the object divided by the total time which the animal spent to explore the two objects (object time / total time of exploring). As it is shown in the graphs control animals have a significant difference between the two objects novel (N) and familiar (F), on the other hand experimental animals had no significant difference between objects which indicates loss of memory. Paired t test was used.

Effects of rTMS on neuronal plasticity and memory:

The following section deals with behavioral studies. The stimulation of the animals with 1 Hz rTMS significantly impaired their performance in the NOR test when evaluated immediately after. While the control mice spent much less time exploring familiar rather than non-familiar objects ($p<0.005$), animals stimulated with the rTMS- devoted approximately the same amount of time investigating both objects (n.s. Fig 3.48). Stimulation with 8 Hz had a similar negative effect on their performance and there was no difference in the recognition index for familiar and non-familiar objects (Fig 3.48). However, the animals exposed to 15 Hz rTMS and tested immediately after, demonstrated an improvement in memory expressed as a significant decrease in the recognition index (Fig 3.49A). These animals spent much less time than the control mice exploring familiar objects and much more time exploring new objects. Nevertheless, this trend quickly disappeared, as the time between exposure and testing increased. The mice evaluated 1 hour and 3 days after stimulation with the 15 Hz rTMS, performed worse than the control group. They apparently lost their complete ability to differentiate between familiar and unfamiliar objects (Fig 3.49B and C).

For the in vitro experiments, (Fig 3.51) illustrates examples of LTP recorded from the slices obtained from the control group (A) and rTMS exposed animals (B). While the upper part of each figure depicts the shape of the population spike recorded before and after high frequency stimulation (HFS), the lower part shows the changes in the amplitude of the population spike during the entire experiment. Several slices obtained from rTMS exposed animals seemed to be very sensitive to electrical stimulation. Although this phenomenon was not systematically investigated, it suggests that rTMS

modifies the threshold of the exposed neurons. The success rate of LTP induction in the group of the slices prepared immediately after rTMS exposure was significantly correlated with the frequency used for rTMS stimulation (Fig 3.50). While all the slices obtained from the animals stimulated with 15 Hz rTMS expressed LTP, the success rate in the slices obtained from the animals stimulated with 8 Hz and 1 Hz on the same day, was lower – only reaching 75% and 50%, respectively. Approximately 60% of the slices obtained from the control animals expressed LTP. The magnitude of LTP was also correlated with the frequency used for rTMS stimulation (Fig 3.50). The largest LTP significantly exceeding the magnitude observed in the control slices (over 150%), was recorded from the preparations obtained from the animals exposed to the 15 Hz rTMS. The LTP magnitude recorded from the slices obtained from the animals stimulated with 1 Hz rTMS and 8 Hz rTMS was similar to that of the control group. It appeared that the influence of rTMS exposure (15 Hz) on the magnitude of LTP was gradually attenuated, as the time intervals between the exposure and the slice preparation increased. The slices prepared 1 hr and 3 days after the exposure to 15 Hz rTMS stimulation expressed 50% and 80% reduction in the magnitude of LTP, respectively, as compared to LTP recorded in the slices prepared immediately after stimulation (Fig 3.50B).

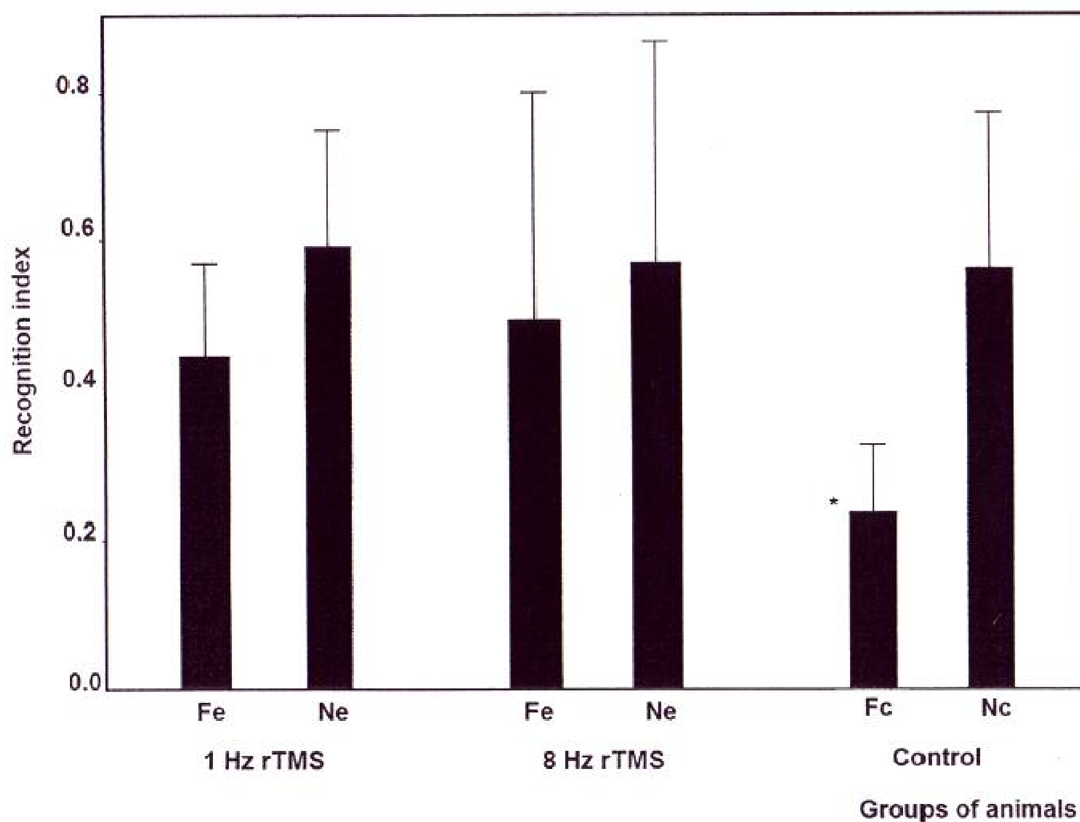
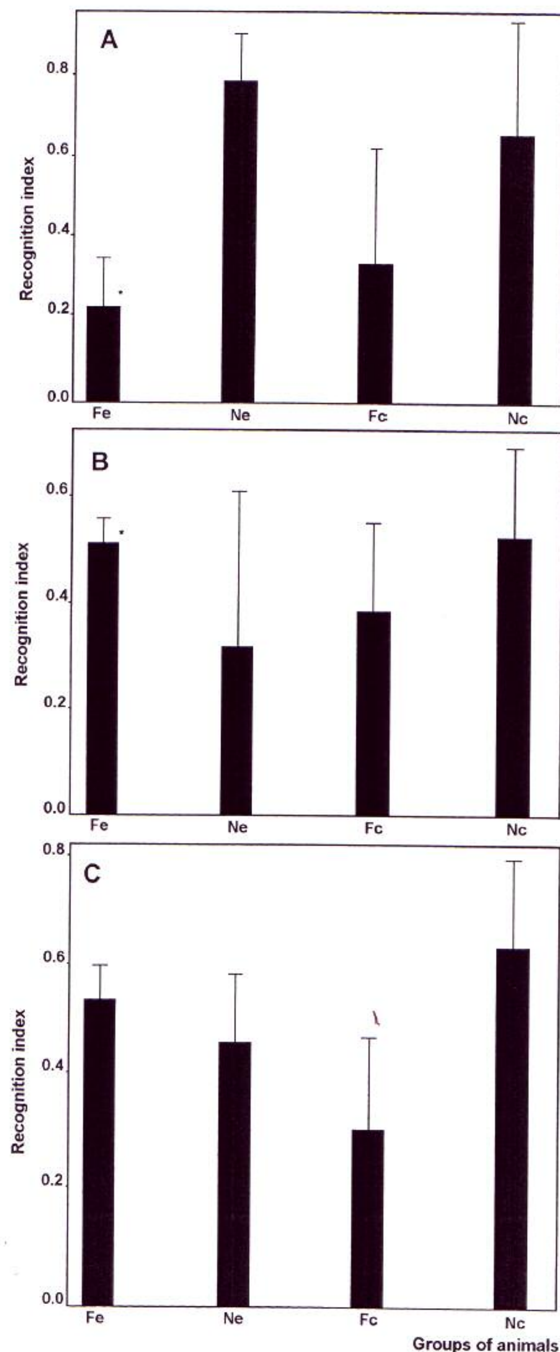


Fig 3.48. A detrimental effect of 1 Hz and 8 Hz rTMS on the performance in NOR. All animals were tested immediately after the exposure to rTMS. The results are expressed as a recognition index which takes into consideration the time spent by control (c) and exposed (e) animals exploring familiar (F) and non-familiar (N) objects. Fe and Fc illustrate recognition index for the exposed and control animals exploring familiar objects, and Ne and Nc marks the recognition index for the animals exploring non-familiar object. While the control animals spent much less time exploring familiar object (the difference between Nc and Fc is statistically significant at $p<0.005$, *), the exposed animals could not differentiate between familiar and non-familiar objects (the difference between Fe and Ne for both rTMS frequencies is not statistically significant, $p< 0.2$; paired t test). The data are expressed as means ± SEM; n = 8 for each group.

