



3 T homogeneous static magnetic field of a clinical MR significantly inhibits pain in mice

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ABSTRACT

Aims: In recent years nuclear magnetic resonance (MR) systems have proliferated worldwide. This imaging/spectroscopy technique utilizes a strong homogeneous static magnetic field, much smaller time-varying gradient magnetic fields, and radiofrequency radiation. Many studies addressed the question of potential adverse side effects induced by MR, but less attention has been paid to its potential beneficial, therapeutical effects. The present study shows that whole body exposure of mice to the 3 T homogeneous static magnetic field of a clinical MR resulted in a statistically significant antinociceptive activity.

Main methods: Antinociceptive activity was studied in the writhing test, where pain was elicited by the intraperitoneal injection of 0.6% acetic acid in the mouse. No imaging sequence of the MR was used during the experiments. Mice could freely move in their cage without any restraint.

Key findings: An antinociceptive activity of $68 \pm 2\%$ ($p < 0.001$, $n = 18$) was found. Subcutaneous injection of naloxone (0.2 mg/kg) in the mice reversed the magnetic field-induced antinociceptive activity. The effect of noise, vibration and lighting stimuli could be neglected. Although motion-induced effects generated in the body of the mice could not be completely excluded, their influence on pain perception was estimated to be below threshold.

Significance: MR's static magnetic field should be regarded as a potential therapeutical tool.

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Introduction

As a continuation of our previous studies (László et al., 2007; Sándor et al., 2007; Gyires et al., 2008), we investigated the influence of the static magnetic field (SMF) component of a clinical nuclear magnetic resonance apparatus (MR) on pain reaction. Our hypothesis was that the strong, homogeneous SMF of the MR could induce a significant antinociception in the writhing test in mice. We aimed to study i) the pain perception of mice in a clinical MR (at rest) using the writhing test; if there is an effect ii) the possible mechanism of the antinociceptive action. During the experiments we had to control the potential effect of noise, vibration and illumination stimuli. We also had to estimate the potential effect of motion-induced current densities and time derivatives of the magnetic flux density.

MR as a diagnostic tool has become an everyday device also in the investigation of small animals. Starting with Hansen et al. (1980), many studies have dealt with rodents in an MR. Good reviews of the state of the art can be read in the paper of Pirko et al. (2005) and in Section 7.2.2 of the report of the World Health Organization (2006). However, with the exception of behavioral studies in almost all

experiments the subjects were immobilized by anesthesia (Lukasik and Gillies, 2003).

Beyond mammalian reproduction and development studies, most of what we know about the complex effects of MR apparatuses on rodents is as follows.

Prato and his group was active in behavioral tests in rats, e.g., in the spatial memory test by Innis et al. (1986), who did not find an effect. They also studied the open field behavior and the passive avoidance test in mice (Ossenkopp et al., 1986), and found no effect. Teskey et al. (1987) examined the survival and stress reactions. They neither found any effect on hormone levels, nor on the weight of animals in a period of 13–22 months following the exposure. They could not identify any change in survivability. The blood brain barrier was investigated by Shivers et al. (1987). They observed a temporary opening of the blood brain barrier, recovered 15–30 min after exposure. Prato et al. (1990, 1994) discovered a significant increase in the permeability of the blood brain barrier. Kwong-Hing et al. (1989) examined the acute exposure effects of a 0.05 T MRI on dentin and bone formation in mice. They found that the exposure caused a significant increase in the synthesis of the collagenous matrix of dentin in the incisors. Levine, Bluni and coworkers made efforts (Levine and Bluni, 1994; Levine et al., 1995) to reveal the effect of 0.3 and 2 T homogeneous SMF of an MR on the left and right discrimination learning ability and the serum melatonin levels of mice with exposure durations of 30 to 100 min. They found a

