

Chicks incubated in hypomagnetic field need more exogenous noradrenaline for memory consolidation

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Abstract

The geomagnetic field (GMF) is one of the essential characteristics of the terrestrial environment but does not apply in outer space. The elimination of GMF may interfere with the normal activities of life in many aspects. Previous behavioral experiments have found that long-term memory is impaired in chicks incubated in a near-zero magnetic environment (i.e. hypomagnetic field or HMF). The present study was designed to evaluate the possible involvement of noradrenergic change in the functional abnormality observed before. A HMF space was produced by nullifying the natural GMF with three pairs of Helmholtz coils. The one-trial passive avoidance learning paradigm was performed on day-old chicks incubated in either the HMF space or the natural GMF. Exogenous noradrenaline was administered by intracerebral injections and the effect on memory consolidation was compared between the two categories of subjects. In the behavioral paradigm, the HMF chicks had a higher elimination rate than the GMF chicks and displayed a significant reduction in overall responsiveness. The administration of moderate doses (0.1–0.5 nmol/hemisphere) of noradrenaline led to fairly good memory retention in GMF chicks but had little effect on HMF chicks. However, long-term memory of HMF chicks could be elevated to the normal level by much higher doses (1.0–1.75 nmol/hem) of the drug. These results suggest that prolonged exposure to HMF may induce disorders in the noradrenergic system in the brain and indicate a potentiality of counteracting the ill-effect of GMF deprivation with appropriate pharmacological manipulation.

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1. Introduction

With continuing advances in astronavigation, increases in both duration and distance of flight from the Earth create more complex challenges for space missions. Since the environment in the cosmos is very different from that on the Earth, the physiological, psychological and behavioral health of astronauts is at potential risk. The geomagnetic field (GMF) constitutes one of the essential characteristics of the terrestrial environment for living beings but does not apply in outer space. Previous studies have demonstrated that the elimination of GMF may lead to struc-

tural and functional abnormalities at molecular, cellular, physiological and behavioral levels in various organisms (Dubrov, 1989; Belyavskaya, 2004). Specifically, exposure to a near-zero magnetic environment (i.e. hypomagnetic field or HMF) interferes with the brain functions of different animals; for instance, HMF exposure results in disruption of circadian activity rhythm in house sparrow (Bliss and Heppner, 1976), reduction of stress-induced analgesia in mice (Choleris et al., 2002; Del Seppia et al., 2000), impairment of long-term memory in day-old chick (Wang et al., 2003), and gradual amnesia in drosophila of successive generations (Zhang et al., 2004). Although it has been shown in golden hamster that the contents of several neurotransmitters in the central nervous system are affected by prolonged exposure to HMF (Li et al., 2001; Zhang et al., 2007), little is known

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about the neural mechanisms underlying the effects of GMF deprivation on animal behaviors. In the long run, an extensive knowledge of the relationship between GMF and life will be important for understanding the nature of life and advancing space exploration.

The one-trial passive avoidance learning task in the day-old chick provides a powerful model for the study of memory mechanisms (Gibbs and Ng, 1977; Rose, 2000). The model is widely used by research groups across the world (see Crowe and Hamalainen (2001) and Gibbs et al. (2008) for a comparison of protocols employed in different laboratories) and has yielded many valuable results. In this model, young chicks are presented with a particular colored bead that is coated with a bitter-tasting substance, methyl anthranilate (MeA). In a single trial, chicks will learn to associate the visual properties of the bead with the unpleasant taste, and will avoid pecking at beads that share similar visual properties. Levels of memory retention can then be tested at different times after the initial learning phase. With strongly reinforced training (100% MeA), the chicks exhibit three stages of memory processing (short-, intermediate- and long-term), which can be distinguished by transient retention deficits at approximately 15 and 55 min after training (Gibbs and Ng, 1979). Conversely, weakly reinforced training (20% MeA) leads to only short- and intermediate-term memory, lasting for about 30 min. Interestingly, this labile memory trace can be changed into a permanent one by intracerebral administration of exogenous noradrenaline (Crowe et al., 1990; Gibbs and Summers, 2000). The effect of noradrenaline appears to be dose-dependent, with moderate doses promoting memory consolidation, whereas higher doses may actually prevent consolidation. These results suggest that noradrenaline can act in multiple ways to influence or modulate memory function and may improve memory under appropriate conditions (Gibbs, 2008; Gibbs and Summers, 2002).