Fig.3.49. The effect of 15 Hz rTMS on the performance in NOR test. A - The performance in the NOR test evaluated immediately after exposure to magnetic field. Note, the significant amount of time spent by the exposed animals exploring non-familiar object. The difference between Fe and Ne is statistically significant at p< 0.001(**); B - The performance in the NOR test determined 1 hr after exposure to magnetic stimulation. Note that the recognition index of exposed animals exploring familiar object (Fe) is significantly higher (p<0.05; paired t test, *), than the recognition index for non-familiar object (Ne); C - The performance in NOR test assessed 3 days after exposure to magnetic stimulation. The recognition index for novel and non-familiar objects is practically the same. All data are expressed as means ± SEM; n = 8 for each group. See the legend for Fig 1 for further details.



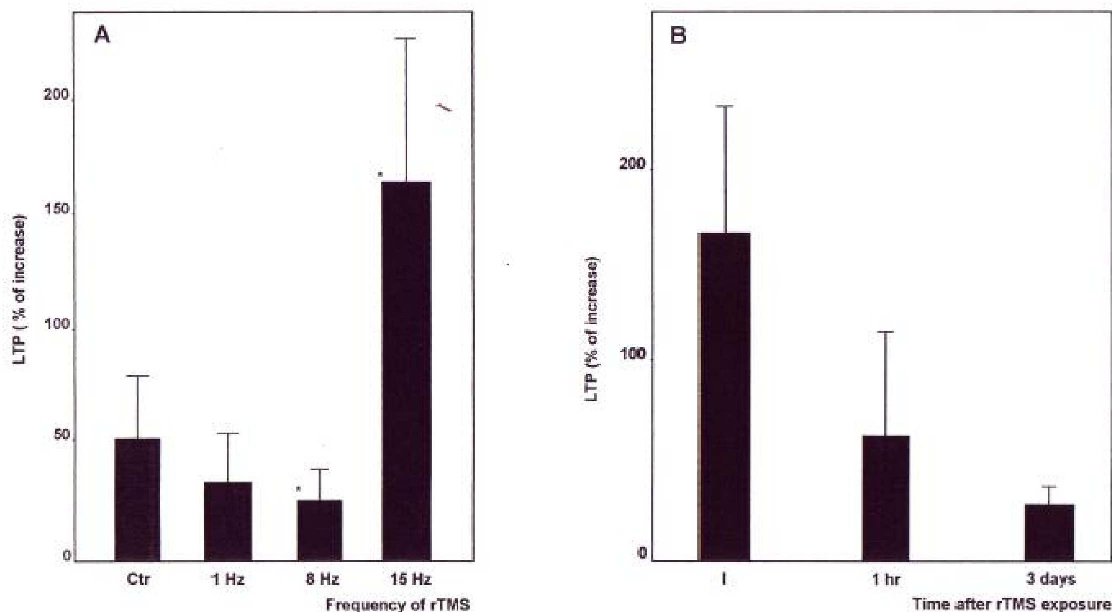


Fig.3.50.The average magnitude of LTP induced in the slices obtained from animals exposed to rTMS. A - The influence of rTMS frequency on LTP. The animals of all groups were decapitated immediately following stimulation by rTMS. Both, 1 Hz and 8 Hz groups demonstrated LTP lower than in the control slices, although the difference was statistically significant for 8 Hz group only ($p<0.035$; paired t test, *). The LTP in 15 Hz group was significantly higher ($p<0.003$, **) than in control slices. Each bar represents the results of 16 experiments (means ± S.E.M.); B - The magnitude of LTP recorded from slices prepared at different time intervals (I - immediately, 1 hr and 3 days) following the exposure of the animals to 15Hz rTMS. The ability of the slices to demonstrate LTP was gradually attenuated, as the time between the stimulation with rTMS and decapitation increased. Each bar represents average results (± SEM) obtained from 16 experiments. The difference between each of these groups is statistically significant (ANOVA, two-way, $p< 0.05$, *).

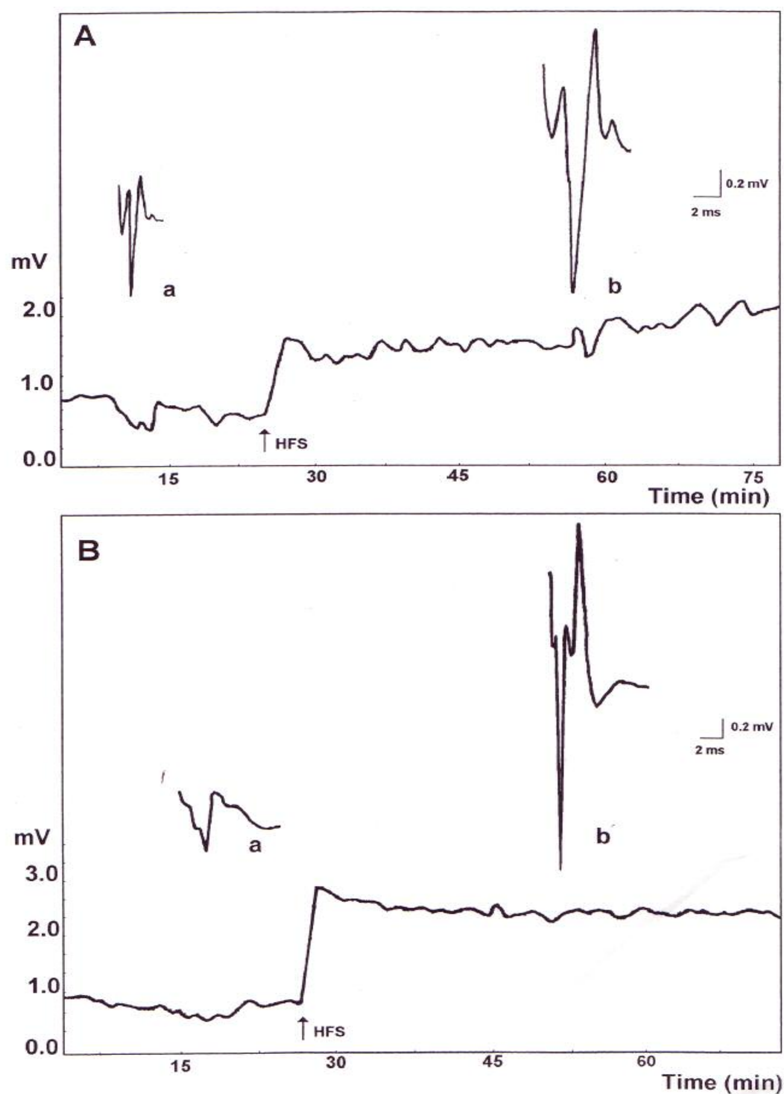


Fig.3.51.The representative examples of Long Term Potentiation (LTP) induced in the slices obtained from the control animals (A) and from the animals exposed to 15 Hz rTMS (B). The slices from the exposed animals were prepared immediately after stimulation with magnetic fields. The upper part of the figures depicts the average of 10 population spikes recorded just before (a) and 40 minutes after (b) application of high frequency stimulation (HFS). The lower part of each figure illustrates the changes in the amplitude of the population spike during the whole experiment.

Discussion and conclusions:

Summary

My findings support the previous reports on the effect of PMF on the nervous system (Semm, et al., 2003; Francis et al., 2003; Wieraszko, et al., 2004). I found that PMF effect depended on frequency, where high frequency caused an increase in excitability and low frequency caused a decrease in excitability. PMF has induced long-term amplification for both population spike (Wieraszko, et al., 2004) and the EPSP recorded from the hippocampal slices. A comparison between the cellular mechanism of LTP and the PMF- induced amplification revealed that the PMF has a different mediatory mechanism from LTP. The LTP expression is localized at the synapse (pre synaptic terminal and post synaptic spine). Although the PMF induced amplification was apparently not localized to an expression place, careful investigation revealed that PMF is mainly expressed in the axonal region of neurons.