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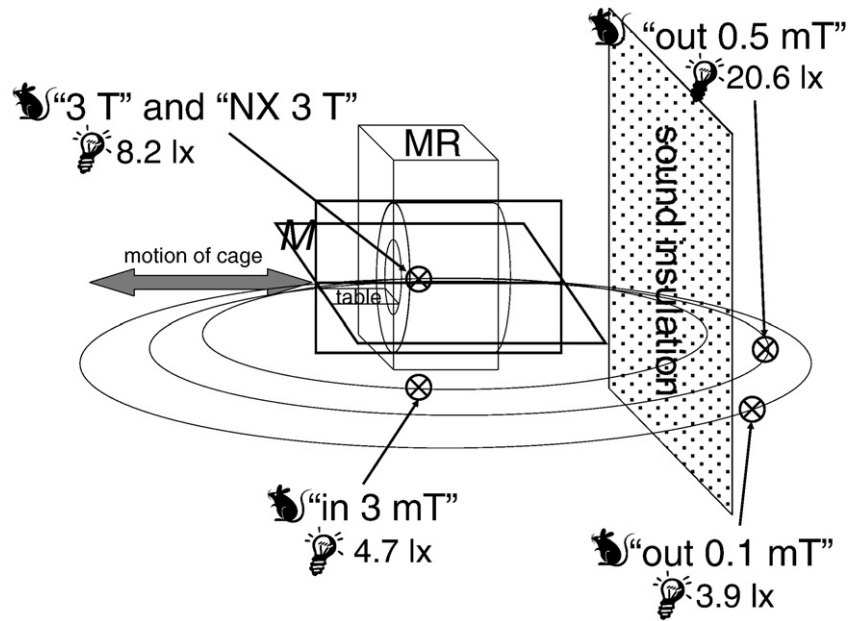


Fig. 1. Scheme of the experimental arrangement. We denoted locations by crossed circles, where the magnetic field has been probed: i) 0 T SMF (control, $n=18$), not seen in the figure, ii) 3 mT inside the MR lab (“in 3 mT”, $n=6$), iii) 0.1 mT outside the MR lab (“out 0.1 mT”, $n=6$), iv) 0.5 mT outside the MR lab (“out 0.5 mT”, $n=6$), v) 3 T inside the bore of the MR (“3 T”, $n=18$), and vi) 3 T inside the bore of the MR following naloxone pretreatment (“NX 3 T”, $n=6$). Double arrow shows the motion of the cage into and out of the bore. M is the median plane of the bore. Dosimetric illumination values in units of lx are also shown.

significant interference of SMF with spatial discrimination learning, but no influence of SMF on serum melatonin levels. Prasad et al. (1984) examined the chromosomes in the bone marrow of mice influenced by 1 hour exposition to homogeneous 0.75 T SMF in an MR and experienced no effects, no chromosomal damage. They also executed the taste aversion paradigm in rats under the exposure of 1.89 T for 30 min (Messmer et al., 1987). They found no effect. Rofsky et al. (1995) examined the stability of chromosomal damage in regenerating liver cells. They used an MR with 1.5 T homogeneous plus gradient magnetic fields for 5–10 min. No effect induced by the MRI alone or in combination with gadopentetate dimeglumine was observed. High et al. (2000) published a substantial contribution to the study of the effects of a strong homogeneous magnetic field (9.4 T) on a wide range of biological endpoints from spatial memory to gross pathologic findings in male and female rats exposed to the field 3 h a day, twice a week for 5 weeks. Their basic conclusion was that no adverse biological effects in either the parent rats or in their progeny could be attributed to the exposure. Weiss et al. (1992), later Nolte et al. (1998) investigated the behavior of laboratory rodents in SMFs equal to or greater than 4 and 9.4 T, respectively. They found that SMFs stronger than 4 T may be unpleasant, may induce aversive responses and conditioned avoidance. Later Houpt et al. (2003, 2007) confirmed these findings, when published a result on rats that could freely move through the 4 T homogeneous SMF of an MR, but avoided to enter the field. The authors related this experience to the action of the motion-induced currents in the vestibular system.

There are only few papers on the low frequency magnetic field-induced analgesic action in experimental animals. Ossenkopp et al. (1985) used a combined exposure of static magnetic and RF fields (simulating the MRI diagnostic procedure) with 0.15 T SMF induction for a duration of 2×22.5 min in mice and found an alteration in the day- and night-time responses to morphine in a thermal stimulus test. Mice in this experiment freely moved in their cage in groups of 5 within the bore of the magnet. The following year Prato et al. (1987) published that the static field component of the same MR had no evident effect on morphine-induced analgesia. More recently Shupak et al. (2004) have used a special pulsed magnetic field with peak flux density 0.1 mT and frequency 60 Hz. Mice had no restraint in their

cage. The authors found a statistically significant difference in the latency period of treated mice compared to control in the hot plate test. The difference was found to be equivalent to a moderate dose of morphine (5 mg/kg).

