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# NEUROPROTECTIVE EFFECT OF WEAK STATIC MAGNETIC FIELDS IN PRIMARY NEURONAL CULTURES

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**Abstract—Low intensity static magnetic fields (SMFs) interact with various biological tissues including the CNS, thereby affecting key biological processes such as gene expression, cell proliferation and differentiation, as well as apoptosis. Previous studies describing the effect of SMFs on apoptotic cell death in several non-neuronal cell lines, emphasize the importance of such a potential modulation in the case of neurodegenerative disorders, where apoptosis constitutes a major route via which neurons degenerate and die. In this study, we examine the effect of SMFs on neuronal survival in primary cortical and hippocampal neurons that constitute a suitable experimental system for modeling the neurodegenerative state *in vitro*. We show that weak SMF exposure interferes with the apoptotic programing in rat primary cortical and hippocampal neurons, thereby providing protection against etoposide-induced apoptosis in a dose- and time-dependent manner. Primary cortical neurons exposed to SMF (50 G) for 7 days exhibited a 57.1 ± 6.3% decrease in the percentage of cells undergoing apoptosis induced by etoposide (12 μM), accompanied by a marked decrease in the expression of the pro-apoptotic markers: cleaved poly ADP ribose polymerase-1, cleaved caspase-3, active caspase-9 and the phospho-histone H2A variant (Ser139) by 41.0 ± 5.0%, 81.2 ± 5.0%, 72.9 ± 6.4%, 42.75 ± 2.9%, respectively, and by a 57.2 ± 1.0% decrease in the extent of mitochondrial membrane potential collapse. Using the L-type voltage-gated $Ca^{2+}$ channel inhibitor nifedipine, which is selective to $Ca^{2+}$ influx through $Ca_v1.2$, we found that the anti-apoptotic effect of SMFs was mediated by $Ca^{2+}$ influx through these channels. Our findings demonstrating altered $Ca^{2+}$-influx in response to thapsigargin stimulation in SMF-exposed cortical neurons, along with enhanced inhibition of KCl-induced $Ca^{2+}$-influx through $Ca_v1.2$ channels and enhanced expression of $Ca_v1.2$ and $Ca_v1.3$ channels, allude to the involvement of voltage- and store-operated $Ca^{2+}$ channels in various aspects of the protective effect exerted by SMFs. These findings show the potential susceptibility of the CNS to weak SMF exposure and have implications for the design of novel strategies for the treatment and/or prevention of neurodegenerative diseases.** **© 2014 IBRO. Published by Elsevier Ltd. All rights reserved.**

**Key words: apoptosis, calcium influx, etoposide, neuroprotection, primary neuronal culture, static magnetic fields.**

## INTRODUCTION

Weak static magnetic fields (SMFs; 0.1–400 mT)[1] have raised a growing interest in recent years due to a wide variety of biological effects reported in both preclinical experiments (Ohkubo and Xu, 1997; Okano et al., 1999; Miyakoshi, 2005; Saunders, 2005), and clinical studies in human subjects (Vallbona et al., 1997; Man et al., 1999; Carter et al., 2002; Weintraub et al., 2003). In the CNS, SMFs were reported to affect numerous functions such as inhibition of sensory nociceptive action potentials (McLean et al., 1995), alteration in gene expression (Hirai and Yoneda, 2004; Tenuzzo et al., 2009), in cell orientation and morphology (Pacini et al., 2003; Teodori et al., 2006), and promotion of neuronal progenitor cell proliferation (Nakamichi et al., 2009). Interestingly, SMFs were also implicated in the modulation of apoptotic cell death in several non-neuronal cell lines (Fanelli et al., 1999; Tenuzzo et al., 2006), notably, in human leukemic monocyte lymphoma cell line (U937) and T lymphocytic CEM cell lines (Fanelli et al., 1999; Chionna et al., 2003; Cerella et al., 2011), lymphocytes and thymocytes (Flipo et al., 1998; Tenuzzo et al., 2006) and glioblastoma (Teodori et al., 2002). However, such modulation of apoptosis has not been demonstrated thus far in primary neuronal cultures such as

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*Abbreviations:* Aβ, amyloid beta; AD, Alzheimer's disease; AIF, apoptosis inducing factor; ANOVA, analysis of variance; APAf-1, apoptotic protease activating factor 1; Ara-c, cytosine arabinoside; BSA, bovine serum albumin; CREB, cyclic-AMP response element binding-protein; EDTA, ethylenediaminetetraacetic acid; ER, endoplasmic reticulum; GusB, glucuronidase beta; H2A.X, H2A histone X variant; pH2A.X, phospho-histone H2A.X (ser139); JC-1, 5,5′,6,6′-tetrachloro-1,1′,3,3′-tetraethylbenzimidazolylcarbocyanine iodide; MF, magnetic field; MMP, mitochondrial membrane potential; NBM, neurobasal medium; NMDA, N-methyl-D-aspartate; PARP1, poly (ADP-ribose) polymerase-1; PBS, phosphate-buffered saline; PBST, PBS-Tween 20; PC12, pheochromocytoma cell line; PM, plasma membrane; qPCR, quantitative real-time PCR; SD, Sprague–Dawley; SMF, static magnetic field; SOC, store-operated channel; ThG, thapsigargin; TUNEL, terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling; U937, human leukemic monocyte lymphoma cell line; VGCC, voltage-gated calcium channel.

[1] 1 mT = 10 Gauss.