The PMF induced an increase in axonal excitability that was reflected as an increase in synaptic chemical and electrical activity. Thus, PMF amplified EPSP increased the synaptic turnover of glutamate, the accumulation of Ca^{+2} (Wieraszko, et al., 2004) and the level of cAMP (Hogan and Wieraszko, 2004). PMF enhanced the cell-cell interaction through gap junction, which in turn increased spontaneous activity.

The PMF induced increase in PPF and PPI. The PPF enhancement implies presynaptic involvement, which reflects the influence of the PMF on the mechanism of neurotransmitter release. Enhancement of PPI on the other hand reflects the influence of the PMF on the efficiency of the recurrent inhibitory loop. The finding that PPI enhancement was accompanied by an increase in excitability shows parallel and

simultaneous amplification of two supposedly antagonistic activities. Moreover, the results of the PPI experiments show that the increase in excitability superseded the increase in inhibition. PMF has a unique effect on the relationship between PS1 and PS2 that differs from the controlled condition and from the effect of increasing the electrical stimulus strength. While the correlation between PS1 and PS2 was negative in the control and after increasing the electrical strength, it was positive after the PMF exposure.

The result from the antidromic slice recording provided the first clue toward the axonal involvement in the PMF-induced increase in excitability. When both synaptic transmission and cell-cell interaction through gap junction were blocked, the PMF was effectively inducing an increase in the antidromically recorded population spike. Further analysis of the result of these experiments indicated that the population spike amplification was not due to an increase in the number of firing neurons but as a result of an increase in excitability of the same population of neurons. The analysis also revealed that the size of the PMF-induced change in hippocampal excitability was predictable. The blocking 4-AP sensitive potassium channels did not alter the effect of the PMF.

The PMF amplified the compound action potential amplitude recorded from the sciatic nerve. This amplification was characterized as long-term, (more than an hour after the field was turned off), and independent of the electrical test stimulus. I also investigated the effect of PMF exposure on STD using the paired pulse paradigm. Similar to the results obtained from PPI and PPF in the hippocampal slices, the PMF increased both CAP1 and CAP2 with the simultaneous and permanent enhancement of STD. This apparent increase in excitation and depression, hints to the involvement of two separate mediatory mechanisms in the PMF-induced effect and also shows the similarity in the

effect in both central and peripheral nervous systems. The source of the axonal excitation and depression is voltage gated sodium and potassium channels, respectively. Therefore, investigating the effect of the PMF exposure on STD at different inter-stimulus intervals uncovered a specific effect on the recovery period of CAP, which implied changes in the properties of the voltage gated sodium channels. The PMF made the recovery period shorter when compared to the control. Combining the PMF exposure with blocking TEA sensitive potassium channels (including voltage gated), made the recovery period even shorter. TEA abolished the STD that may suggest that the Potassium channels are accountable for the expression of STD and therefore the PMF-induced increase in STD. However, this explanation does not discount other possible interpretation like that TEA may leads to generalized increase in resistance of axons so that the shunting of Na^+ current is reduced. In four out of the nine experiments STD was turned into facilitation by PMF exposure in the presence of TEA.

PMF reduced the threshold of CAP recorded from the sciatic nerve. One important observation in the results of the PMF-induced reduction in axonal threshold is that the plateau of CAP-stimulus relationship was similar in those exposed to the PMF and of those within the control. This implies that the PMF-induced increase of axonal excitability was due to an increase in excitability of the same population of axons and not by the recruitment of new axons.

Axons in a nerve bundle such as the sciatic nerve can influence each other by axon-axon interaction through gap junctions (Debanne, 2004). However, PMF-induced CAP amplification was not altered by the blocking gap junctions. The PMF-induced effect on CAP was not altered by the combined treatment of both PKA and PKC kinases

inhibitors. In the bio-electromagnetic area of research, there is great speculation with regard to the mediatory mechanism of magnetic field exposure through free radical accumulation. However, the PMF induced effect was not influenced by the incubation of the nerve with the three strong free radical scavengers (vitamin E, C and Dpi).

TTX and lidocaine, and VrD are specific sodium channel blockers and activator, respectively. PMF has shown to reverse the depressive action of TTX and lidocaine and then amplified the CAP at a later time in the experiment. VrD on the other hand blocked the PMF-induced amplification of CAP. The combined treatment of both veratridine and PMF showed the greater enhancement of STD that was caused by the specific reduction of the second CAP.

The elevation of the extra-cellular potassium up to 12mM, depressed the CAP amplitude, which spontaneously recovered within 10 minutes and that also blocked the PMF-induced CAP amplification. Elevation of the extra-cellular potassium up to 10 mM, only, caused no changes in either CAP amplitude or STD, however. Subsequent application of the PMF did not lead to CAP1 amplification but instead greatly increased STD. These results imply that the PMF exposure might permanently altered potassium ions homeostasis.

The influence of the PMF exposure on mice behavior was tested using the novel object recognition task (NOR). PMF impaired the mice's memory as that related to the NOR tasks. This effect was tested and found to remain positive for three days from the day of exposure.

We tested the effect of rTMS on hippocampal plasticity and on behavior (NOR). There was a correlation between the effects of rTMS on the hippocampal plasticity (LTP

induction and amplitude) and its effect on behavior. In slices obtained from animals exposed to high frequency rTMS (15 Hz), the probability of LTP induction increased up to 100% compared to control 60% and also increased its amplitude. The effect of the same treatment on the behavior of animals' was an immediate increase in the NOR score indicating an improvement in this type of memory. Both effects on the LTP and the behavior were gradually deteriorating. Other types of rTMS frequencies were tested such as 1 Hz and 8 Hz. Both frequencies worsened the LTP expression and memory test scores.

The following is a detailed discussion of each section of the results. As seen in that section, the PMF-induced an increase of neuronal excitability that may be due to either the increase in the synaptic efficiency, the amplification of the action potential, the involvement of electrical synapses, or all of the above.

PMF and hippocampal plasticity (LTP):

The PMF induced effect and long-term potentiation (LTP) are two distinct phenomena. When LTP was induced and a saturated level of potentiation was reached, the PMF turned on for thirty minutes. The PMF application elicited an additional amplification to the already potentiated PS. The first intuitive conclusion is that the PMF and high frequency stimulation HFS have separate mechanisms to influence neuronal excitability.

The APV experiments illustrated that PMF and HFS induced augmentation were indeed distinct. The APV uncoupled the two mechanisms by hindering the HFS induced augmentation. It not only did not affect the PMF augmentation, but also made it more readily inducible (early appearance of PMF-induced effect compared to control). PMF

usually elicited an epileptic field potential (EFP) in 50 to 60% of the exposed slices. Although, that NMDA blocker was used primarily in some experimental models to prevent epileptic field potential, (Mody et al., 1987; Lee and Halbitz, 1990), the PMF, in the presence of APV, induced EFP in about 100% of the exposed slices. Moreover, 40% of the PMF exposed slices showed signs of sudden cell death (abrupt disappearance of potentials after intensive EFP).

The mechanism that has the same interaction with the NMDA blocker (APV) is low extra-cellular potassium concentration (2.5 mM), (Gorji et al., 2001). These authors found that blocking the NMDA receptor intensified the increase in neuronal excitability as induced by low $[K^+]_o$. Comparing this action to the action of the PMF, it can be concluded that changes in the steady state membrane potential may play a role in PMF induced hyper-excitability. One should realize that reducing the extra-cellular potassium is in fact a way of making the resting membrane potential more hyperpolarized. Similarly, hyperpolarization might occur due to PMF exposure, which will trigger the same type of interaction as low extracellular potassium concentration. This will be discussed later.

<u>Effects of PMF on PPF and PPI:</u>

PPF and PPI expressions are due to the accumulation of calcium at the pre-synaptic terminal and the activation of the recurrent inhibitory loop, respectively.

Collectively, from the PPF and the PPI experiments, the following are the main findings:

- An increase in the PPF after the exposure to PMF.

- No correlation existed between the initial PPF and the change in PPF after the PMF exposure. The change is the average potential after PMF exposure minus the average potential before (baseline) PMF exposure.
- An increase in the PPI after the PMF exposure.
- A positive correlation between initial PPI before the PMF exposure and the change in PPI.
- A positive correlation between PS1 and PS2 in the PMF exposed slices in both types of PPI and PPF experiments.
- A negative correlation between PS1 and PS2 in the control groups.

In addition to the above findings it was observed that PMF amplified PS1 and PS2, as well as a simultaneous increase in PPI. This observation was very interesting because it gives a new way of looking at the relationship between excitability and inhibition in the nervous system. The current view of that relationship is antagonistic, as inhibition increases, excitation decreases and vice-versa. In other words there is a balance between excitation and inhibition. Here, on the other hand, we see the inter-dependability between the two components. We can see an increase in excitability, shown as an increase in PS1 and PS2, and a simultaneous increase in PPI (inhibition).