Materials and methods

Magnetic exposure conditions

A commercial Philips Achieva 3T MR with active shielding was used in our experiments. It has a 3 T homogeneous, horizontal SMF parallel to the axis inside the bore of the MR. The gradient system and the RF radiation were not used in our measurements.

The animal cage is made of Plexiglas with air holes on all sides except the bottom; its size is $140 \times 140 \times 46$ mm. The side walls of the cage are transparent, the top is covered with an opaque, air permeable material, and the support under the cage was always plane. The lighting conditions inside the cage were basically independent of the cage location (locations are described below). The cage was illuminated with halogenous lights from above during the experiments. The shaded area was always bigger than 94×140 (*46) mm during the experiments. The lamps generated a scattered light in the shaded area of the cage between 3.9 and 20.6 lx. The dosimetric illumination values are shown in Fig. 1.

No efforts were made to magnetically shield the experimental setup from the geomagnetic field. Creatures throughout the hundred millions of years of phylogenesis on Earth have adapted to this field. Therefore, shielding might have introduced new effects (c.f., Prato et al., 2005).

In order to check the possible effect of noise stimulus originating from the Cold Head of the helium pump of the MR regarded as background noise (64 dB equivalent continuous A-weighted sound pressure level at the isocenter, c.f., Moelker et al., 2002; Sesay et al., 2007) as a stress source for mice, 3 additional locations (tested with groups of 6 animals each) were applied. The cage was placed in the service area outside the MR lab, where the MR's stray field had either 0.1 mT or 0.5 mT horizontal flux density component denoted as “out 0.1 mT” and “out 0.5 mT”, respectively, see Fig. 1. The vertical field

component could be neglected since the cage was in the median plane of the bore (denoted by M in Fig. 1). Since gradient fields were not on, the only horizontal field components were the axial ones that certainly could be regarded homogeneous within the cage. In the third group (denoted by “in 3 mT”) animals were exposed to SMF inside the MR lab in the median plane (M), where the horizontal magnetic flux density originating from the MR was 3 mT, but noise and vibration were similar to those in the bore. The 0.1, 0.5 and 3 mT flux density values were taken from the magnetic field map of the MRI as provided by Phillips showing the main horizontal components of the stray field in the median plane (M).

In order to minimize the generation of motion-induced currents in the animals, the groups of mice at locations “3 T” and “NX 3 T” were moved into the experimental position in the bore along its axis (c.f., double arrow in Fig. 1) with a constant speed not exceeding 0.5 m/s. The way of the cage out of the bore did not influence the measurement.

Animals, assessment of pain

Male CFLP mice (24–26 g) were used in the experiments. The animals were housed in groups of 5, the room was held under a 12:12 light/dark cycle at 20 ± 2 °C, feed and water was provided ad libitum.