Although the avian brain looks very different to the mammalian brain, recent molecular genetic and anatomical studies have shown major organizational and structural resemblances between the two structures (Jarvis et al., 2005; Reiner, 2005). The memory system of the chick is very similar to that of mammals in terms of brain regions recruited in memory processing and in the ways memory is modulated by noradrenaline (Gibbs, 2008; Gibbs and Summers, 2002; Rose, 2000). Therefore the day-old chick model may provide important clues for research in mammals including humans. Experiments involving this animal model have found that long-term memory becomes labile and vulnerable in chicks incubated in a hypomagnetic environment (Wang et al., 2003). To further explore the underlying mechanisms, the present study was conducted to examine whether exogenous noradrenaline can diminish the memory impairment caused by HMF exposure, and to demonstrate the potential influence of GMF deprivation on the noradrenergic system in the brain.

2. Materials and methods

2.1. HMF space

A HMF space was produced by nullifying the natural GMF ($\sim 52.21 \mu\text{T}$ in our laboratory) in vertical, north/south, and east/west directions, respectively, with three pairs of mutually orthogonally nested Helmholtz coils (2.01, 1.80 and 1.61 m in diameter). An incubator (HJ-LC212, Beijing, China) made of non-ferromagnetic materials was placed in the center of the HMF space (see Fig. 1). The residual magnetic field intensity within the incubator, measured at 40 discrete positions with a fluxgate magnetometer (CTM-5W01, Beijing, China), ranged from 0.02 to $1.99 \mu\text{T}$, averaging $0.74 \pm 0.18 \mu\text{T}$ (mean \pm standard deviation).

2.2. Animal preparation

The experiments, in which care was taken to minimize any suffering of the chicks, were approved by the Animal Ethical Committee, Institute of Biophysics, Chinese Academy of Sciences. Breeding eggs (*Gallus domesticus*) were obtained from a local farm at Chinese Agricultural University. From the beginning of the incubation period to the start of the behavioral session (22–24 days in total), the experimental groups were held in the non-ferromagnetic incubator in the HMF space while the control groups were in an ordinary incubator placed in the natural GMF. All eggs were incubated and hatched in nearly identical conditions for all other factors (temperature, humidity, illumination, ventilation, etc.).

2.3. Behavioral paradigm

The protocol described by Gibbs and Summers (2000, 2002) was implemented to perform the one-trial passive

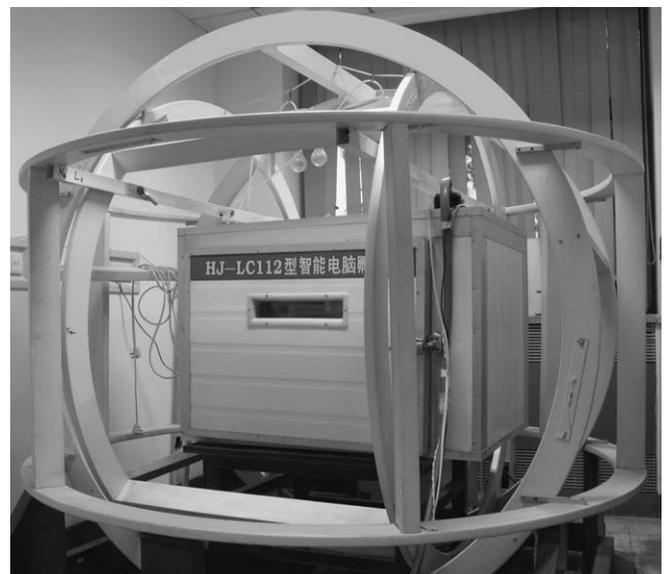


Fig. 1. HMF system and non-ferromagnetic incubator used in the present study.

avoidance learning paradigm, so that the results would be directly comparable to the previously reported effect of noradrenaline. Briefly, chicks were removed from their incubation environment (exposure to GMF or HMF) at 30–40 h old, randomly sorted into pairs, placed in wooden pens (18 × 25 × 20 cm) and maintained in this situation throughout the protocol. After approximately 1 h of acclimatization, the chicks were presented in turn with a white, a red and a blue bead (3 mm in diameter, dipped in water in advance) for pre-training of pecking behavior (10 s presentation for each bead, 3 min interval in between). Five minutes later, the chicks were presented with a red bead dipped in 20% MeA (Fluka, Switzerland; diluted in ethanol) for 10 s (training for avoidance reaction to the red bead). Memory retention for the aversion was tested at 120 min after the training, by presenting a red bead (now tasteless) and a blue bead for 10 s each. Chicks that did not peck at the red bead during training or did not peck at the blue bead during test were eliminated from data analysis, since the behavioral paradigm was not successfully established. The memory retention level was evaluated for each qualified subject as: discrimination ratio (DR) = NB/(NB + NR), where NB was the number of pecks at the blue bead during the test trial, and NR, the number of pecks at the red bead.