PPI is a measure for the inhibitory loop efficacy. Postsynaptic neurons of the CA1 receive axoaxonic, axodentritic and axosomatic contacts from the inter-neurons that complete a recurrent feedback inhibitory loop. Measuring the effect of this loop at two different sites could give us a more comprehensive idea on its involvement in the PMF induced effect. Therefore, recording fEPSP in paired pulse (less than 10 ms intervals) was conducted to test the effect of PMF on the efficiency of axodentritic component of

the recurrent loop. Recording the population spikes with the same inter-stimulus interval was conducted to test the efficiency of the axosomatic contacts. However, PMF increased the efficiency of both contacts, which was expressed as an increase in PPI. In contrast to what occurred in the control slices after PMF exposure, the PS1 and PS2 were negatively correlated. That implies that PMF may activate a mechanism for excitability that shifts the balance between inhibition and excitation. PMF may also activate a common cellular mechanism shared by all neurons, that when activated causes elevation in all background activity. Another explanation is that PPI is a result of more than one mechanism. That was clear when GABAa receptor was blocked. This treatment reduced PPI but did not abolish it, which means that other mechanisms may be involved in the expression of PPI, not just the inhibitory loop. Using the inter-stimulus delay for less than 10 milliseconds brings refractory periods of action potential into play. At this inter-stimulus delay, refractory periods and changes in background ions concentration of about 80% are accounted for, (the portion of PPI that was not eliminated by the GABAa blocker) of the inhibition seen.

The results of the PPF experiments can add to our view of the nervous system excitability. In addition to that, the PMF amplified PPF increases the amplitude of PS1 and PS2. The fact that increasing PS1 and PS2 did not impinge on PPF expression may mean that excitability in the nervous system has many independent forms and mediatory mechanisms. What also supports this idea, is the additive relationship between LTP and PMF-induced long-term PS amplification mentioned early. Neither blocking nor the not blocking of the expression of LTP has an effect on the expression of the PMF-induced

long term PS amplification. As we see from this discussion the use of PMF sheds light on some mechanisms of the nervous system.

The PMF-induced PPF amplification indicates a pre-synaptic effect. The fact that there was no correlation between the initial PPF and the change in the PPF implies that the PPF-amplification occurred in the same population of neurons and not due to the recruitment of new ones. PMF-induced PPF amplification may be due to: decreased buffering, increased release from the internal stores, and/or increase of the influx through calcium channels at the pre-synaptic terminal.

A previous report (Wieraszko, et al., 2004) showed that PMF, with the same parameters as the field used here, increases synaptosomal Ca^{+2} accumulations. This is an indication of decreased Ca^{2+} buffering, increased Ca^{2+} release to the synaptic terminal, and neurotransmitter releases. In the same report slices were exposed to PMF showed reduction in glutamate uptake (Wieraszko et al., 2005). Thus, our finding that PMF increases PPF is in agreement and support of the results previously obtained in our laboratory (Wieraszko et al., 2004).

PMF and gap junctions:

Gap junctions are ubiquitous in the central nervous system. There are gap junctions between neurons (principle and inter-neuron) and between glial cells. Between neurons Gap junctions transmit electrical impulses and therefore they synchronize the neuronal activity and contribute to EFP (Carlen et al., 2000). Being that PMF increased the rate of spontaneous firing and induced EFP, it made the electrical gap junction a target for my studies. Gap junctions found between glial cells can also modulate the neuronal activity indirectly (Walz 2000). The blocking of a gap junction using the

nonspecific gap junction blocker cbx, reduced the PMF induced effect when added after its application. In addition, cbx reduced the spontaneous firing rate significantly. However, when hippocampal slices were incubated with cbx before being transferred to the recording chamber and subjected to the PMF treatment, they died off. Cbx treatment was deleterious to hippocampal slices and that effect was time dependent. I investigated that effect of cbx separately under controlled conditions by following recordings from slices in a bath containing the same concentration of cbx as was used with the PMF experiments. In these experiments the following evoked potentials were gradually reduced by cbx until they disappeared giving an indication of the deleterious effect of cbx. However, when cbx was added after the slices were exposed to PMF, the evoked potentials were stable for a long period. The conclusion from these experiments is that gap junctions in general mediate the spontaneous activity seen after the PMF exposure. However, further investigation of this phenomenon has been hampered by harmful effect of cbx on the hippocampal slices.

The PMF effect on evoked population spikes recorded antidromically:

Although PMF made synaptic changes such as the one seen as an increase in PPF and partially PPI, the bulk of the increase could not be justified by synaptic changes. Therefore, I decided to use another model to investigate the matter. The action potential was investigated while the contributions of synaptic transmissions were eliminated by the blocking glutamatergic receptors, and performing the experiments in a Ca^{+2} free medium.

In these experiments, the PMF dramatically increased the excitability of the hippocampal pyramidal cells. This was an indication of a synaptic independent mechanism mediating the PMF induced effect. Additionally, the PMF induced multiple

firings (increased burst duration) in response to the electrical testing pulse and intensified the spontaneous activity. I also observed a faster response to the PMF exposure: the slices began to show an increase in the evoked population spikes within the first 10 minutes of PMF exposure compared to over 20 minutes in the control.

Again, in order to show that the PMF induced effect could be predicted, scatter plot and histogram analyses were used. These were shown in the results section. Here, the PMF-induced effect was not dependent on the initial amplitude of the population spike since it has a mean quantity of 390 μV. This suggests that PMF activates a uniform mechanism that has a similar ceiling of excitability in between the slices taken from different animals. In all experiments with hippocampal slices – orthodromically or antidromically recorded – the PMF induced effect had a saturation level that the amplitude of population spike could not supersede. At this saturation level the amplitude curve was horizontal. Although the size of the slices were approximately 0.350 ± 0.205 mm, the slight differences in slice sections may account for the variability in the saturation levels between them because of the differences they have in the amount of neuronal tissue. Even if the size of the slices were similar, the number of neurons differed significantly between sections.

Blocking 4-AP sensitive potassium channels increased the amplitude of the antidromically evoked population spikes significantly. However, this treatment did not block the PMF induced increase in hippocampal excitability thus pointing out that modulation of potassium channels was not a factor in the PMF induced increase in excitability. This does not exclude their probable role in the PMF induced increase of short-term inhibition (PPI). As discussed previously, GABAa could not account for all

the value of PPI. At short time intervals in paired pulse, potassium channels can have a main role in reducing the amplitude of the second response.

In summary, PMF induced a predictable increase in the amplitude of the evoked population spike. The 4-AP sensitive potassium channels play no role in the PMF induced excitability. The most conclusive outcome of these results is that the mediatory mechanism of PMF may lay in the axons.

The effect of PMF on compound action potential recorded from the sciatic nerve:

As previously concluded, the axon was the source of the PMF-induced increase in excitability. To start with, axons with no pharmacological treatment were exposed to the same PMF protocol of exposure. The PMF reliably induced hyper-excitability, which was shown as increase in the amplitude of compound action potential (CAP). The experiments also showed that this effect was independent of the electrical stimulus.

Compound action potential is a collection of single action potentials generated in different axons. Axons in the sciatic nerve have different sizes and contain different combination of channels to serve different functions. The sciatic nerve carries afferent and efferent, myelinated, unmyelinated, somatosensory and autonomic fibers. However, extra-cellular recording cannot differentiate between these fibers. Axonal action potential is mainly generated by sodium channels activation and terminated by sodium channels inactivation and fast potassium channels activation. There is an electro-chemical gradient across the membrane, which is the most important factor in generating action potential. The sodium ions concentration is as high in the extra-cellular space as 10 times the intercellular (140 vs 14 mM); the potassium ions concentration is as high in the intracellular as 30 times the extra-cellular space (150 vs 5 mM). The chloride ions

gradient is more complex in the PNS. It was hypothesized that the chloride gradient is reversed (Nishi et al., 1974) in the axons of peripheral sensory neurons because GABAa receptors activation on the PNS axons led to depolarization (Brown and March, 1978). The concentration gradients of charged ions generated two opposing forces chemical and electrical called driving forces. The resulting membrane potential of most important ions in the nervous system (Na^+, K^+, Cl^-, Ca^{+2}) is driven by the potassium ions efflux because a significant number of potassium selective ion channels are open during rest. The two driving forces: chemical and electrical that are exerted on sodium ions, have the same direction of force to move sodium ions into the cell. On the other hand, in the case of potassium ions, the two driving forces are acting opposite each other and almost balance each other out. The relation between ion concentration and the resting membrane potential can clearly be demonstrated by the GHK equation:

$$E_{m,K_xNa_{1-x}Cl} = \frac{RT}{F} \ln \left(\frac{P_{Na^+}[Na^+]_{out} + P_{K^+}[K^+]_{out} + P_{Cl^-}[Cl^-]_{in}}{P_{Na^+}[Na^+]_{in} + P_{K^+}[K^+]_{in} + P_{Cl^-}[Cl^-]_{out}} \right)$$

Where the E_m is the membrane potential, P_{ion} is the permeability for that ion, $[ion]_{in}$ is the intracellular concentration of that ion, $[ion]_{out}$ is the extracellular concentration of that ion, R is the noble gas constant, T the temperature in Kelvin, and F is Faraday's constant.

