# Effects of Non Uniform Static Magnetic Fields on the Rate of Myosin Phosphorylation

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The effect on myosin phosphorylation from exposure to a magnetic field generated by an array of four permanent magnets was investigated. Two lateral positions in the non uniform field over the array were explored, each at four vertical distances the surface of the device. The rate of myosin phosphorylation was found to depend on the position laterally over the array as well as the distance from the device surface. The square magnet array was comprised of axially magnetized, cylindrical NdFeB permanent magnets arranged with poles of alternating polarity in a plane (MagnaBloc<sup>TM</sup> therapeutic device). Detailed dosimetry of the magnet array was compiled: the magnetic flux density averaged over the exposure volume spanned the range 0.7–86 mT for the eight different exposure positions. The corresponding range for the absolute field gradient was 0.4–20 T/m. Comparing the dosimetry to the experimental outcome, our results imply that magnetic field amplitude alone is not sufficient to describe the influence of the field in this preparation. Bioelectromagnetics 00:1–5, 2002. © 2002 Wiley-Liss, Inc.

Key words: gradient; dosimetry; therapeutic magnet array

# INTRODUCTION

The MagnaBloc<sup>TM</sup> magnetic therapeutic device [Holcomb, 1994; Source: Amway Corporation, Ada, MI] is clinically successful in reducing various forms of pain: wrist pain, lower back pain [Holcomb et al., 1991], and arthritic knee pain [Segal et al., 2001]. The device consists of four NdFeB permanent magnets arranged with alternating polarities in a  $2 \times 2$  array (Fig. 1). There is some understanding of the biological mechanisms at the cellular level from studies of action potential blockade [McLean et al., 1995], as well as cell swelling [McLean et al., 2000], but the physical mechanism underlying these observations remains elusive.

Myosin phosphorylation as a tool to study biological effects initiated by magnetic fields was pioneered by Shuvalova et al. [1991]. The magnetic sensitivity of myosin phosphorylation has since been studied by one of us (M.S.M.) for 10 years, including work to establish optimal conditions for successful execution of the assay [Markov and Pilla, 1997]. The same fundamental process has been examined with focus on a wide range of parameters: static fields [Markov et al., 1993; Markov and Pilla, 1997], field shielding with high permeability materials [Markov et al., 1993], extremely

low frequency magnetic fields [Markov et al., 1993; Markov and Pilla, 1994b], as well as chemical properties [Markov and Pilla, 1994a]. The canonical low field magnetic field experiment was recently the subject of an unsuccessful replication attempt [Coulton et al., 2000]. The present study marks a continuation of the use of this system for characterization of biological effects of magnetic fields, this time for relatively strong gradient fields.

The biophysical interaction of the field has previously been modeled as a factor in the binding of calcium ions to calmodulin binding sites [Pilla et al., 1997], but it is not clear that this model carries over to the present exposure levels. It has been previously observed that the spatially inhomogeneous nature of the field surrounding the MagnaBloc device may be part of the physical metric responsible for biological effects seen at different locations in its magnetic field

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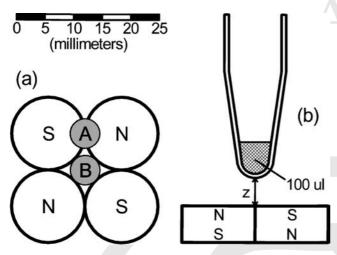


Fig. 1. **a**: The exposure device. Experiments were done above positions A, the point where two neighboring magnets touch, and B, the geometrical center of the array. **b**: Four different heights were investigated: z=0, 5, 10, 15 mm. The hatched area represents the 100  $\mu$ I experimental exposure volume in the bottom of a 1.5 ml Eppendorf tube.

[Cavopol et al., 1995], and for this reason we contrast two lateral positions over the device in this investigation.

Another issue regarding the therapeutic usefulness of a magnetic device is its ability to penetrate tissue and produce a clinically beneficial field at a physiological target. Evidence using single cell action potentials suggests an effective distance threshold of 25 mm for the present device. Information from the present alternative biological assay is valuable in understanding the range in which one may expect biological effect of the device. The myosin phosphorylation assay has two properties that makes it particularly well suited for studying the spatial characteristics of a gradient field: (1) the exposed volume is not very large (100 ul), providing a relatively localized field and gradient distributions; (2) the physical target ensemble is in solution and thus very likely isotropically distributed in space, allowing for some simplifying assumptions for the field description.