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cortical and hippocampal neurons, whose progressive degeneration and death in neurodegenerative diseases such as Alzheimer's disease (AD) have been suggested to occur via apoptosis (Hartmann et al., 2000; Tatton, 2000; Mattson, 2006), and therefore serve as a suitable model for the study of neurodegenerative diseases. Theoretical models aimed at explaining the interaction of SMFs with biological systems often hypothesize a primary physical interaction of the field with a particular molecular target, preceding subsequent events that ultimately affect the movement of charged particles, such as $Ca^{2+}$, in the cell (Rosen, 1993b). Indeed, studies examining the effect of magnetic fields (MFs) on numerous, mostly non-neuronal cell lines, consistently point to the central involvement of a disrupted ionic equilibrium driven by altered $Ca^{2+}$ fluxes (Bawin and Adey, 1976; Bian et al., 1997; Gobba et al., 2003; Liboff et al., 2003; Grassi et al., 2004; Tenuzzo et al., 2006; Piacentini et al., 2008; Wang et al., 2010). For instance, Fanelli et al. (1999) showed that the anti-apoptotic effect elicited by 0.6-mT SMFs in U937 cells was mediated by modulated $Ca^{2+}$ influx from the extracellular medium. A static field strength of 6 mT was reported to inhibit apoptosis and to affect $Ca^{2+}$ influx from the extracellular medium in human glioblastoma cells (Fanelli et al., 1999; Teodori et al., 2002), and pheochromocytoma-derived (PC12) cells exposed to 230–280-mT SMFs were shown to exhibit a decreased $Ca^{2+}$ efflux from the cells to the extracellular medium (Wang et al., 2010). It has been suggested that MF-induced altered $Ca^{2+}$ oscillations are mediated by a selective modulation of $Ca^{2+}$ influx through L-type voltage-gated $Ca^{2+}$ channels (VGCCs) (Grassi et al., 2004), although SMFs' effects on $Ca^{2+}$ fluxes operating through other $Ca^{2+}$ channels such as store-operated channels (SOCs), have also been reported (Cerella et al., 2011).

In the present study, we examine the pro-survival effect exerted by weak SMFs in primary neuronal cultures that model the neurodegenerative state *in vitro*. Effects of SMF exposure in primary neuronal cultures are described in a few studies that do not directly address the pro-survival aspect of weak SMFs' action, but rather focus on cellular signaling cascades or alterations in cellular morphology affected by strong SMFs (Pacini et al., 1999; Prina-Mello et al., 2005, 2006; Teodori et al., 2006), which utilize different mechanisms of action than those activated by weak SMFs (Rosen, 1993b).

We show that SMF exposure reduces apoptosis in primary cortical and hippocampal neurons subjected to either etoposide or amyloid beta $(A\beta)^{1-42}$ toxicity. The reduction in apoptosis in primary cortical neurons is associated with a marked down regulation of caspase-3, caspase-9 and other pro-apoptotic proteins and with the stabilization of the mitochondrial membrane potential (MMP), and is mediated by calcium influx through the voltage-gated calcium channels (VGCCs) whose expression was enhanced by SMF exposure in the absence of neurotoxins.

## EXPERIMENTAL PROCEDURES

### Animal procedures

All procedures with animals were authorized by the Technion Animal Care and Use Committee, whose ethical standards are based on those detailed in the National Institutes of Health (Bethesda, MD, USA) Guide for the Care and Use of Laboratory Animals (NIH Publications 80-23 revised 1996), and whose general procedures for animal welfare comply with Israeli law on animal experimentation.

### Materials

Unless otherwise specified, all reagents and chemicals were purchased from Sigma–Aldrich (St. Louis, MO, USA), all primary antibodies were purchased from Cell Signaling Technology Inc. (Danvers, MA, USA) and all secondary antibodies were purchased from Jackson Laboratories (Bar Harbor, ME, USA).

### Cell culture

*Primary cortical and hippocampal neurons.* Dissociated neurons were prepared from the brain cortices or hippocampi of postnatal day 1 Sprague–Dawley (SD) rats (Harlan laboratories, Jerusalem, Israel), plated on poly-D-lysine coated 24-well plates ($2.5 \times 10^5$ cells/well) and maintained in neurobasal medium (NBM) supplemented with B27 (Gibco, Grand Island, NY, USA).

*Primary glial cells.* Dissociated glial cells were prepared from the brain cortices of postnatal day 1 SD rats, plated on un-coated 24-well plates and maintained in minimal essential medium supplemented with 40 mM L-glutamine. Two days post-plating, cultures were washed with phosphate-buffered saline (PBS) with gentle shaking for 5 min then returned to the medium. Medium was changed after 72 h.

*SMF exposure system.* SMFs were generated using an array of 24 $Ne_2Fe_{14}B$ magnetic disks of 11.2-mm diameter and known intensities, placed below the 24-well culture plates containing the cells. Different intensities of SMFs were achieved by placing the magnetic array at varying distances beneath the culture plates, and the intensity of the field in each of the 24 wells was measured at five constant points using a Hirst GM08 Gaussmeter equipped with a transverse Hall probe (Fig. 1b). In all experiments, data were collected from six (for apoptosis detection) or 24 (for qPCR) wells per experimental condition, in which the intensity of the measured field was comparable. The culture plates were kept in an incubator with 5% $CO_2$ atmosphere at 37 °C. Unless otherwise specified, cells were exposed to SMFs over the 7-day culture period.

*Time-course experiments.* In order to determine the effect of different times of culture in the presence of SMFs, cultures were incubated in the presence of SMFs for one (DIV6–DIV7), four (DIV3–DIV7), five (DIV2–DIV7), six (DIV1–DIV7) or seven (DIV0–DIV7) days, treated with 12 μM etoposide on DIV6, and apoptotic cells were quantified on DIV7 using Hoechst-33342 as described in Section 'Induction, detection and quantification of apoptosis' (see Fig. 2c). In order to

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