The amplitude of action potential depends directly on the resting membrane potential, which in turn depends on the concentration gradient of ions across the membrane and their permeability. First, sodium ions concentration have a direct relation to the amplitude of action potential, because the sodium ions permeability is at its highest

during action potential, therefore the membrane potential would be driven toward the sodium equilibrium potential. As it is shown in the GHK equation, increasing the extra-cellular sodium ion concentration increases the amplitude of E_m or the peak of action potential. On the other hand, potassium ion gradient almost has no direct effect on action potential amplitude during the process of generating the overshoot. Nevertheless, potassium concentration gradient has an indirect relation to the amplitude of action potential. That is, it sets up the amplitude of the action current. For example, starting action potential at – 90 is larger than starting at – 60 mV. Because the difference between the chemical and electrical driving forces is narrow in the case of potassium that makes the potassium current more sensitive to changes in the extra or intra cellular concentration. Increasing the extra-cellular potassium concentration reduces the chemical driving force on potassium ions that in turn reduce the leakage rate of potassium ions (permeability) and depolarizes the membrane potential. Depolarization of the membrane potential will in turn reduces the action current and consequently the amplitude of the action potential. Increasing the extra-cellular potassium ions concentration decreases the driving force acting on the sodium ions during the action potential as well. Decreasing the extra-cellular potassium concentration does the opposite.

Other factors affecting the amplitude of action potential such as the sodium and potassium channel gating systems are the permeability of the ions during an action potential. This will be discussed later in this chapter.

In order for the PMF to influence the amplitude of compound action potential, it has to modulate the homeostasis of the aforementioned factors. Increasing the permeability constant in the GHK equation would consequently increase all other

factors, in turn increasing the stabilizing activity of transporters and exchangers such as Na^+/K^+-ATPase pump. A scenario that can occur is that the PMF increases the permeability of potassium ions to exit the cell and in turn hyperpolarizes the cell membrane and that in turn increases the sodium ion permeability that increases the activity of the Na^+/K^+-ATPase pump. This would set the steady state of the membrane potential to a lower value because both the increase in potassium leakage and the increase in the Na^+/K^+-ATPase pump activity work against the sodium leakage. Increasing the potassium leakage would change the potassium ions extra-cellular concentration to higher values, and decrease the threshold. That could happen by setting a new balance point between an increase of the leakage rate of potassium ions (electrical driving force) and their concentration gradient (chemical driving force) at a lower membrane potential. Consequently, Na^+/K^+-ATPase pump has to compensate for the new balance point by setting its activity at a higher rate changing the sodium ions concentration gradients to higher values outside rather than inside the cell. Increasing the sodium ions concentration gradient outside rather than inside the cell would increase the chemical driving force acting on them. That and the increase in the electrical driving force created by increasing the leakage rate of potassium ions would sum up to increase the electrochemical driving force acting on sodium ions. Increasing the electrochemical driving force on sodium ions consequently increases the amplitude of action potential.

There are two pieces of evidence in the results those support the aforementioned argument: 1) PMF exposure led to an increase in both CAP1 and CAP2 when maximal stimuli were used, and 2) PMF did not reduced the axonal threshold for the maximal CAP (fig. 3.31 and 3.32), although PMF significantly increased the CAP amplitude. However

at sub-maximal stimulus intensities, new axons might be recruited. It means that PMF has set up a new balance point (steady state) for the membrane potential and the amplitude of action potential. These postulated changes by PMF exposure needed parallel changes in voltage gated sodium and potassium channels.

There were miscellaneous experiments that excluded other possible factors and supported the argument mentioned above. Incubating the axonal segments with cbx did not affect the PMF induced increase in axonal excitability, excluding any role for gap junction. Similarly, incubating the nerve segments with PKA and PKC inhibitors had no effect on PMF induced excitability, which made the involvement of sodium channels phosphorylation in PMF-induced effects very unlikely. Activation of both PKA and PKC was found to reduce the peak of the macroscopic Na^+ current (Numann et al., 1991; Li et al., 1992; Smith and Golden, 1996). Logically, disinhibition would increase the Na^+ current. Next, we tested the hypothesis that PMF may increase the concentration of free radicals, which in turn leads to the observed effect. However, incubating the nerve segments with known strong free radical scavengers did not block the PMF induced excitability. The summative result of these experiments is a supportive one for the argument mentioned above.

PMF and sodium channels:

According to (Meek and Mennerick, 2004) the single axon measurement is a microscopic reflection of the fiber volley (compound action potential). Therefore, we can make inferences about sodium and potassium channels by measuring compound action potential (CAP).

Starting with figure 3.30, blocking some of potassium channels with TEA would prevent the modulatory effect of these potassium channels on the action potential. Many things happened in that preparation. First, without any changes in the amplitude curve dynamic, PMF caused shorter CAP recovery time (75 ms faster than baseline). The recovery time is the inter-pulse interval needed for CAP2 to have amplitude equal to the one of CAP1. The fact that PMF led to shorter recovery time cannot be interpreted as changes in sodium channels dynamic. In order to do this properly, the size of the inhibition has to be scaled up, which is smaller after PMF in TEA, and then fit an exponential. However, these results could be useful as a justification for either voltage clamp or single axon intracellular recordings. Second, there is an increase in the amplitude of CAP1 and CAP2. Third, PMF caused facilitation at 50 ms inter-pulse intervals. The STD was not significant either before or after PMF exposure. The facilitation at 50 ms intervals may be due to an increase of the intracellular sodium ions accumulation, elevating $[Na^+]_i$ after exposure to PMF . In this preparation some TEA sensitive potassium channels were blocked and therefore, the bulk potassium outside had little change after the action potential. On the contrary, intracellular sodium ions concentration got few folds higher after action potentials. The activity of Na^+/K^+-ATPase depends on the intracellular concentration of sodium ions therefore, higher $[Na^+]_i$ increases the activity of Na^+/K^+-ATPase, (see a review by Bers et al., 2003). Accumulation of the cytoplasmic sodium ions increases the activity of the Na^+/K^+-ATPase pump which in turn hyperpolarizes the membrane and increases the amplitude of action potentials. Hyper-polarization, in this speculative mechanism, does not cause reduction in the second compound action potential, perhaps because it was compensated

for by the reduction of the membrane threshold. As the resting membrane potential and the threshold have moved downward on the y-axis, so has the relative distance between them. Neither changed the reduction of threshold, For example, if normally the resting membrane potential is – 98 mV and the threshold is – 55 mV; the difference is – 98 - (– 55) = - 43 mV. Presumably, after the PMF exposure, the resting membrane potential moved downward to – 112 and the threshold to – 75 mV, the difference became – 37. Although, the membrane potential is hyperpolarized to -112, the difference is lesser than normal showing, the reduction in the threshold. Another possible consequence for the addition of TEA is that, TEA may simply be increasing the resistance of the sciatic nerve preparation so that the shunting of the Na^+ current is reduced.

Another possible explanation for the increase in action potential amplitude might be that, it is a continuous function of pulse strength (Clay, 2005). At a narrow range between the sub-threshold and the supra-threshold electrical stimulus strength, the action potential amplitude is graded. Thus, the fixed axonal threshold is not fundamentally present (Cole et al., 1970). The overshoot of the action potential ranges in amplitudes from +20 to +45 mV (Clay, 2005). The proceeding results demonstrate that PMF reduces the threshold of the sciatic nerve axons, which could turn a sub-threshold response into a supra threshold response at the same axon. This means that, instead, the axons were responding to a stimulus by an overshoot of + 20 mV before PMF exposure, they might respond by an overshoot of + 45 mV after the exposure to PMF. Summing up the effect over a larger number of axons leads to a significant final increase of compound action potential. The experimental testing stimulus that was used in our experimental design was supra-threshold, which argues against this explanation. Except that if the PMF exposure

caused changes in the sodium channels that led to the subsequent increase in their activation rate and availability. Thus, the axonal response to the same stimulus was higher. It was as if the PMF exposure moved the saturation level of sodium channels availability to an upper value. One can consider in this argument that the amplitude was dependent on two factors 1) the reversal potential of sodium ions across the axoplasma membrane and 2) the availability of sodium channels. Therefore, if the reversal potential of sodium ions was fixed at +58 mV for example, the availability of sodium channels was divided into low, moderate and high. If the availability of sodium channels was at a low level, it restricted the number of available sodium ions ready to cross the membrane that would reduce the amplitude of the resultant action potential. On the other hand, at a moderate or high availability of sodium channels, the overshoot of the action potential would be closer to the reversal potential of sodium ions. One can then consider that the availability factor here was the rate of activation of sodium channels since increasing the rate would presumably increase the availability of sodium channels. Therefore, the amplitude of the action potential is a function of the rate of activation.