Preclinical pain models can be separated into 3 functional divisions based on their potential mechanism. Nociception can be induced acutely by thermal, mechanical or chemical stimulus (e.g., hot plate test, tail flick test), it can be based on tissue injury (e.g., writhing, formalin, inflamed knee, joint, paw test) or can originate from nerve injury (Yaksh, 1999). In our experiments we applied the writhing test in mice, where pain is elicited by the intraperitoneal (i.p.) injection of a slightly irritating agent. The pain sensation manifests itself in a characteristic stretching, writhing movement of the animal, the number of writhings per time period reflects pain intensity. Both opioid and non-opioid analgesics are able to exert antinociceptive activity in this pain model. This animal model is widely spread and it counts as a good predictor of human studies (Le Bars et al., 2001). Numerous agents have been described to cause abdominal pain (Collier et al., 1968) and depending on the irritant, inflammatory or non-inflammatory pain can develop (Gyires and Torma, 1984). In our experiments we used 0.6% acetic acid in a volume of 0.2 ml/mouse as an irritant. Acetic acid induces a well reproducible, relatively long-lasting, mild pain reaction. We used the method as described by Wende and Margoli (1956), modified by Witkin et al. (1961). Two mice were placed in the cage at the same time and the number of writhings was monitored during the 0–5, 6–20 and 21–30 min time intervals following the acetic acid challenge. Mice in control groups were treated the same way as those in experimental groups; they were placed in identical cages, but were not exposed to the magnetic field. Since pain sensation is known to be socially modulated in mice (Langford et al., 2006), the same number of mice (2) was placed simultaneously in the cage both in the control as well as in the magnetic field exposed cases. Mice could freely move in their cage. Treated mice were exposed to the magnetic field with their whole body during the experiment. In order to reduce experimental stress, an adaptation period was introduced for treated as well as for control mice: 2 mice together were placed in the plastic cage (where they were kept during the experiments) for 30 min for 4 consecutive days before the experiment. Mice were observed for 48 hours after the experiments, their behavior and appetite was checked. No difference between treated and untreated mice could be observed. Neither morbidity, nor mortality was observed.

In order to examine the potential involvement of an opioid component in magnetic-field induced analgesia naloxone was injected subcutaneously (s.c.) in a dose of 0.2 mg/kg 20 min before the acetic acid challenge (denoted as “NX 3 T”). Naloxone was purchased from Sigma-Aldrich (St. Louis, MO).

Ethics

Altogether 60 naïve mice participated in 6 types of experiment. Twenty-four animals were exposed to the 3 T 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), complied with the recommendations of the International Association for the Study of Pain (Zimmermann, 1983) and the Helsinki Declaration. The studies were in harmony with the Ethical Codex of Animal Experiments and were approved by the Animal Care Committee of Semmelweis University, Budapest (permission number: 1810/003/2004).