# **METHODS**

The experiments described in this study were performed by using myosin light chains (MLC) and myosin light chain kinase (MLCK) isolated from turkey gizzard, kindly donated by M. Ikebe (University of Massachusetts, Amherst MA). The reaction involves a basic solution composed of 40 mM HEPES buffer, pH 7.0, 0.5 mM magnesium acetate, 1 mg/ml bovine serum albumin, 0.1% (w/v) Tween 80, and 1 mM EGTA as well as 2.5 µM free Ca<sup>2+</sup>, 70 nM

Calmodulin, 160 nM MLC, and 2 nM MLCK. The concentration of free calcium (2.5 µM) was achieved by adding 875 µM calcium. The low MLC/MLCK ratio was chosen to obtain linear time behavior in the minute range [Markov and Pilla, 1997]. This provided reproducible enzyme activities and minimized pipetting time errors. All chemicals used in these experiments were obtained from Sigma (St. Louis, MO).

The reaction mixture was dispensed in 100 µl aliquots into 1.5 ml Eppendorf tubes (Hamburg, Germany). The experiment was conducted within a specially designed plexiglass chamber which was maintained at  $37.0 \pm 0.1$  °C by constant perfusion of water prewarmed by passage through a heat exchanger (Model 900, Fisher Scientific, Pittsburgh, PA). Temperature was monitored with a traceable thermometer (Fisher Scientific) immersed in the temperature regulated chamber during the experiments. The reaction mixture temperature was allowed to equilibrate for 10 min, after which the reaction was initiated by adding 2.5 µM  $^{32}$ P ATP (2000–6600 cpm/pmol) to the solution. The reaction was allowed to run for 5 min and was then stopped with 100 µl of Laemmli Sample Buffer solution containing 30 µM EDTA.

Once the reaction was stopped,  $20 \,\mu l$  of the solution was aliquoted on six  $2 \times 2$  cm squares of 3M filter paper (St. Paul, MN). Unreacted ATP, as well as the ADP product of the reaction, were removed by washing the filter paper in diluted TCA. Then each square was placed in a  $20 \, ml$  scintillation vial filled with  $15 \, ml$  distilled water, and the Cherenkov emission was counted for each sample/vial. At least six blank samples were counted in each experiment. Blanks consisted of the total assay mixture minus calcium, one of the active components. When blank counts were higher than  $500 \, cpm$ , the washing was considered incomplete and the whole experiment was rejected; this happened once in this series of experiments.

Phosphorylation was evaluated using a liquid scintillation counter (Model LS 6500, Beckman, Fullerton, CA) that counted <sup>32</sup>P incorporated into myosin light chains. For each position of the tube in the magnetic field, 5 or 11 independent runs of the experiment were conducted (Table 2). The internal repetition rate was 6 readings for each independent run.

#### **MAGNETIC FIELD DOSIMETRY**

The exposure device is a  $2 \times 2$  array of axially magnetized cylindrical NdFeB magnets configured with the magnetic pole faces in a plane in which adjacent magnets have alternating polarities (Fig. 1a). Previous studies of this device [e.g. McLean et al., 1995] indicate that field magnitude alone is not

sufficient to fully describe the exposure [Cavopol et al., 1995]. For this reason our dosimetry includes measures of the field gradient in addition to the field magnitude.

Although the exposure volume is relatively small ( $100 \mu l$  in a 1.5 ml Eppendorf tube), the magnetic field generated by the exposure device is inhomogeneous and a range of field conditions exists throughout the volume. A detailed field map was obtained by numerical continuation of magnetic field information in a plane close to the surface of the magnetic device. Briefly, the magnetic field and its gradient was calculated by the following formulae [Engström, 2001]:

$$\begin{split} B_p(x,y,z) &= \frac{1}{2\pi} \iint \frac{p}{r^3} B_z(x',y',z_0) \ dx'dy' \\ \frac{\partial B_p}{\partial q}(x,y,z) &= \\ &- \frac{1}{2\pi} \iint \frac{3p^2 - r^2}{r^5} B_z(x',y',z_0) \ dx'dy' \quad (p = q) \\ \frac{\partial B_p}{\partial q}(x,y,z) &= \\ &- \frac{1}{2\pi} \iint \frac{3pq}{r^5} B_z(x',y',z_0) \ dx'dy' \quad (p \neq q) \end{split}$$

where p and q are one of the of three Cartesian coordinates (x, y, z),  $r^2 = (x - x')^2 + (y - y')^2 + (z - z_0)^2$ , and  $z = z_0$  is a plane in which we know the  $B_z$  component of the field out to a low cutoff level.

