Analysis of Inhomogeneous Static Magnetic Field-Induced Antinociceptive Activity in Mice

János F. László¹ and Klára Gyires²
¹Institute for Research Organization, Hungarian Academy of Sciences
Nádor u. 18, 1051 Budapest, Hungary
²Department of Pharmacology and Pharmacotherapy, Semmelweis University, Budapest, Hungary

Abstract—The effect of inhomogeneous static magnetic field (SMF) on visceral pain elicited by the intraperitoneal injection of 0.6% acetic acid (writhing test) was studied in mice in an environment, where animals could freely move. 30 min, whole-body exposure of mice to SMF (permanent NdFeB N50 grade 10×10 mm cylindrical magnets with alternating poles) following the nociceptive challenge resulted in a 74% inhibition of the pain reaction (p < 0.001). With the help of several inhomogeneous SMF configurations, where magnets were grouped in partitions and a 2D model of ambulation, motion-induced electric current density, MR-equivalent switching, and slew rate were estimated. Their potential contribution to peripheral nerve stimulation is discussed in correlation to pain inhibition.

1. INTRODUCTION
An increasing number of evidence suggest that static magnetic field (SMF) can induce analgesic action in humans — as tested mostly in chronic pain. The beneficial effect of SMF was observed in patients under different pain conditions [1–5]. SMF exposure also shortened the inflammatory period [6].

Beside human data preclinical studies demonstrated the antinociceptive activity (AA) of SMF under experimental conditions [7–15].

Motion in an inhomogeneous SMF can induce electric currents in the moving body. The most sensitive parts of a mammal to electrical stimulus are peripheral nerves. If an electric current is high enough to stimulate visceral nerves, it certainly stimulates peripheral nerves. Consequently, pain inhibition in the viscera will definitely be effective, if induced electric currents remain below peripheral nerve stimulation (PNS) threshold.

The aim of the present study was to answer the following questions concerning motion-induced electric current density, MR-equivalent switching, and slew rate in the mouse ambulating freely while exposed to an inhomogeneous SMF: (i) Do these quantities correlate with AA? (ii) Can they be made responsible for PNS?

2. MATERIALS AND METHODS
2.1. Magnetic Field Exposure
SMF was generated by an exposure system described by László et al. [10]. This system contained two magnet holding matrices, one below and another on top of the animal cage. The exposure volume was 140×140×50 mm. The individual magnets used in the matrices were NdFeB N50 type (Bₚ = 1.47 T), axially magnetized, cylindrical magnets (d = 2r = 10 mm, h = 10 mm) provided by ChenYang Technologies GmbH & Co. KG, Finsing, Germany. The poles below and above the cage facing each other were of opposite polarity allowing field lines cross the cage, i.e., the main SMF was vertically directed. SMF between the matrices was adjusted by magnetically connecting the opposite poles of the lower and upper magnets in a horseshoe-like coupling. When magnets sat one next to another with alternating polarity, the resultant SMF was inhomogeneous also laterally with a matrix constant (λ =)10 mm. It had a peak-to-peak amplitude of 754 ± 7 mT at 3 mm from the surface of the magnets, 109 ± 1 mT at 10 mm and 3 mT at 15 mm measured along the axis of a magnet and averaged over all pairs of neighbouring magnets. Magnetic induction (flux density) as well as its gradient have horizontal components, but they were a minimum of one order of magnitude below the vertical components at any position in-axis and therefore, were neglected as fringe field/gradient components. For the same reason, the vertical components off-axis were ignored.
This arrangement allowed us to insert a $140 \times 140 \times 46$ mm Plexiglas animal cage with air holes into the exposure chamber. An air permeable opaque material covered the cage on four sides to make illumination conditions similar in the exposure chamber and in the sham experiment.

A special arrangement called $M$-SMF denoted that the above magnet arrangement was modified in the sense that some magnets sat in the matrix with identical polarity in quadratic groups, while the neighbouring groups were of opposite polarity. We used the square root of the number of magnets ($M$ partitioning number) in each group for distinguishing between partitionings. The notation of these arrangements follows the rule: $2 \times 2$ for the quadratic groups of 4 magnets ($M = 2$), $3 \times 3$ for groups of 9 magnets ($M = 3$), etc. The original SMF corresponds to $1 \times 1$ ($M = 1$) in this notation.