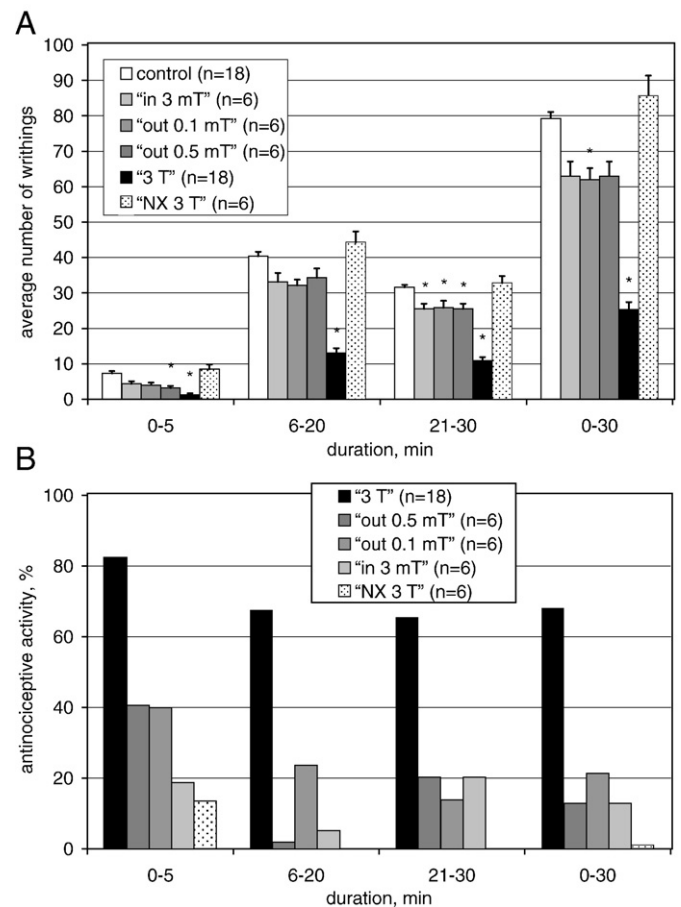


Fig. 2. A) The average number of writhings (mean \pm S.E.M.) in mice in different time intervals following the intraperitoneal (i.p.) injection of 0.6% acetic acid. The locations, where the magnetic field has been probed are the same as in Fig. 1: i) control ($n=18$), ii) 3 T inside the MR lab (“in 3 mT”, $n=6$), iii) 0.1 mT outside the MR lab (“out 0.1 mT”, $n=6$), iv) 0.5 mT outside the MR lab (“out 0.5 mT”, $n=6$), v) 3 T inside the bore of the MR (“3 T”, $n=18$), and vi) 3 T inside the bore of the MR following naloxone pretreatment (“NX 3 T”, $n=6$). n denotes animal numbers. Statistical analysis of the data was evaluated by means of one-way ANOVA completed with Dunnett’s test between pairs of data series. Equal numbers of animals were compared. A probability of $p < 0.05$ was considered statistically significant. ANOVA resulted $p_{\max} < 0.001$ by $F_{\min} > 18.7$, $F_{\text{crit}} = 2.4$ for the four the time intervals 0–5, 6–20, 21–30 and 0–30 min. In case of the comparison between “3 T” group and control, we used pooled data; in every other case we compared the treated group to the daily control. The p and η^2 values of the pairwise comparisons are listed in Table 1. B) Antinociceptive activity in the writhing test in mice in different time intervals following the acetic acid challenge. The results in Fig. 2B are derived from those in Fig. 2A according to the definition of the antinociceptive activity. The antinociceptive activity in group “NX 3 T” proved to be -1% and 0% in the 6–20 and 21–30 min time interval, respectively and thus remain hidden in the graph. If we did not pool the results for group “3 T” and control, we would have $68 \pm 5\%$, $p_{\max} < 0.001$ as the average antinociceptive activity for 3 groups of 6 mice each instead of the pooled $68 \pm 2\%$ ($p < 0.001$, $n=18$).

Table 1
 p and η^2 values as calculated with Dunnett's test for pairwise comparisons between different treated groups and control

Time period (min)	" 3 T" (n=18)		" in 3 mT" (n=6)		" out 0.1 mT" (n=6)		" out 0.5 mT" (n=6)		" NX 3 T" (n=6)	
	p	η^2	p	η^2	p	η^2	p	η^2	p	η^2
0–5	<0.001 *	0.2	>0.1	0.1	>0.1	0.1	<0.05 *	0.0	>0.1	0.2
6–21	<0.001 *	0.3	>0.5	0.1	>0.1	0.0	>0.5	0.1	>0.5	0.2
21–30	<0.001 *	0.4	<0.01 *	0.1	<0.05 *	0.1	<0.05 *	0.1	>0.5	0.1
0–30	<0.001 *	0.3	>0.05	0.1	<0.05 *	0.1	>0.05	0.1	>0.5	0.2

Equal numbers of animals were compared. In case of the comparison between group "3 T" and control, we used pooled data ($n=3*6$), in every other case we compared the group to the daily control ($n=6$). * denotes significant difference at the 5% risk level ($p<0.05$).

Statistical analysis

The results are expressed as means of number of writhings \pm S.E.M. Statistical analysis of the data was evaluated by means of one-way ANOVA completed with Dunnett's test between pairs of data series. A probability of $p<0.05$ was considered statistically significant. Equal numbers of animals were compared. p and η^2 values of the pairwise comparisons are summarized in a table. In case of the comparison between "3 T" group and control, we used pooled data; in every other case we compared the treated group to the daily control.

The antinociceptive activity of a treatment in a given time interval was expressed in % as follows: $100\left(1-\frac{\bar{y}}{\bar{x}}\right)$, where \bar{x} and \bar{y} are average numbers of writhings in the treated and in the control group of animals, respectively.

Results

The average number of writhings \pm S.E.M. in different time intervals following the acetic acid challenge in the writhing test in mice can be seen in Fig. 2A and the corresponding antinociceptive activities in % in Fig. 2B. The results in Fig. 2B are derived from Fig. 2A according to the definition of the antinociceptive activity. The experiments were performed according to the following protocol:

- no SMF (control, $n=18$),
- 3 mT inside the MR lab ("in 3 mT", $n=6$),
- 0.1 mT outside the MR lab ("out 0.1 mT", $n=6$),
- 0.5 mT outside the MR lab ("out 0.5 mT", $n=6$),
- 3 T inside the bore of the MR ("3 T", $n=18$), and
- 3 T inside the bore of the MR following naloxone pretreatment ("NX 3 T", $n=6$).