2.4. Drug administration

Following the protocol described by Gibbs and Summers (2000, 2002), intracranial injections of noradrenaline bitartrate (Sigma, St. Louis, MO, USA) were made into the intermediate hyperstriatum ventrale (IMHV) region of the chick brain, an area considered to be involved in memory formation and storage (Csillag, 1999; Kossut and Rose, 1984; Stewart and Rusakov, 1995). Briefly, free-hand injections were made into both hemispheres of loosely restrained, conscious chicks at 20 min after training. The site for injection was approximately 2 mm from the midline and 3 mm from the suture between the forebrain and the cerebellum, at a depth of 3.5 mm. Each batch of chicks was treated with several different doses of noradrenaline, which was prepared in physiological saline to a total injection volume of 10 µl per hemisphere. A group of sham-control chicks were injected with saline to measure a baseline discrimination ratio. The experimenter conducting the behavioral test was blind to the treatment of the chicks.

3. Results

3.1. Incubation of chicks

In total, 13 batches of HMF chicks (~100 eggs per batch) and 10 batches of GMF chicks (~150 eggs per batch) were incubated and hatched (some of the newborn birds were used for other purposes). Consistent with that in a previous study (Wang et al., 2003), there was no obvious difference between the two categories in terms of hatch

Table 1

Hatch rate, incubation period and elimination rate for GMF and HMF chicks (presented as mean ± standard deviation).

	Hatch rate (%)	Incubation period (day)	Elimination rate (%)
GMF chick	81.8 ± 5.4	21.87 ± 0.13	17.4 ± 4.8
HMF chick	78.0 ± 6.5	21.79 ± 0.31	39.7 ± 6.8

The values were calculated for each batch of chicks and averaged across batches.

rate and incubation period (see Table 1), but the HMF groups appeared to have a higher proportion of weak or crippled birds. These ‘sick’ birds often exhibited apparent behavioral disorders such as inactivity or dyskinesia and tended to die shortly, thus were excluded from further experiments. Nevertheless, the elimination rate (the proportion of subjects being disqualified in the behavioral test) in the HMF groups was significantly higher than that in the GMF groups (see Table 1; Student’s *t*-test, $p < 0.0001$). No relationship was found between the elimination rate and the dose of noradrenaline being injected into IMHV, for both the HMF and GMF chicks.

3.2. Memory retention level

Chicks incubated in the presence of two different magnetic exposures (GMF or HMF) were tested for the memory retention level at 120 min after training, in order to examine the effect of exogenous noradrenaline over a range of doses (0–3.0 nmol/hemisphere). Each bird was treated only with one of the doses (0.25, 0.5, 0.75, 1.0, 1.5, 2.0, and 3.0 nmol/hem as primary doses, see below for additional doses) or saline (as the sham-control). In total, for each exposure, 10 groups of different chicks were involved in the experiments. A multiway ANOVA (the regular two-way ANOVA is inapplicable due to the unbalanced sample size) revealed a significant main effect for drug ($F_{7,1020} = 2.707$, $p = 0.009$), a non-significant main effect for magnetic exposure ($F_{1,1020} = 0.112$, $p = 0.738$) and a non-significant interaction effect ($F_{7,1020} = 1.414$, $p = 0.196$). Post hoc comparisons with the sham-controls found significant increases of discrimination ratio at 0.25 and 0.5 nmol/hem in GMF chicks, and at 1.0 and 1.5 nmol/hem in HMF chicks. In order to determine the effective dose range of noradrenaline more closely, additional doses were applied alternatively (0.05 and 0.1 nmol/hem for GMF chicks, 1.25 and 1.75 nmol/hem for HMF chicks) and the data were compared with the corresponding control by performing one-way ANOVA and post hoc tests. The results of these analyses are displayed in Fig. 2.