The  $B_z$  field was mapped over a  $100 \times 100 \text{ mm}^2$  surface at 0.2 mm resolution using a planar magnetic scanner (Redcliffe Magtronics, Bristol, UK). This data was extended in 0.2 mm increments above the scan plane (near the device surface), and data subsets were extracted as appropriate to model the actual exposure volume (Fig. 1b) in the different positions and heights over the surface of the magnetic device. At 0.2 mm linear resolution, the 100  $\mu$ l exposure volume is represented by approximately 12500 volume elements.

Since the exposure targets may safely be assumed to be isotropically distributed in the exposure volume because the enzyme reaction sites are in solution, a complete local description of the field and its gradient is given by the absolute field magnitude and two components of the field gradient, here chosen as the components parallel and perpendicular to the local field vector. We denote these quantities  $B_a$ ,  $B_p$ , and  $B_q$ , respectively. In support of the isotropy assumption, it has been shown experimentally that the effects of magnetic fields in the geomagnetic range on myosin phosphorylation are independent of the direction of applied field [Markov et al., 1993].

In order to assess the effects as a function of the magnitude and gradient of the applied magnetic field, a custom made fixture was designed and manufactured (R. Jones, Inland Technical Services, CA). This allows experiments to be carried out at four heights (z = 0, 5, 10, 15 mm) over the surface at two different locations (Fig. 1): over the point where two adjacent magnets touch (A) and over the geometrical center of the four magnets (B). The four heights were chosen in order to cover a good fraction of the device separations known to be effective from the previous action potential experiments [McLean et al., 1995].

Controls were kept under ambient laboratory field conditions in the same exposure chamber and thermal conditions as the exposed samples, using a dummy device instead of the active permanent magnetic device. The physical dosimetry of these locations is summarized in Table 1.

## **EXPERIMENTAL RESULTS AND DISCUSSION**

The rate of myosin phosphorylation depends on the lateral position of the sample over the magnet array, as well as the distance from the surface of the device (Table 2, Fig. 2). The procedural variance was very low and the results after multiple replications are highly statistically significant. Day to day variations of

TABLE 1. Field and Gradient Dosimetr	v for Positions 'A' and 'B' (Fig	g. 1) and Four Heights Over the	ne Exposure Device (z)

Position	z (mm)	$B_a^{a}$ (mT)	$D_a^{a}$ (mT)	$B_p$ (T/m)	$D_p$ (T/m)	$B_q$ (T/m)	$D_q$ (T/m)
A	0	85.64	39.14	9.73	7.08	23.70	12.81
A	5	21.65	8.55	2.09	1.45	5.28	2.34
A	10	6.62	2.33	0.56	0.37	1.47	0.57
A	15	2.36	0.75	0.18	0.12	0.49	0.16
В	0	30.75	15.89	13.42	9.72	14.29	9.22
В	5	7.10	3.30	2.96	1.95	3.15	1.84
В	10	2.06	0.89	0.82	0.51	0.88	0.48
В	15	0.71	0.29	0.27	0.16	0.30	0.15

 $<sup>{}^{</sup>a}B_{a}$  is the field magnitude,  $B_{p}$  is the mean absolute value of the gradient component parallel to the local field vector, and  $B_{q}$  is the mean of the local perpendicular gradient component over the 100  $\mu$ l exposure volume (Fig. 1b).  $D_{x}$  represents the standard deviation of field property ' $B_{x}$ '. Means and standard deviations were calculated from a 12500 voxel decomposition of the exposure volume.

TABLE 2. Results for Control and Exposed Samples

Position	z (mm)	Rep. (N)	Mean (counts)	SD (counts)	t-test P <sup>a</sup> (vs. ctrl)
Ctrl	n.a. <sup>b</sup>	11	1905	126	n.a.
A	0	11	3418	481	$5 \times 10^{-7}$
A	5	11	4255	283	$6 \times 10^{-13}$
A	10	5	3494	177	$2 \times 10^{-6}$
A	15	5	3066	243	$5 \times 10^{-3}$
В	0	11	2642	308	$5 \times 10^{-6}$
В	5	11	2427	176	$2 \times 10^{-7}$
В	10	5	2317	101	$5 \times 10^{-5}$
В	15	5	2126	105	$2 \times 10^{-4}$

<sup>&</sup>lt;sup>a</sup>Welch's two sided approximate *t*-test.

the absolute reaction rates were also small, allowing the data to be presented as raw counts from the radioactive assay.