Now, how does PMF increase the rate of activation of sodium channels? Sodium channel has three sub units α, β1, and β2. The α subunit consists of four homologous domains, each containing six trans-membrane segments S1 – S6. Segment four (S4) is a voltage sensor (Horn, 2002). S4 contains positive charges and it is connected to a gating particle. S4 moves toward the extra-cellular space in response to a depolarizing stimulus. This movement occurs in steps (Keynes, 2002). The outer mouth and the surrounding space of a sodium channel molecule contain negative charges that are carried by proteins. These negative charges are normally neutralized by divalent ions such as calcium or

magnesium that are bound. Calcium ions in the extra-cellular space screens the local potentials generated by the negative charges, therefore it indirectly affect the movement of S4 and of course the channel gating. Calcium ions in the extra-cellular space serve as channel gating modifier, high [Ca2+]o shift the sodium channel activation curve to positive potentials, and low $[Ca^{2+}]_o$ shift the curve to negative potentials. Thus, low extra-cellular calcium concentration increases neuronal excitability (Hille, 2001). The negative charges present at the extra-cellular side of voltage-gated channels produce negative potential, which is called local potential (Hille, 2001).

Now that I have discussed that, how is that relevant to sodium channels and PMF induced effect? PMF shifted the recovery curve to shorter time recovery, which indicates faster recovery from inactivation and / or shifted activation curve of sodium channels to negative potentials. One thing could account for that effect, and that is that the PMF changed the binding affinity of the local potential negative charges to calcium ions. Therefore, PMF precluded the screening effect of calcium ions. For that to be true, PMF has to change the confirmation of sodium channel so that it reduces or prevents its affinity for binding molecules. The experiments with lidocaine and TTX showed that PMF changed the affinity of these molecules significantly. In the presence of lidocaine (150 µM), the PMF reversed the depressing effect of lidocaine. Similarly, the PMF reversed the depressing effect of TTX (10 nM). This PMF effect appeared to be dependent on the sodium channel state. When PMF preceded the addition of lidocaine, it accelerated the lidocaine-depressing effect."

Local anesthetic drugs such as lidocaine can shed more light on how PMF induced hyperexcitability. In the presence of lidocaine sodium channel inactivation is

greatly amplified. Hyperpolarization of the cell membrane is needed to relieve inactivation of the channel (Hille, 2002). Theoretically, lidocaine binds to a site inside the channel pore and its binding is voltage dependent. Depolarization is needed for the drug to block the channel. The conclusion is that lidocaine binds more tightly to an inactivated sodium channel (Hille 2002). These phenomena can explain why the PMF relieved the sodium channels from the lidocaine effect. Hyperpolarization of the cell membrane by PMF leads to several sequential processes. First, it opens the inactivated gate more readily, second, it loosens drug binding and finally, makes the drug more prone to site dissociation. However, hyper-polarization cannot alone explain all the PMF induced effects mentioned above, but also PMF evidently makes changes to the channel protein confirmation.

PMF reversed the action of 10 nM of TTX. This toxin binds to a receptor in the extra-cellular site of the sodium channel. The action of TTX is not voltage dependent. It blocks stimulated and non-stimulated axons. TTX does not react with the channel gating system (Heggeness and Starkus 1986; Keynes et al., 1991). Therefore, hyperpolarizing the cell membrane would not affect the action of TTX, which is an indication of another mechanism of the PMF induced effect. A change in the protein conformation is the most probable mechanism. Moreover, that change in conformation can account for all PMF induced effects including hyper-polarization of the cell membrane.

PMF induced effect was blocked by veratridine. Veratridine reduces the conduction of sodium channels by 15% of normal and prevents inactivation (Hille 2002). The action of veratridine is activity-dependent and it does not affect the activation gate of sodium channels. Veratridine modifies the inactivation gate of sodium channels and the

lifetime of that modification is in milliseconds. The reaction of the drug (binding and unbinding) develops from the open state of sodium channel (Hille, 1968; Ulbricht 1969; Vijverberg et al. 1982; Sutro 1968; Leibowitz et al. 1986; Barnes and Hille 1988; Wang et al. 1990). The dependence of veratridine action on the open state of sodium channel explains the cooperative effect between the PMF and veratridine actions. The PMF increased the rate of activation of sodium channels and that in turn intensified the action of veratridine. Thus, veratridine together with PMF reduced the amplitude of CAP2.

Being that PMF did not reverse the action of veratridine, it means that the PMF effect on the shape of the channel protein is specific. If the change in the channel complex is general, it would have affected its affinity to all antagonists. Instead, the PMF changed the affinity of lidocaine and TTX and reversed their actions, it did not affect the affinity for veratridine, and it intensified veratridine action. This observation points to the probability of the presence of particular site (or sites) in the channel complex that is more sensitive to magnetic field changes.

The effect of $[K^{+}]_o$ on PMF-induced effect

After action potential, the extra cellular potassium rose dramatically from the resting concentration. In the squid axon, the extra-cellular potassium rose up to 16 mM in the later part of the re-polarization phase of the action potential (Clay, J. R. 2005), and up to several millimolar by strong stimulus trains (Ransom and Goldring, 1973; Ballanyi et al., 1987). The basement membrane and glial cell layers are physical barriers that prevent the fast diffusion of K^+ ions from the extra-cellular space to the bath solution. The concentration of the $[K^+]_o$ during action potential was activity dependent. And it also seemed that the highest the action potential peak, the longer the potassium channels held

open, thereby, making more K^+ ions available to cross the membrane. More extra-cellular accumulation of potassium ions occurred with larger action potentials. The brain adapted certain mechanisms to clear abruptly the elevated extra-cellular potassium after action potential; the main mechanism and the most established one is the clearance of $[K^+]_o$ by astrocytes (see a review by Walz 2000). The results here suggested that the PMF effect on $[K^+]_o$ was independent of neural activity and the amplitude of CAP, even though PMF increased both significantly.

If one assumes that the PMF increased the leakage rate of potassium ions during rest, it would follow that PMF would hyperpolarize the membrane to a lower voltage. Hyper-polarization of the membrane sets the driving force high for potassium ions. However, the driving force is not a variable in the case of potassium channels activation because that activation is voltage gated (Cole and Moore 1960). Nevertheless, starting at a lower voltage increases the size of the action potential. According to Ohm's law, $\boldsymbol{I_k = g_k\ (V_m\text{-}E_k)}$. Which it means $\boldsymbol{I_k}$ is positively proportional to the membrane potential ($\boldsymbol{V_m}$) and negatively proportional to the equilibrium potential for potassium ($\boldsymbol{E_k}$), but because $\boldsymbol{I_k}$ is an outward current it is in fact negatively proportional to the membrane potential ($\boldsymbol{V_m}$). Therefore the electrochemical force – expressed as the term ($\boldsymbol{V_m\text{-}E_k}$) – would increase approaching the $\boldsymbol{E_k}$ in result to an increase in the leakage current of potassium ions ($\boldsymbol{I_k}$). As a result, the resting membrane potential hyperpolarizes into a low steady state that would give –if induced – higher amplitude for action potential. In the results section of this dissertation, PMF increased the amplitude of the compound action potential which may be interpreted as the fact that PMF hyperpolarized the membrane potential. If that assumption held true, then adding elevated extra-cellular potassium

would oppose the action of the PMF. Elevation of the $[K^+]_o$ decreases the electrochemical force and depolarizes the membrane potential. That would lead to reduction in the action potential amplitude. That's exactly what happened, elevating the $[K^+]_o$ blocked the action of PMF.

