Static magnetic field effects on cells. A possible road to cell differentiation.

Adriele Prina-Mello[*], V. Campbell[**], and J.M.D. Coey[***]

[*] SFI Trinity Nanoscience Laboratory, Trinity College Dublin, Dublin 2, Ireland, prinamea@tcd.ie.
[**] Trinity College Institute of Neuroscience, Trinity College Dublin, Dublin 2, Ireland, vcmpbll@tcd.ie.
[***] Department of Physics, Trinity College Dublin, Dublin 2, Ireland, jcoey@tcd.ie.

ABSTRACT

The use of higher uniform magnetic field intensity, such as those used in magnetic resonance imaging, is thought to exert little influence at the cellular level. There are several experimental studies that indicate that magnetic fields are unlikely to be cell proliferation/differentiation initiators although further investigations are needed to clarify potential factors acting together with magnetic field to eventually promote changes in the signaling cascade and therefore cancer onset or neurodegenerative diseases.

In this work, we report on the modifications of the signaling cascades in rat cortical neurons cultured for one hour in magnetic fields of up to 5 tesla (T). The results show a maximum at 0.75 T (~10 %) for the activation of the extra cellular-regulated kinase (ERK). These results indicate a magnetic induction of the signaling cascade events associated with cell differentiation. A possible *magnetohydrodynamic effect* mechanism is also discussed at the porous-membrane interface.

Keywords: **Static magnetic field, cell signaling cascade, rat primary cortical neurons, cell differentiation, magnetohydrodynamic effect.**

INTRODUCTION

The use of higher uniform magnetic field intensity has been reported, to date, to exert little influence at the cellular level. This is because at cellular level the picture is much less clear. No magnetic field effects are reported on the aggregation of melanophores [1], proliferation of human breast cancer cells [2], axonal outgrowth and proliferation of motoneurons in chick embryos [3,4], alignment of cortical neurons in mouse embryos [5], regeneration in rat sciatic nerve [6], early embryonic development of frogs eggs [7], behavior of normal lymphocytes and monocytes [8] or growth of T-cells under normal cell-culture conditions [9]. On the other hand, several experimental studies showed the potential factors acting together with magnetic field to eventually promote changes in the signaling cascade and therefore cancer onset [10,11] or neurodegenerative diseases [12]. In other cases it has allowed proven therapeutic effects on bone disorders, particularly in bone fractures [13]. More recently, several studies seem to indicate a possible wider medical role for magnetic field. Among them, some reported the induction of apoptosis in human leukemic cells [14], while others demonstrated the positive benefits over bacteria's growth [15].

The biological effects linked to the possible differentiation induced by static magnetic fields such as those found in the magnetic imaging environment, and the underlying mechanisms are being widely debated.

In the present study, the effect of an extracellular magnetic field as an extracellular stimulus to trigger intracellular signaling cascades is investigated in rat primary cultured cortical neurons. Although, in the past evidence of biochemical stimulation of specific intracellular signaling cascades (i.e., MAP1, MAP2, MAP3) on different cell types have been reported [16,17] it was unknown whether a static magnetic field could trigger an intracellular kinase activation. Extracellular regulated kinase (ERK) and c-Jun N terminal kinase (JNK) are intracellular kinases usually activated in cell survival [17] and cellular response to stress [16,17].

In this work, we report on the modifications of the signaling cascades in rat primary cortical neurons cultured for one hour in magnetic fields of up to 5 tesla (T). The analysis of the influence of strong static magnetic field strength on the activation of activated ERK in neurons was measured by immunocytochemistry. This technique was used to quantify the expression of phosphorylated ERK (i.e., active) undergoing cell differentiation. In addition, the intracellular Ca^{2+} concentration was also investigated to identify any correlation between changes in the voltage-dependent calcium channel and magnetic field strength.

METHODS

Primary cortical neurons were prepared from 1-day old Wistar rats and maintained in neurobasal medium (Gibco, UK) [18]. The rat cortices were incubated in phosphate-buffered saline (PBS, Sigma-Aldrich, UK) with trypsin (0.25 μg ml-1) for 25 min at 37.0 °C. After trituration and filtering, the suspension was centrifuged and the pellet suspended in warm neurobasal medium supplemented with horse serum (10 %), penicillin (100 U/ml), streptomycin (100 U/ml) and glutamax (2 mM) (Sigma-Aldrich, UK). Suspended cells were plated at a density of 0.25×10^6 cells on circular 10 mm diameter coverslips, coated with poly-L-lysine (60 μg/ml), and incubated (5 % CO_2, 37.0 °C) for 2

hrs. Media were exchanged for fresh media every 3 days and cells were grown in culture for up to 14 days before exposure to the magnetic fields.