The "NX 3 T" group achieved an average antinociceptive activity of $\sim 1\%$ and 0% in the 6–20 and 21–30 min time interval, respectively and thus these values remain hidden in the graph. ANOVA resulted $p_{\max}<0.001$ by $F_{\min}>18.7$, $F_{\text{crit}}=2.4$ for the four time intervals 0–5, 6–20, 21–30 and 0–30. The p and η^2 values of the pairwise comparisons with the control can be seen in Table 1.

Discussion

It was previously shown that an inhomogeneous SMF with 783 ± 8 mT (mean \pm S.E.M.) maximum peak-to-peak magnetic flux density and 10 mm lateral periodicity caused an antinociceptive activity in the acetic acid – as well as in the epsom salt-induced writhing tests in mice (László et al., 2007; Gyires et al., 2008). Since the inhomogeneous SMF-induced antinociceptive activity was reversed by the opioid receptor antagonist naloxone, an opioid-mediated mechanism was raised (Gyires et al., 2008). Moreover, capsaicin-sensitive nerves were also supposed to be involved in the antinociceptive activity of the same inhomogeneous SMF in formalin-, and carrageenan-induced hyperalgesia in mice (Sándor et al., 2007). Namely, mice were pretreated with resiniferatoxin in order to

desensitize the C fibers. In this group the antihyperalgesic effect of the inhomogeneous SMF was abolished (Sándor et al., 2007).

Our earlier investigation including homogeneous SMFs (László et al., 2007) showed that a 84 ± 3 mT homogeneous SMF could be associated with an antinociceptive activity of $50 \pm 2\%$ ($p<0.05$, $n=10$) in the writhing test in mice during a 30 min observation period. In the present experiment the 3 T homogeneous SMF of a clinical MR causes an antinociceptive activity of $68 \pm 2\%$ ($p<0.001$, $n=18$) in the same test. (If we did not pool the results for group "3 T" and control, we would have $68 \pm 5\%$, $p_{\max}<0.001$, as the average antinociceptive activity for 3 groups of 6 mice each.)

In our present experiments naloxone pretreatment reversed the pain-inhibitory effect of SMF again confirming the hypothesis that an opioid component may be involved in the action. Since the dose of naloxone (0.2 mg/kg s.c.) has been considered selective for μ -opioid receptors, we might conclude that μ -opioid receptors may play a role in the antinociceptive activity of MR's SMF.

If we compare the results of the writhing test in the group of mice close to the bore of the MR ("in 3 mT"), or in the proximity of the MR, but outside the range of the background noise ("out 0.1 mT" and "out 0.5 mT"), we can conclude that noise stimulus did not significantly affect the pain-inhibition. Even if it did, noise has never been shown to produce analgesia. (A practical concern that occurs is rather noise protection.)

The effect of vibration stimulus that is known to produce anesthesia (e.g., Smith et al., 2004) and analgesia (for references see Chapter 19 in Core curriculum for professional education in pain, 2005) could be excluded, since the vibration of the table of the MR at rest (without imaging sequence) is negligible. The magnets' mass is 6 tons, while the moving table is less than 20 kg. We optically checked this by simply positioning a glass of water at the spot of measurement on the table and watched for surface waves, disturbances. We failed to find any sign of vibration. This control is reliable in the frequency range between 10 and 100 Hz.

Another potential confound was the effect of illumination. It has been long known that light can be a source of aversive stimulus for rodents (Kaplan et al., 1965; Pinel et al., 1994; Stern and Laties, 1998), even an anxiety model (de Jongh et al., 2002), and a light/dark test (Takao and Miyakawa, 2006) were proposed on this basis. Garcia et al. (2005) published that 3 lx seemed to be the threshold of aversion in the exploratory behavior of rats in an elevated plus maze, but not in their locomotor activity. Some animals use the polarization of sunlight for their orientation, even the million-time dimmer polarization of the moonlight proved to be a sufficient source to orient the African dung beetle (Dacke et al., 2003). Therefore, in our experiments care was taken to produce similar illumination at all experimental locations: inside the bore of the MR and outside. A big enough dimmed (<20.6 lx) area was produced inside the cage by screening light emitted from halogenous lamps above the cage. The highest illumination was provided at the location of the control experiments.