Those potassium channels are voltage gated and their activation does not depend on the driving force of potassium ions. Therefore, the lowering of the resting membrane potential should not be due to activation of voltage gated potassium channels but rather to increasing the leakage rate of cations efflux or anions influx. Chloride ion current, persistent sodium ion current, and non-selective cation current are leakage currents. Additionally, there are sodium potassium pump and sodium – calcium exchanger. Potassium ions have specifically selective leakage channels such as flickering K^+ channels (Koh et al., 1992), Ca^{2+} -activated K^+ channels, ATP sensitive K^+ channels and sodium-activated potassium channels. The role of K^+ leakage currents can be recognized from the leakage equation $\boldsymbol{I_L = g_L (V - V_L)}$ (Frankenhauser and Huxley, 1964) the potassium $\boldsymbol{I_L}$ is responsible for setting the membrane resting potential. Note that the $\boldsymbol{I_L}$ is the sum of all leakage currents. I assumed that PMF exposure increased the rate of the above mentioned potassium leakage channels and in turn the $\boldsymbol{I_L}$, which subsequently increased the amplitude of the action potentials. Note that increasing the $\boldsymbol{I_L}$ K^+ increases the action current, which is responsible for the amplitude of the action potential. The action current can be defined as the range of the action potential: from the point it starts to the point it ends. PMF increased the $\boldsymbol{I_L}$ K+, which increases the distance between the two points of the action current.

Although TEA was added to the recording bath, it may not be enough to block all K^+ channels and the action of the PMF. The concentration of TEA (500uM) that was used in our experimental setting was enough to block some of potassium channels such as K_1, K_{s2}, and K_{ca} but not others such as K_f, K_s, K_{ATP}, K_{Na}, or $K_{flicker}$ (see a review by Vogel et al., 1995). The compound action potential (CAP) was amplified significantly after the exposure to PMF in the presence of 500μM of TEA. Thus, the PMF probably increased the leakage current through these unblocked leakage potassium channels as well as other leakage channels and transporters. TEA presence in the recording bath abolished the STD between the CAP1 and CAP2. This showed that the increase in the STD was due to potassium channels that are TEA sensitive. Prolonged activation of potassium channels, as mentioned previously, lead to an increase in the number of potassium ions available to cross the membrane. That would increase two parameters: first, it increased the potassium ions concentration in the extra-cellular space, and second, it increased the re-polarizing effect of the fast potassium channels. Accumulation of the extra-cellular potassium in sub-threshold concentration could lead to an increase in the neuronal excitability, as observed in my experiments shown in figure 3.47. Increasing the re-polarizing effect of the fast potassium channels, showed the increase in the STD between CAP1 and CAP2 which was done mainly through depressing CAP2.

Elevating the extra-cellular potassium alone neither caused changes in the CAP1 nor on the CAP2 shape or amplitude. The combined action of both the 10 mM $[K^+]_o$ and the PMF remarkably diminished the amplitude of CAP2. This observation shows a parallel action of in both, the elevated $[K^+]_o$ and the PMF. A moderate elevation of $[K^+]_o$ alone led to an increase in the action potential failure (Meeks and Mennerick, 2004) and

(Debanne, 2004) the PMF alone led to an increase in the STD between CAP1 and CAP2 which can be interpreted as a relative increase in action potential failure of axons those are activated in the CAP2. Therefore, combining the two actions led to a significant increase in action potential failure. Thus the conclusion here is that the PMF increased $[K^+]_o$ accounting for both, an increase in the STD (CAP1 – CAP2) and partial axonal excitability. The difference between the effect of 12 and 10 mM $[K^+]_o$ was significant. The effect of 12 mM was depressive in the first 15 minutes, on the other hand 10 mM was a facilitator at the first 15 minutes. That means the effect of $[K^+]_o$ on compound action potential is concentration dependent.

The mechanism of how elevated $[K^+]_o$ increased the difference between CAP1 and CAP2 is that it the elevated $[K^+]_o$ inactivates sodium channels (Meeks and Mennerick, 2004) and (Debanne, 2004). Therefore, increasing $[K^+]_o$ by PMF after the first impulse could increase the action potential failure (CAP2) and account for the depression along with the increase in voltage gated potassium channels activation rate.

From the elevated $[K^+]_o$ experiments we could conclude that the mechanism that mediates the effect of PMF on STD (CAP1 – CAP2) is different from the mechanism that mediates the increase of the amplitude of both CAPs. That is because the elevated $[K^+]_o$ blocked the amplification of the amplitude of both CAPs. On the other hand, it amplified STD.

<u>PMF effect on animal behavior and learning:</u>

In vivo, the situation was complex and the stimulation of the whole animal and its whole brain can induce a more complex effect than the one that was observed in vitro. The magnetic stimulation in vivo is assumed to be uniform all around the brain. I used

the same paradigm that I have used in vitro, to help in making sensible conclusions of the functional consequences of the PMF induced excitability. Now, the exposure of mice to 30 minutes of PMF impaired the mice's ability to recognize a novel from a familiar object. It can be suggested that PMF induced the same effect in vivo as in vitro – increased the neuronal excitability. That would create a noise, which in the presence of that noise, will be hard to retrieve specific memory signal. I recalled that, the PMF induced hyper-excitability in the hippocampal slices superseded in amplitude and in time the LTP induced excitability, would make the induction and detection of LTP difficult. Therefore, it can be deduced that the PMF induced effect overshadow the signal-processing system. Keep in mind that the nature of the PMF exposure was uniform and random and it did not differentiate between brain regions or between different parts of the cell. Especially if it was mediated by a shared mechanism by all type of neurons and probably glial cells as well. However, the orientation of neurons and their axons in respect to magnetic field lines may lead to unequally distributed stimulation, and in turn responses. That may bias the effect of the PMF between certain brain regions, which can lead to a simultaneously disruptive or facilitative effect. However, these assumptions cannot be assessed for the methodological difficulty. Finally, these finding support the finding of (McNuaghton et al., 1996; Moser et al., 1998) that LTP saturation in vivo impaired special learning. Those authors persuasively argued that saturating the synaptic weight with repeated tetanization would make them reach a neural state where LTP no longer inducible. The similarities between those findings and the findings here – with PMF – is that the PMF induced excitability may saturate the neural pathways so that LTP cannot be induced. Although, in the findings and outcomes of electrically tetanizing LTP

saturation and PMF, LTP saturation is similar, they are mechanistically different. LTP is mainly a synaptic phenomenon, which is a process of driving synaptic weight to maximal; on the other hand, PMF increases the axonal excitability to maximal, which may obscure the LTP induction and detection as a memory signal. That suggests that neurons may adjust their axonal excitability accordingly to allow differences in synaptic weights to be unmasked.

The effect of rTMS on the neuronal plasticity and animal behavior:

We have demonstrated that exposure to rTMS modulates the expression of the learning ability of animals and alters the neuronal excitability in the hippocampal slices obtained from stimulated animals. The influence of rTMS on the behavior of the animals tested immediately after exposure is mostly neurophysiological since we did not notice any other behavioral changes that are related to other body systems.

However, the effects observed in the slices prepared from exposed animals are long-lasting and may involve protein biosynthesis. Apparently, the processes initiated by the exposure to rTMS in vivo outlasted the period of stimulation and were expressed in the tissue tested in vitro. This conclusion supports previous data showing improvement in LTP recorded from the slices obtained from the animals exposed to long-lasting (7 days) stimulation with rTMS (Levkovitz, 1999; Ogiue-Ikeda et al., 2003), and corroborate the experiments demonstrating increased binding to NMDA receptors in the brain slices obtained from the animals exposed to rTMS (Kole et al., 1999).

The effects of rTMS, which extended beyond the period of stimulation could be explained by the activation of specific genes (Fujiki and Steward, 1997; Schlaepfer and Rupp, 2002; Kudo et al., 2005) and subsequent generation of the molecules modulating

neuronal activity. The biosynthesis of the new molecules induced by rTMS exposure was demonstrated convincingly by Anschel and collaborators (2003). The cerebrospinal fluid from humans exposed to rTMS injected into rats changed their response to kindling. While CSF from patients exposed to 10 Hz rTMS enhanced kindling-induced seizures. Reduced incidents of seizures were observed in animals injected with CSF from patients exposed to 1 Hz rTMS. Apparently, depending on the frequency of rTMS, molecules, which could either enhance or inhibit seizures, have been synthesized in the human central nervous system. The concentration of these molecules was high enough to make them transferable and effective in animals. This may explain the most intriguing result of our studies yet, showing a significant correlation between the impact of rTMS stimulation on mice learning ability, and the quality of LTP recorded from the slices prepared from exposed animals. I assumed that exposure to rTMS of either higher (15 Hz) or lower (1 and 8 Hz) frequencies generated molecules that could improve learning and facilitate the expression of LTP or were detrimental for both of these processes, respectively. These rTMS-evoked changes in neuronal excitability occur in analogy to the modifications induced by electrical stimulation. While high-frequency stimulation amplified neuronal excitability initiating LTP, the application of low frequency stimulation (1Hz for 15 min) depressed the synaptic responses inducing Long-Term Depression (LTD). LTP and LTD, which are prominently expressed in the hippocampus, are currently considered important models for studying the mechanisms of persistent alteration in synaptic efficiency in the central nervous system, leading to learning and memory formation (Bliss and Collingridge, 1993; Teyler 1999; Morris et al., 2003; Bear and Linden, 2004). The NOR behavioral test used in our research was originally introduced in 1988 (Ennanaceur et al.,