The switching rate of an MR machine is often connected to the time-varying gradient field component. We defined a similar quantity called MR-equivalent switching rate ($MEWR$). Its definition is the maximum peak-to-peak amplitude of the magnetic induction at the boundary of two partitions, divided by the average duration it takes the mouse to move through the boundary. This boundary means a distance of 10 mm, if $M = 1$, 20 mm, if $M = 2$, etc. for a mouse crossing it perpendicularly. We also defined a quantity similar to MR’s slew rate, the MR-equivalent slew rate ($MESR$). This is similar to MEWR, but instead of the peak-to-peak amplitude of the magnetic induction it has magnetic induction gradient between two partitions in the numerator.

The magnetic induction in all experimental setups was measured with a calibrated 5 V Hall probe with 12.3 mV/T sensitivity (model UGN3503, Allegro Micro Systems, Inc., MA, USA). These measurements were executed separately from the animal experiments.

### 2.2. Animals, Pain Essay, Ethics

Male CFLP mice (24–26 g) were used in the experiments. Animals were housed in groups of five, the room was held under a 12 : 12 light/dark cycle at 20 ± 2°C.

The writhing test as described by Wende and Margoli [16] and modified by Witkin et al. [17] was applied. The visceral pain was elicited by the intraperitoneal (i.p.) injection of 0.6% acetic acid in a volume of 0.2 mL/mouse. As a result of chemical irritation a characteristic stretching and writhing movement could be observed. $AA$ of a treatment in a given time interval was defined as $AA = 100(1 - \bar{x}/\bar{y})\%$, where $\bar{x}$ is the average of the number of writhings for the treated and $\bar{y}$ is that for the control animals. Immediately after the administration of acetic acid some mice were exposed to SMF for 30 min. Two mice were placed in the plastic cage simultaneously and the number of writhings was monitored in 0–5, 6–20 and 21–30 min following the acetic acid challenge.

In all experiments a daily control was used, when the mice were given acetic acid, placed to the sham cage, but were not exposed to SMF.

All experimental procedures were carried out according to the 1998/XXVIII Act of the Hungarian Parliament on Animal Protection and Consideration Decree of Scientific Procedures of Animal Experiments (243/1988) and complied with the recommendations of the International Association of the Study of Pain [18] and the Helsinki Declaration. The studies were approved by the Animal Care Committee of Semmelweis University, Budapest (permission number: 1810/003/2004).

### 2.3. Ambulation Model — Approximation of $B(x; y; z)$, Mouse Motion and Induced Electric Current Density

When approximating the $m$ measured values of the SMF distribution along the $x$ axis (in plane with the magnets’ surface), we fitted normal (Gaussian) probability density functions to the individual peaks, where the contribution of the $k$-th peak to the distribution was $\frac{1}{\sigma \sqrt{2\pi}} e^{-\frac{(x - \sigma_k)^2}{2\sigma^2}}$, with $k = 1, 2, 3, \ldots, m$, $\sigma_k$’s (standard deviations) were considered equal for all peaks, and $\lambda$’s (matrix constants) equal in the lateral directions. For symmetry reasons this simple 2D approach holds for the $y$ axis as well. This model is widely used for finding the spatial resolution in laterally or vertically periodic systems [19]. $B(x; y)$ can then be approximated by the linear combination of the periodic functions for $x$ and $y$, c.f., the Error Function Superposition Approximation (EFSA) introduced by Marton et al. [20]:

$$B(x; y) = B_x \frac{1}{\sigma \sqrt{2\pi}} \sum_{k=1}^{m} e^{-\frac{(x - \sigma_k)^2}{2\sigma^2}} + B_y \frac{1}{\sigma \sqrt{2\pi}} \sum_{k=1}^{m} e^{-\frac{(y - \sigma_k)^2}{2\sigma^2}}$$  \hspace{1cm} (1)
with \( B_x \) and \( B_y \) magnetic induction in the \( x \) and \( y \) directions, respectively. In the present model \( B_x = B_y \equiv B_0 \) and we further approximate \( B(x; y) \) with:

\[
B(x; y) \approx \frac{B_0}{\sigma \sqrt{2\pi}} \sum_{k=1}^{m} e^{-\frac{(\Delta z + (k-1)\lambda)^2}{2\sigma^2}} \left\{ \sin \left[ \pi \left( \frac{x}{M\lambda} - 1 \right) \right] + \sin \left[ \pi \left( \frac{y}{M\lambda} - 1 \right) \right] \right\}
\]

(2)

where \( M \) is the partitioning number. The limit of this approximation is the finiteness of the resolution, namely the following condition for the resultant derivative of \( B(x) \) in the range \( 0 \leq x \leq M\lambda \) must hold:

\[
\frac{\partial B(x)}{\partial x} = B_0 \frac{(k-1)\lambda - x}{\sigma \sqrt{2\pi}} \sum_{k=1}^{m} e^{-\frac{(\Delta z + (k-1)\lambda)^2}{2\sigma^2}} \left\{ \right. = 0, \quad \text{if } x = \frac{M\lambda}{2} \left. \right\downarrow \neq 0 \quad \text{elsewhere}
\]

(3)

and similarly for \( \partial B(y)/\partial y \). This gives a non-analytic solution for the \( \sigma(\lambda) \) function (and thus for the resolution, \( \Delta z = 2\sigma \) with the geometrical constraint \( \lambda \geq d \).