One parameter of the circadian cycle is the change of lighting. Rodents are subject to the circadian cycle in almost all areas of their life (e.g., Cain et al., 2004). The experiments were therefore carried out in the same period of time of the day, between 9 and 12 a.m.

Although we cannot exclude the possibility that pain perception of mice was influenced by motion-induced currents in their body, we think that this effect is also negligible from the viewpoint of the present study. Magnetohydrodynamic forces are related to the power product: flux density times gradient of the magnetic field in the direction of the field (see e.g., Schenck, 2000, 2005). Motion-induced currents in a homogeneous SMF are usually regarded small (Crozier et al., 2007). The difference between magnetic susceptibilities (or relative magnetic permeabilities) at interfaces of biological materials are small and therefore, may not imply an effect (Schenck, 2005; Collins, 2008). The question to be answered is rather the motion of the cage (and the mice inside) into the bore of the MR, along which the flux density changes from practically zero to 3 T. In order to control the effect of the induced currents due to the gradient of the magnetic field, we moved the cage along the axis of the

bore with a constant speed not exceeding 0.5 m/s. By accepting numerical results extrapolated from the literature (Crozier and Liu, 2005), and applying a size conversion from human to mouse according to the inverse-square law (e.g., Dawson et al., 2003), we found an induced current of 0.6 mA/m² in mice. The threshold is now considered to be 480 mA/m² for humans. This threshold value has been long discussed in the last 2 decades (c.f., Section 8.3 in International Programme on Chemical Safety, 1987; International Commission on Non-Ionizing Radiation Protection, 1994; Kangarlu and Robitaille, 2000; Zhao et al., 2002; Tenforde, 2005; Vecchia, 2007; Wood, 2008; Dimbylow, 2008). The size-corrected value for mice is 1.6 mA/m². On the other hand, motion-induced effects, primarily on peripheral nerve stimulation (PNS) are closely related to the time derivative of the flux density (Gandhi and Chen, 1999; Schenck, 2000; Formica and Silvestri, 2004; Bencsik et al., 2007). Although this effect is often connected to gradient switching in MRI, it should also be estimated when moving the cage into the strong magnetic field. The threshold for PNS is accepted to be at 40 T/s (Schenck, 2000; Formica and Silvestri, 2004; Tomasi and Wang, 2007; Safety guidelines, 2007). Since in our experiments this derivative never exceeded 1.5 T/s, this effect can be ignored. Also, it is difficult to imagine that induced currents would decrease pain, data of the literature suggest the opposite (e.g., Shellock and Crues, 2004). There is also evidence that exposure to SMFs greater than about 1 T induces flow potentials around the heart and major blood vessels, but the physiological consequences of this remain unclear (Tenforde, 2005). Several hours of exposure to very high flux densities of up to 8 T in the heart region however, did not result in any cardiovascular effects in pigs (Kangarlu et al., 1999). Neurobehavioral studies suggest that the movement of laboratory rodents in SMFs equal to or greater than 4 T may be unpleasant, inducing aversive responses and conditioned avoidance (Weiss et al., 1992; Nolte et al., 1998; Hout et al., 2003, 2007). We did not experience any efforts of the mice to avoid the 3 T magnetic field in the bore of the MR; mice made no attempts to escape the cage. Van Rongen et al. (2007) wrote about all so far animal studies: "In all these cases however, the endpoints are rather labile, a situation that may have been complicated by pharmacological manipulation, including anesthesia in some cases and immobilization."

Conclusions

The present study shows that the 3 T homogeneous SMF of a clinical MR induces a significant pain-inhibitory effect in the writhing test in mice. Since the antinociceptive activity for the whole 30 min observation period is 68% ($p < 0.01$, $n = 18$), MR's SMF should be regarded as a potential therapeutic tool. Naloxone pretreatment reverses the pain inhibitory effect of SMF indicating that the effect is likely to be mediated by opioid receptors. Noise, vibration, lighting stimuli as well as motion-induced effects were estimated not to contribute to pain sensation in a significant manner in the present experiments.

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