1988). Since it takes the advantage of the rodent's natural drive to explore new environment, it eliminates stress related to stimulation of the animals in behavioral tests. I was fully aware, that during in vivo exposure to rTMS the hippocampus would not be the only structure to be affected. Although other brain regions, activated simultaneously with the hippocampus could influence the mice's response, there is a lot of evidence suggesting that the hippocampus was essential for the NOR test (Reed and Squire 1997; Myhrer, 1988; Mumby et al., 1996). Therefore, the correlation between the influence of rTMS on performance in NOR testing and the properties of LTP reinforced the general view that LTP represents the mechanism of memory, and further supports the notion about the usefulness of rTMS in studying complex brain mechanisms. Regardless of the type of changes, it is clear that rTMS stimulation in vivo is "priming" the tissue making it more/less susceptible to subsequent electrical stimulation and expression of synaptic plasticity in vitro. One can assume that rTMS stimulation changed the threshold of tissue excitability, and modified the neuronal response to consequent activation. This type of higher form of plasticity, called "metaplasticity" is defined as "…activity-dependent modulation of subsequent synaptic plasticity…" (Abraham and Tate, 1997). Since the neurons activated by rTMS change their excitability in response to electrical stimulation, rTMS exposure can be considered, as a procedure that adjusts neuronal plasticity according to the principles accepted for meta-plasticity.

In summary, our results demonstrate that stimulation of the nervous tissue with rTMS significantly modifies properties of the neurons. The effects are closely correlated with the properties of applied magnetic fields and persist past the period of stimulation. The research aimed at neurochemical processes initiated by rTMS is essential to apply

this relatively new technology to the benefit of patients and to use this technology effectively in psychological research.

Conclusion and future research

This study characterized the effect of PMF as an increase in neuronal excitability. PMF also increased neuronal inhibition through its effect on inter-neurons. The mediatory mechanism of the PMF-induced effect is expressed as changes in channels and transporters of neurons, whose presence is in every neuron. The bulk of the PMF-induced effect is taking place in the axons and through voltage gated sodium and potassium channels. Although this study implicated very strongly voltage gated sodium and potassium channels, it has no direct evidence on their actual changes. Thus a future research would be on using other techniques such as voltage clamp or X-ray crystallography to determine the nature of these changes.

Although the PMF used in this study is a very specific form of magnetic field spectrum, the findings and their analysis that took place in this dissertation can be applied as a basis for other magnetic field forms biological effects. As previously mentioned in the introduction, magnetic field in general is approved to be beneficial as an adjunct therapy in medicine (see review by Shupak, 2003). The lack of mechanisms of magnetic field action hindered its research toward therapeutics (Rubik, 1997). Here we introduced a mechanistic basis for their effect, which would help and guide clinicians and researchers to the best possible utilization of magnetic field in the treatment of the pathology. A clinical based research would be to test the effect of PMF on the peripheral nervous system on humans. Using surface electrodes to measure the compound action potential from a peripheral nerve such as the median nerve is a very simple and safe

procedure. Then applying the same form of PMF or introducing some changes to the field parameters (intensity or frequency) to suite the new model, would bridge the gap between animal research and its therapy application in humans.

Appendix A

Physical properties of magnetic field:

Magnetic field has many different parameters. Those parameters are the magnetic field strength or so-called the magnetic field flux density, the magnetic field force, the polarity and the inclination. Magnetic field is a descriptive concept that demonstrates the effect of magnetic field on moving electrical charges on the empty space around them. The magnetic field can be created around a bar magnet or when electric current passes through a wire. It has a north and a south pole. The field's lines diverge from the south and re-enter the magnet through the north. Therefore, the magnetic field circulates and never converges at a given point, whereas the electrical field converges at a point and never circulates. The two components of the magnetic field, north and south poles, cannot be isolated the same way electrical charges can (positive and negative). Regardless of how big (earth) or small (electron) the magnetic field of an object is, it always has two components north and south. Electrons circulating around the atomic nucleus have a magnetic field that follows the right hand rule direction. So the electron circular motion can be considered as a loop of wire that carries current. The north and south poles of the magnetic field can be considered as positive and negative, respectively. The two poles have a similar effect on a test charge that only differs in the direction of their force vector. For example a moving positive testing charge will be turned away from the North Pole and turned toward the South Pole.

The magnetic field force:

The magnetic field exerts a force on the moving charges. It was found that the force acting on a moving charge (*Q*) in a magnetic field (*B*) was directly proportional to the velocity of the charge (*V*) and the strength of the magnetic field.

$$Fm = Q \times V \times B.$$

Where *Fm* is the magnetic field force and *Q* is the charge, *V* the charge velocity and *B* the magnetic field strength. This equation is satisfied when the magnetic field is perpendicular to the charge axis. Otherwise, it is equal to the right hand term of the above equation multiplied with sin theta θ where theta is the angle between the two vectors *B* and *V*.

$$Fm = Q\times V \times B\times \sin\ \theta$$

If the angle θ is zero (when the magnetic field lines are parallel with the charge axis) the magnetic force would be zero as well. The maximum force applied by a magnetic force on a moving charge is that when the angle between the charge axis and the magnetic lines is normal. Starting at zero increasing θ would increase the applying magnetic force and vice versa.

Current-carrying wire

When a wire-carrying current is placed at a uniform magnetic field, the moving charges will experience a magnetic force. The direction of that force is perpendicular to the plane of the magnetic field and the wire. If the angle between the wire and the magnetic field is θ, the force on the charges (electrons) is equal:

$$Fm = B \times L \times I \times \sin\ \theta,$$

Where *I* (the current) is the number of charges passing a particular point per unit time *t*.

$I = q / t$. Where q is the electron charge.

Induction:

Induction occurs when there is a relevant motion between the current carrying wire and the magnetic field. This phenomenon is time dependent. If a loop that is connected to an ammeter is moved across a magnetic field, the charges carried inside that wire would move and generate a current. The velocity of the charges is a vector that is perpendicular to the magnetic field force and it follows the right hand rule. The movement that generates that current is relevant. It does not matter, if the wire moves in relation to the magnetic field or the magnetic bar that is moving. Similarly, using an electrical magnet, with a coil wound around ferromagnetic material, the magnetic field generated by that magnet did not become a field instantaneously, but increased with time. Therefore, the change in the magnetic field density or flux by time resembles the relevant motion of the wire or the magnet, which produces electromotive force in the loop and moves the charges, which creates a current, appears on the ammeter.

Lenz's law

Lenz's law explains why the current through a coil does not reach its final steady state instantaneously. It says that if a current passes through a coil, this current generates a magnetic field generating an electromotive force that opposes the original current. This phenomenon is called self-induction. The same thing happened between two coils set close together. A current passing through one coil generates a magnetic field flux that crosses the other in a time dependent way, producing an electromotive force that opposes the direction of the original electromotive force (EMF).

To quantify the magnetic field flux through an area, we should think of it as the number of lines crossing this area per units of time. We call it the magnetic field flux. The magnetic field flux (ϕ) through a uniform area equals the product of the field strength B and the surface area A.

$\phi = B \times A$ where B and A are perpendicular.

Faraday concluded that the EMF induced in a conductor is equal to the rate of change of the magnetic flux through that conductor.

EMF = (the change ϕ) / (the change of time).

<u>The Current (I) and the Current density (J):</u>

Current, is the total number of charges that pass through a cross sectional area per unit time. If a number of charges Q pass through a defined cross sectional area per unit of time t then the current I equals the passing rate of charges ΔQ / the rate of change in time Δt.

$$I = \Delta Q / \Delta t$$

Current density is the rate of charge flow per unit area in a uniform conductor. Unlike current, which is scalar, current density calculation is a vector that has to take into account the direction of the charges.

$$J = I / A$$

$$J = \text{v} \times Q \times N_q$$

Where v is the velocity of the charge, N_q is the number of charges per unit volume. And the current can be obtained by:

$$\text{I} = v \times Q \times N_q \times A$$

Where A is the area perpendicular to v (the velocity), the density of the free electron N_q of a substance, can be calculated as follows:

Nq = (number of atoms per Mole) × (number of free electron per atom × the mass density) / (the molar weight).

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