The cultured neurons where subsequently exposed to static magnetic fields using a wide-bore superconducting magnet (Cryogenic Ltd, UK). The magnetic field strength varied up to 5 T with 0.01 % variation of the field strength over the culture chamber.

Duplicates of coverslips containing neurons were flooded with pre-warmed medium solution in plastic petri-dishes, RPMI 1640 (Life Technologies, UK).

Each experiment included two Petri dishes inserted into a dedicated chamber, which was attached via inflow tubing to a thermostatically-controlled water bath [19]. The temperature inside the chamber was monitored and set to 37. 0 ± 0.5 °C. Cultured neurons were then exposed to one of six different static magnetic field strengths, (0.1, 0.5, 0.75, 1, 2, 5 T), for an exposure time of one hour. Control samples were placed under the same experimental conditions (i.e., CO_2-free medium solution in Petri-dishes at 37.0 °C), but with no magnetic field (sham). Briefly, after the exposure each sample was fixed with 4% paraformaldehyde in TBS (tris-buffered saline, Sigma-Aldrich, UK), incubated overnight at 4 ºC with a mouse polyclonal anti-phospho-specific ERK (Santa Cruz Biotechnology Inc, Santa Cruz, CA, USA) and mounted on microscope slides and viewed with a light microscope under x40 magnification for quantitative analysis [18,19].

The experimental data was measured for each static magnetic field strength by quantifying the amount of p-ERK positive response over 400 cells counted. Ten series of neurons were analyzed and counted by experienced observers. A statistical analysis gave the mean and standard error of the mean, and Student's t-test was used to establish the significance of the data obtained.

Analysis of the intracellular calcium level $[Ca^{2+}]_i$ was carried out using the Ca^{2+}-sensitive indicator, Fura-2AM (Molecular Probes, USA). 20 batches of neurons were prepared, following the same protocol described above, and loaded with Fura-2AM (2 μM) for 40 min. The cells were initially centrifuged and washed with 0.5 ml of hanks balanced salt solution (HBSS) and then with 10 ml of HBSS. Subsequent to that a 10 min incubation guaranteed a sufficient hydrolysis of the Fura-2AM. Spectrophotometer measurements were then carried out by using a Cairn spectrophotometer (Cairn, UK). Each sample (2 ml) was excited for short time by an alternating filtered Xenon UV laser light, λ = 340 and 380 nm). The signal was then filtered, amplified and digitally stored. Baseline recording ratio (340/380) was calculated and then cells were depolarized with 50 mM KCl to allow Ca^{2+} influx through voltage-dependant calcium channels. The $[Ca^{2+}]_i$ was then calculated according to the equation of Grynkiewicz et al. [10]. Samples were exposed to static magnetic field strengths (0.1, 0.5, 0.75, 1 T), for two minutes; control samples were also measured under the same technique.

The calculation of the $[Ca^{2+}]_i$ level related to each exposed field, the increase of intracellular calcium level related to KCl activation and the variation between control and exposed fields were carried out. Subsequently, statistical analysis was performed to calculate mean, standard error of the mean; finally Student's t-test was used to establish the significance of the data obtained.

Magnetization measurements were carried out using a 5 T Quantum Design MPMS SQUID magnetometer (Quantum Design, USA). Following the procedure mentioned above, freshly-harvested neurons were placed in a 250 μl RNA extraction tube which contained about 40 mg of dry mass of neurons. The tubes were mounted in a plastic drinking straw for the SQUID measurements, which were made within one hour to maintain cell viability.

RESULTS

The results of this work demonstrate an increase in the activation of p-ERK between each cell groups exposed to different static magnetic field strengths and the corresponding controls. This could be associated with the differential effect of the magnetic field applied on the cells. In particular, it was found that the activated form of the phosphorylated ERK was significantly increased compare to the matched control for neurons exposed to a static magnetic field of 0.75 T for 1 h. This was subsequently confirmed by the quantitative analysis of the batches investigated (Fig. 1). In addition, strong positive phosphorylation activation was also found for those neurons exposed to 1 T magnetic field strength. For magnetic field of 0.75 T the variation between exposed neurons versus control samples was statistically significant (10.4 ± 1.8 %, $p < 0.01$); where at 1 T magnetic field strength a variation of 7.9 ± 1.0 % ($p \cong 0.05$) was measured.

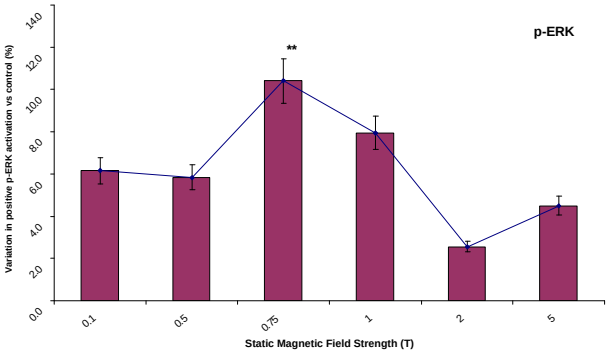

Figure 1. Variation in positive p-ERK activation over different magnetic fields (calculated over control/sham samples). Each histogram is based on 10 runs; each run is based on a standard sample size of 400 cells. Results are expressed as mean ± SEM (** $p < 0.01$).