In the \( z \) direction of the arrangement (perpendicular to the cylindrical magnets’ surface), the magnetic induction along the axis of an individual magnet in the isocenter of the arrangement is primarily \( z \)-dependent and can be expressed in an analytic form from the integration of the Poisson equation:

\[
B(z) = \frac{B_x}{2} \left\{ \frac{h+z}{(R^2+(h+z)^2)^{1/2}} - \frac{z}{(R^2+z^2)^{1/2}} + \frac{h+D-z}{(R^2+(h+D-z)^2)^{1/2}} - \frac{D-z}{(R^2+(D-z)^2)^{1/2}} \right\}
\]

(4)

Its gradient is:

\[
\frac{\partial B(z)}{\partial z} = \frac{B_x R^2}{2} \left\{ \frac{1}{(R^2+(h+z)^2)^{3/2}} - \frac{1}{(R^2+z^2)^{3/2}} + \frac{1}{(R^2+(h+D-z)^2)^{3/2}} - \frac{1}{(R^2+(D-z)^2)^{3/2}} \right\}
\]

(5)

taking into account two (lower and upper) magnets along their common axis (in-axis) in a distance of \( D = 50 \text{ mm} \) from each other. We fitted \( B_0 \) so that at specifically \( z = 20 \text{ mm} \), where \( AA \) is supposed to take place in the mouse, \( B(z) \) should well estimate the measured vertical values in a first-neighbour approximation.

We also introduced \( N \), the number of gradients per distance as the number of inflection points of the vertical magnetic induction component per average linear ambulation distance.

If SMF is also inhomogeneous laterally, mice sense a time-dependent magnetic flux (\( \phi \)) by crossing the field lines during their ambulation in the cage. Our assumption is that the mouse moves in a 2D plane always along a straight line with constant speed (\( v \)) as long as it reaches a boundary/wall (at \( 0 \leq x \leq a \) or \( 0 \leq y \leq b \), then changes direction semi-randomly (in a degree of \( \alpha \) — pointing into the cage) and continues its motion. This simple model can be justified by the fact that the mouse’s body is 3–4 times shorter than the side length of the cage. The iterative equations of motion of the \( i \)-th linear section are as follows:

\[
x_i = \Delta t |v| \cos \alpha_i + x_{i-1}; \quad y_i = \Delta t |v| \sin \alpha_i + y_{i-1}
\]

(6)

where \( \Delta t \) is an equidistant time step. Since \( x = x(t) \) and \( y = y(t) \), then \( B(x; y) = B(t) \) as if SMF would in effect be time-dependent.

As described by Maxwell’s 4th (Faraday’s) law, the induced electromotive force (\( \varepsilon m f \)) in the mouse is:

\[
\varepsilon m f = \oint_{\ell_0} \vec{E} \cdot d\vec{l} = -\frac{d}{dt} \int_{A_0} \vec{B} dA
\]

(7)

where \( \vec{E} \) is the induced electrostatic field in the mouse, \( d\vec{l} \) is an infinitesimal section of the circumference \( (\ell_0) \) of the mouse’s area exposed to SMF. We assume that the surface of a mouse perpendicular to the magnetic induction is \( A_0 \) (with the assumption \( \vec{B} \| dA \)), \( \vec{B} \) can be regarded
as constant on $A_0$, and the homogeneous specific electric resistivity of the mouse is constant ($\rho$). Then the motion-induced current density is:

$$\vec{j}(t) = -\frac{1}{\rho \ell_o} \frac{d\vec{B}}{dt}$$  \hspace{1cm} (8)

Using Eqs. (2) and (6), the absolute value of the induced current density of the $i$-th section can be expressed as:

$$j_i = -\frac{2\pi |v| B_0}{\rho \ell \lambda} \left\{ \cos \alpha_i \cos \left[ \frac{2\pi}{\lambda} \left( \Delta t |v| \cos \alpha_i + x_i \right) \right] + \sin \alpha_i \cos \left[ \frac{2\pi}{\lambda} \left( \Delta t |v| \sin \alpha_i + y_i \right) \right] \right\}$$  \hspace{1cm} (9)

Data used for the estimate were: $|v| \approx 1$ m/s [21], $\rho \approx 1.82 \ \Omega m$ (in point 7.4.1 in [22]), $\ell_0 \approx 100$ mm, $B_0 = 0.5$ mT (after fitting to actual dosimetric values), $\lambda = 10$ mm, $k = 20\pi \ \text{mm}^{-1}$, $-1 \leq \cos \alpha_i \leq 1$, $x_i \leq \min(a; b) \approx 140$ mm, $y_i \leq \min(a; b) \approx 140$ mm.