The phosphorylation response was consistently larger for all samples exposed to magnetic fields. The exposures over position A, the point where neighboring magnets touch, reached a maximum at 5 mm distance from the surface and then declined toward the control value. Position B, magnet array center, responded with a smaller increase in the rate of myosin phosphorylation, but this position also showed a decline toward control levels as the sample was removed from the device surface.

The MagnaBloc device has been shown to inhibit single cell action potentials, and the experimental response as a function of lateral position is consistent with these studies [McLean et al., 1995]. The maximum at

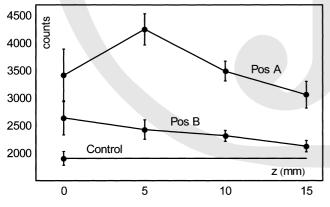


Fig. 2. Experimental results (Table 2) reported as raw counts of <sup>32</sup>P incorporated into myosin light chains as a function of distance from the device for the two exposure positions. While each run had its own control, the control levels were very consistent and have here been pooled into one overall average value. Error bars represent one standard deviation

5 mm separation over position A is consistent with unpublished results that note that the field effect is maximal several millimeters away from the device surface [M. McLean et al., unpublished<sup>Q1</sup>].

The most striking result of the experiments at hand is that position A produces a larger rate of phosphorylation than that produced in position B for all explored distances to the device surface. This is counterintuitive since the average field values drop off much more rapidly with distance from the device (a factor of 30 for 0–15 mm separation), than it does with lateral positioning (roughly a factor 3 for A to B). See the field dosimetry in Table 1 for a detailed comparison.

This observation can be formalized if we focus on the magnetic field amplitude as a putative physical metric for the results. There is a fairly good agreement between the field amplitude at position A when compared to the level observed 5 mm closer to the surface at position B (Table 1). With this in mind, consider the null hypothesis:

The distribution of magnetic field amplitudes is the sole determining factor for the experimental outcome.

This hypothesis is not consistent with the dosimetry and experimental results of the pairs {A @ 10 mm/B @ 5 mm} and {A @ 15 mm/B @ 10 mm}. For these two pairs in particular, we note that while the field amplitudes are quite similar (field distributions are also very similar), the experimental outcomes for the experimental pairs are very different, suggesting that the null hypothesis above is rejected for both of these two experimental pairs ( $P = 5 \times 10^{-6}$  and  $P = 1 \times 10^{-3}$ , respectively [two-sided t-test]). Rejecting the hypothesis does not tell us what the relevant exposure parameter is, but it refutes the notion that magnetic field amplitude is the only parameter that determines the outcome of the experiment.

Although there is no similar match in the locally defined gradient properties (Table 1), sole dependence on these quantities seems unlikely, since their variation is also ten-fold larger from the vertical displacement than it is from the lateral positioning, yet the lateral positions produce comparable results for the four vertical distances considered.

The previously published results [e.g. Markov et al., 1993; Markov and Pilla, 1997] on this experimental system have focused on lower field strengths ( $< 200 \mu T$ ), and there is no information from those studies as to what to expect at the predominantly higher exposure levels used in the present study. One concern was that the system response might saturate at relatively low field strengths. If this were the case, the low end of the field distribution would be crucial in

<sup>&</sup>lt;sup>b</sup>Control values (ctrl) did not vary with position of dummy unit and were combined.

determining the expected biological response. However, the wide stratification of the exposure levels corresponding to heights over the surface of the device carries over to an analysis of this possibility, and saturation is not an adequate explanation of the obtained results.

The results could conceivably be explained by a series of very sharp response peaks as a function of field and/or gradient values (a testable, but untested hypothesis), but we feel that a sounder inference from our data is that we have not uniquely identified the physical factor responsible for our results, despite the fact that we have a complete local description of the field and its gradient character. A remaining possibility is that the proper physical metric that describes the response is a combination of the elementary properties we have mentioned, e.g., a combination of field and gradient values.

The absolute field levels used here are generally larger than the exposures considered previously by Markov and collaborators, yet the response is apparently smaller than previously observed. One reason for this behavior may be a nonmonotonic dependence on field amplitude, but it is also possible that the gradient component of the present exposure is a sufficiently strong factor in determining the assay response in the present set of experiments.

The question of the relevant physical exposure metric can be addressed by experiments using gradient fields designed to change only one property at a time, and a series of such experiments is planned. Such an analysis might be extremely important for the needs of clinical medicine, where the proper choice of the parameters of the acting physical modality determines the success of the therapy.

### **ACKNOWLEDGMENT**

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