A smaller variation was calculated for all the other field strengths ($p > 0.05$) as shown in Fig. 1. Whereas, consistent, but smaller, activation was measured on the remaining fields examined (i.e., 0.1, 0.5, 2, and 5 T). These

were around a variation of 6.0 ± 1.0 % for magnetic field strengths smaller than 0.5 T ($p > 0.05$) and 3.5 ± 0.75 % for magnetic field strengths bigger than 1 T ($p > 0.05$).

In order to identify any triggering effect induced by the magnetic field the measurement of the changes in the free cytosolic calcium concentration was carried out to investigate the intracellular calcium response. Initially, several independent batches were measured to determine the physiological variation of the $[Ca^{2+}]_i$ resting concentration. These were measured for sample of neurons exposed to magnetic field (0.75 T) and for control samples (sham). In fact, if for control samples the intracellular Ca^{2+} was 300 ± 26 nM, for the samples exposed to 0.75 T this was significantly increased to 370 ± 13 nM ($p < 0.05$) as shown in Table 1. These results are statistically significant and confirm the triggering activation of the voltage dependent calcium channel. When KCl was added to the samples, the subsequent activation of voltage- dependent Ca^{2+} channels induced a Ca^{2+} influx and consequently an increase in the free calcium concentration. The depolarization induced by KCl, increased $[Ca^{2+}]_i$ by 46 ± 7 % in control samples and 35 ± 2.9 % in samples exposed to 0.75 T ($p < 0.05$), as reported in Table 1.

Intracellular calcium level $[Ca^{2+}]_i$		
	0 T (control)	***Exposed to 0.75 T***
Resting concentration (Mean ± s.e.m.). [nM]	301 ± 26	370 ± 13
Increase in $[Ca^{2+}]_i$ with KCl. [%]	47.3 ± 5.5	35.2 ± 2.8

Table 1. Variation of the intracellular calcium level $[Ca2+]_i$ between control and exposed samples to 0.75 T magnetic field.

The susceptibility measurements were made on five independent batches at room temperature. Measurements were also made of medium solution (HBSS) and of simple DI water for comparison. Susceptibility results were $\chi = -10.0 \times 10^{-9} \pm 1.0 \times 10^{-9}$ m^3 kg^{-1} for deionized water (S > 30 MΩ); $\chi = -1.0 \times 10^{-9} \pm 0.1 \times 10^{-9}$ m^3 kg^{-1} for HBSS medium, and $\chi = -2.0 \times 10^{-9} \pm 0.2 \times 10^{-9}$ m^3 kg^{-1} for the cultured cortical neurons.

DISCUSSION

The results presented in this work show the activation effect that a static magnetic field of 0.75 T triggers on primary cortical neurons cultured under standardized conditions. This was determined by the investigation of the ERK signaling cascades that have been extensively characterized for different agents associated with cell differentiation for the production of second messengers (e.g., cAMP, cGMP, diacylglycerol, and Ca^{2+}) [17]. Therefore, an understanding of the magnetic field triggering mechanism could be used as a new path to regulate the intracellular signaling and modify cell differentiation. These results are consistent with the literature available where the ERK pathway is known as one of the stress-activated protein kinases because of its sensitivity to environmental stresses (e.g., radiation, tumor necrosis) [17]. This leads us to speculate that a magnetic field of about 0.75 T might trigger in primary cortical neurons the same ERK signaling cascades that have been associated to cellular differentiation [17].

When cells were exposed to a static magnetic field strength of 0.75 T, the resting Ca^{2+} concentration was significantly increased and this may account for the increase in ERK activity induced by 0.75 T since MAPK activation has a Ca^{2+}-dependent component [21]. In contrast, magnetic stimulation (0.75 T) resulted in reduced Ca^{2+} influx following KCl-depolarization. This suggests that the static magnetic field alters the activation kinetics of voltage-dependent calcium channels.

Therefore, different magnetic fields can lead to different signal responses and consequent downstream kinase regulation. However, the molecular mechanisms of this specific extracellular signaling activation remain unclear and uncorrelated to the regulation of downstream protein kinases phosphorylation (e.g., serine/threonine proteins).

Regarding the mechanism by which an intense static magnetic field influences the cellular response and activates a signaling pathway, in this work, it can be found that a possible shift in the rest potential might have occurred. This can be correlated to an increase in the convective hydrodynamic forces, evoked by the magnetic field applied, acting at the membrane level and consequently augmenting the ions internal-external activity (Fig. 2).