3. RESULTS

3.1. The Effect of Static Magnetic Field on Acetic Acid-induced Abdominal Pain in Mice

The effect of SMF ($M = 1$) on the writhing response measured in 0–5th, 6–20th and 21–30th min following i.p. injection of acetic acid resulted in a decrease from $9 \pm 0.7$, $44 \pm 0.9$ and $32 \pm 0.7$ (sham exposed group) to $1 \pm 0.3$, $12 \pm 1.0$ and $8 \pm 0.9$, respectively, when mice were exposed to SMF (Fig. 1). The data shown are pooled averages for multiple experiments. During the total 30 min observation period the number of writhings $85 \pm 1.4$ decreased to $22 \pm 1.6 \ (p < 0.001)$. The average number of writhings correspond to $85, 72, 75\% \ AA$ in the three time intervals, respectively, and $AA=74\%$ for the complete 30 min time period.

![Figure 1](image-url)

Figure 1: The effect of static magnetic field (SMF) on the writhing response induced by the intraperitoneal injection of 0.6% acetic acid measured in the 0–5, 6–20, 21–30 and 0–30 min following the administration of the acetic acid in mice. Open columns: pooled average number of writhings and S.E.M. in control animals that were not exposed to SMF. Dark columns: pooled average number of writhings and S.E.M. in mice exposed to SMF for 30 min after the administration of acetic acid. Animal numbers are shown as $n$. $p < 0.05$ already means significant difference to control by ANOVA-test.

3.2. The Effect of Partitions of Static Magnetic Field on Acetic Acid-induced Abdominal Pain in Mice

Figure 2 shows the average number of writhings of mice exposed to SMF ($M = 1, 2, 3, 4, 5,$ and 6) for a total of 30 min exposure time. The corresponding $AA$ values are listed in Table 1. $p$ values were always below 0.05.
Figure 2: The average number of writhings and S.E.M. in the writhing test in mice for 30 min exposure to SMFs with different partitionings (M = 1, 2, 3, 4, 5, 6). For the meaning of partitionings see the text. * denotes p < 0.05 significant difference to control by ANOVA-test.

We also simulated MEWR and MESR in the M-SMF system. Table 1 presents how dosimetric data and data from the ambulation model correlate with the pain assay results.

Table 1: Correlation of the antinociceptive activities (AA) to the measured values of the peak-to-peak amplitudes of the magnetic induction, to their gradients (\(\nabla B\)), to the numbers of gradient per distance (N), to the MR-equivalent switching and slew rates (both MEWR and MESR as estimated via the ambulation model) in the M-SMFs. Vertical dosimetric data measured at \(z = 20\) mm are shown with the assumption that this is the average target distance from the magnets’ surface, where AA takes place in the mouse. The absolute values of induction gradient are averaged values in first-neighbour approximation. N reflects numbers of inflection points of the vertical magnetic induction component per average linear ambulation distance (140 mm). For \(v = 1\) m/s average ambulation speed MEWRs are equal in their numerical value to the magnetic induction gradients.

<table>
<thead>
<tr>
<th>partitioning number, (M)</th>
<th>M-SMF dosimetry</th>
<th>ambulation model</th>
<th>SMF-induced antinociceptive activity, AA, % for 30 min exposure time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>peak-to-peak amplitude of the magnetic induction at (z = 20) mm, mT</td>
<td>absolute value of induction gradient at (z = 20) mm, (\nabla B), mT/m</td>
<td>numbers of gradient per distance, (N)</td>
</tr>
<tr>
<td>1</td>
<td>1.6</td>
<td>162</td>
<td>14</td>
</tr>
<tr>
<td>2</td>
<td>17.7</td>
<td>46</td>
<td>7</td>
</tr>
<tr>
<td>3</td>
<td>64.9</td>
<td>90</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>133.8</td>
<td>140</td>
<td>3</td>
</tr>
<tr>
<td>5</td>
<td>191.2</td>
<td>164</td>
<td>2</td>
</tr>
<tr>
<td>6</td>
<td>212.5</td>
<td>154</td>
<td>2</td>
</tr>
<tr>
<td>correlation to AA</td>
<td>-3%</td>
<td>70%</td>
<td>45%</td>
</tr>
<tr>
<td>correlation to AA excluding case (M = 5) and (6)</td>
<td>-1%</td>
<td>91%</td>
<td>62%</td>
</tr>
</tbody>
</table>

4. CONCLUSION

Present data confirm earlier observations [10–12] that a 30 min, whole-body, inhomogeneous SMF exposure exert AA up to 74% against visceral pain elicited by acetic-acid in the mouse.