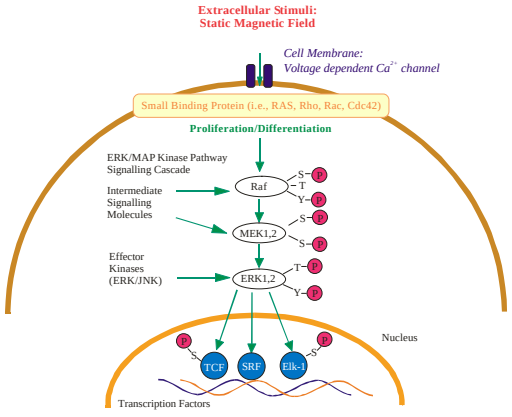

Figure 2. Extracellular regulated kinase pathway (ERK) triggered by static magnetic field as extracellular stimuli. This signaling cascade pathway is associated with cell differentiation.

In neurons there are additional ion currents associated with signal transmission. Typically, these are in the range 1-20 pA [22]. If these relate to a single ion channel, local current densities as great as 10 A cm^{-2} are present. Magnetic-field induced shifts of rest potential are therefore to be expected and it is likely that they will influence the signaling pathways. However, it will be a challenge to observe the effects directly since patch-clamp methods will impede the field-induced microscale convective flow which is needed to produce a rest potential shift.

CONCLUSIONS

This work is the first experimental evidence for any static magnetic field on intracellular signaling where the responses of the ERK signaling pathways in primary cortical neurons were differentially modified. This study also opens the prospect of using static magnetic field as a new physical tool for activating cellular differentiation.

ACKNOWLEDGEMENTS

This study was supported by Science Foundation Ireland (SFI) as part of the CINSE program and by the Programme for Research in Third Level Institutions (PRTLI) as part of the Trinity Centre for Bioengineering (TCB) program. The authors would also like to thank Mr. E. Farrell, and Prof. P.J. Prendergast from TCB for their help and financial support, respectively.

REFERENCES

[1] M.F. Testorf, A.P. Oberg, M. Iwasaka, S. Ueno, Bioelectromagnetics, 23, 444-449, 2002.
[2] S. Pacini et al., Oncol Res, 11, 265-271, 1999.
[3] Y.P. Yip, C. Capriotti, J.W. Yip, J Magn Res Imaging 5, 457-462, 1995.
[4] Y.P. Yip, C. Capriotti, S.G. Norbash, S.L. Talagala, J.W. Yip, J. Magn Res Imaging, 4, 799-804, 1995.
[5] G. Konermann, H. Monig, Radiologe, 26, 490-497, 1986.
[6] P.G. Cordeiro, B.R. Seckel, C.D., Miller, P.T. Gross, R.E. Wise, Plast Reconstr Surg, 83, 301-308, 1989.
[7] S. Ueno, M. Iwasaka, K. Shiokawa, J Appl Phys, 75, 7165-7167, 1994.
[8] P. Aldinucci et al., Bioelectromagnetics, 24, 109-117, 2003.
[9] T. Norimura, H. Imada, N. Kunugita, N. Yoshida, and M. Nikaido, J UOEH, 15, 103-112 1993.
[10] S. Koifman. Med Hypothesis, 41, 23–27, 1993.
[11] W.R. Heende, J.C. Boteler, Health Phys, 66, 127–136, 1994.
[12] A. Ahlbom, Bioelectromag Suppl, 5, S132–S143, 2001.
[13] C.A. Basset, S.N. Mitchell, S.R. Gaston, JAMA, 247, 623–628, 1982.
[14] T. Hisamitsu, K. Narita, T. Kasahara, et al., Jpn J Physiol, 47, 307–310, 1997.
[15] Y. Liu et al., Chem Commun, (online), 2005.
[16] S.V. Kyosseva, Int Rev Neurobiol, 59, 201-220, 2004.
[17] G. Pearson et al., Endocr Rev, 22, 153-183, 2001.
[18] M.P. Fogarty, E.J. Downer, and V. Campbell, Biochem J, 371, 779-798, 2003.
[19] A. Prina-Mello, E. Farrell, P.J. Prendergast, V. Campbell, J.M.D. Coey, Physica Scripta, 71, 1-3, 2005.
[20] G. Grynkiewicz, M. Poenie, R.Y. Tsien, J Biol Chem, 260, 3440-3450, 1985.
[21] J.M. Mulvaney, T. Zhang, C. Fewtrell, M.S. Roberson, J Biol Chem, 274, 29796-29804, 1999.
[22] O.P. Hamill, and B. Martinac, Physiol Rev, 81, 685-740, 2001.