When the mouse crosses a boundary between SMF partitions, it undergoes a change of the magnetic induction in a short time. From the physical point of view this effect is identical to...
either, when (i) a living object is moved into the bore of a high field MR apparatus from practically 
stray field, or (ii) the MR proceeds gradient switching on a static living object inside its bore. 
Applying the ambulation model for the motion-induced current densities (Eq. (9)), two conclusions 
could be drawn: (1) its long-time average in the mouse moving in a periodic SMF is zero, since the 
trigonometric terms result in zero in the integration. This fact can be generalized for every 
rectilinear motion in a laterally periodic SMFs, supposing that the typical “wavelength” of the 
periodicity is not bigger than the typical linear ambulation distance of the animal, the resolution 
obeyes the rule defined in Eq. (3), and the decay time of PNS is much shorter than the time it takes 
for an animal to cross a boundary with strong SMF induction gradient. The partitioned SMF's are 
periodic with wavelengths $\lambda = 10, 20, 30, \text{and} 40 \text{mm}$. The periodicity in case of $M = 5$ and 6 is 
questionable. The boundary widths are shorter than the typical linear ambulation distances in the 
cage (140 mm) with the exception of $M = 5$ and 6. Thus, the discrepancy of the $AA$ results for 
these cases may be due to the failure of the 2D model assumptions. According to models of electric 
signal propagation along the axon (Chapter 3 in [22]), we estimate that an impulse strong enough 
to stimulate a peripheral nerve would decay at a certain location in less than 2 ms, meanwhile a 
mouse needs 10–60 ms to intersect a boundary. (2) For a single linear path the time dependence 
of the motion-induced current density can be non-zero. Can this non-zero induced current density 
contribute to $AA$?

The threshold of the motion-induced current density for PNS was first estimated to be at 
0.01 mA/m$^2$ [23], later 0.48 A/m$^2$ was proposed for human nerves [24]. Now the threshold values 
are accepted to be at 1–10 mA/m$^2$ [25]. The size-corrected value for mice is 0.16–1.6 mA/m$^2$. In 
our ambulation model the absolute value of the motion-induced current density remains below 
1.8 mA/m$^2$ — not excluding an effect. We must assume that the $AA$ effect of SMF exposure based 
on the endogenous $\mu$-opioid receptors is either more effective and/or has a preventive manner as 
compared to the nerve stimulatory effect leading to pain sensation.

The clinically determined PNS threshold for the MRs’ switching rate is between 50 [26] and 
60 T/s [27]. The mean pain nerve stimulation threshold is at 90 T/s, the mean cardiac stimulation 
threshold is by 2 orders of magnitude higher [27]. In our experiments $MEWR$ lay between 46 and 
164 mT/s, 2 orders of magnitude below threshold. Switching rates were shown not to contribute 
to pain inhibition in clinical studies, rather the contrary was found [28]. On the other hand 
Table 1 suggests a strong correlation between $AA$ and $MEWR$. We may resolve this contradiction 
remembering that the inhomogeneity of SMF was demonstrated earlier not to be necessary to 
achieve $AA$: the homogeneous SMF of a 3 T clinical MR also exerts 69% $AA$ in the writhing test in 
mice [12]. Although the peak-to-peak amplitude of the magnetic induction does not have a strong 
correlation directly to $AA$ (Table 1), but it may have through its gradient. We may interpret these 
facts that $AA$ may not exclusively be related with the inhomogeneity of SMF.

The 1D single axis slew rates of clinical MRs are typically 20 T/m/s and above. $MESR$ calculated 
in our model for case (i) above remains well below threshold, at cca. 1 T/m/s for a clinical MR. 
The estimated $MESR$ values in Table 1 are all below typical slew rates. From the correlations of 
the $M$-SMF-induced $AA$ to the $MESRs$ (Table 1), we may conclude for case (ii) that the higher 
the $MESR$, the more expressed the $AA$.

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