As shown in Fig. 2A, the GMF chicks exhibited dose-dependent responses to noradrenaline. Only moderate doses (0.1–0.5 nmol/hem) could produce distinct increases

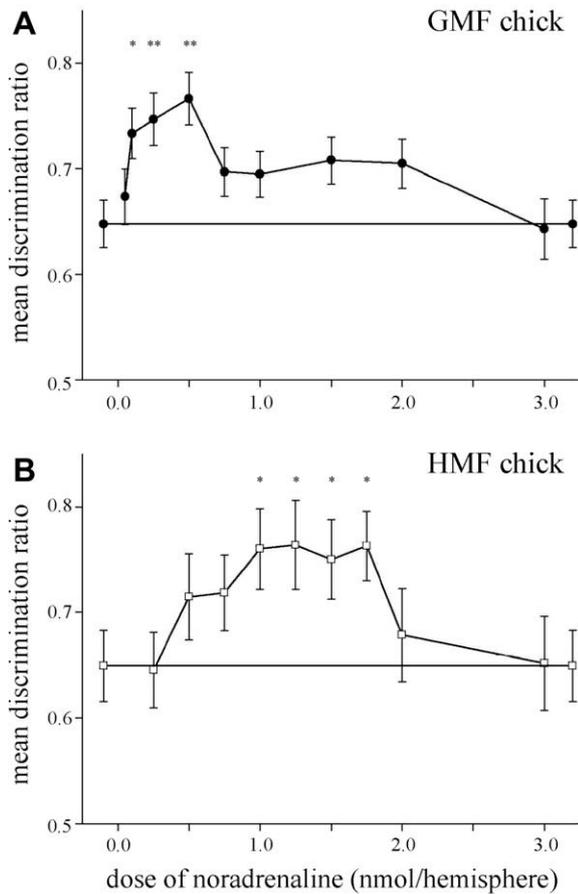


Fig. 2. Effect of exogenous noradrenaline on long-term memory of day-old GMF (A) and HMF (B) chicks. The memory retention level was evaluated for each subject with the discrimination ratio at 120 min after weakly reinforced training (20% MeA). Each data point represents the mean discrimination ratio for a group of chicks and the standard error is shown with error bar. The drug was administered into IMHV of both hemispheres by intracranial injections at 20 min after the training. In each panel, the baseline discrimination ratio obtained from saline-treated chicks is depicted with horizontal line. The number of subjects in each group was 72–112 for GMF chicks and 37–49 for HMF chicks. Significance levels: * $p < 0.05$; ** $p < 0.005$.

in the level of memory retention, as the DR values were significantly higher than the baseline. The effect of noradrenaline became non-significant at either lower (0.05 nmol/hem) or higher doses (0.75–2.0 nmol/hem), and was completely lost at the highest dose (3.0 nmol/hem). These results were generally consistent with what had been reported in the literature (Gibbs and Summers, 2000, 2002), though in Gibbs and Summers (2000) noradrenaline could produce an increase of DR value at doses up to 1.0 nmol/hem (but as discussed in Gibbs and Summers (2002) and Gibbs et al. (2008), this disparity was most likely due to differences in the source and strain of chicks and the experimental conditions). Thus, the experimental model was successfully established in our laboratory and could be used to examine the effect of HMF exposure.

The dose-dependent effect of noradrenaline on memory retention was also observed in the HMF chicks (see

Fig. 2B). The dose–response curve for HMF chicks was similar in shape to that for GMF chicks, with nearly identical DR values for both the baseline and the maximum achieved by noradrenaline administration. However, the mean DR value for HMF chicks was just around the baseline level at 0.25 nmol/hem and had merely a limited rise at 0.5 nmol/hem. At higher doses, the memory retention level was further elevated in HMF chicks but began to fall in GMF chicks. As a result, the two curves were clearly differentiated from each other along the abscissa, and the effective dose range for HMF chicks (1.0–1.75 nmol/hem) was found to be much higher than that for GMF chicks (0.1–0.5 nmol/hem).

3.3. Pecking behavior

The above-mentioned statistical analyses were conducted again to test the effect of exogenous noradrenaline on the numbers of pecks during the test trial (NB and NR), and the results are displayed in Fig. 3. For pecks at the blue bead, the multiway ANOVA revealed a significant main effect for magnetic exposure ($F_{1,1020} = 46.557$, $p < 0.0001$), a non-significant main effect for drug

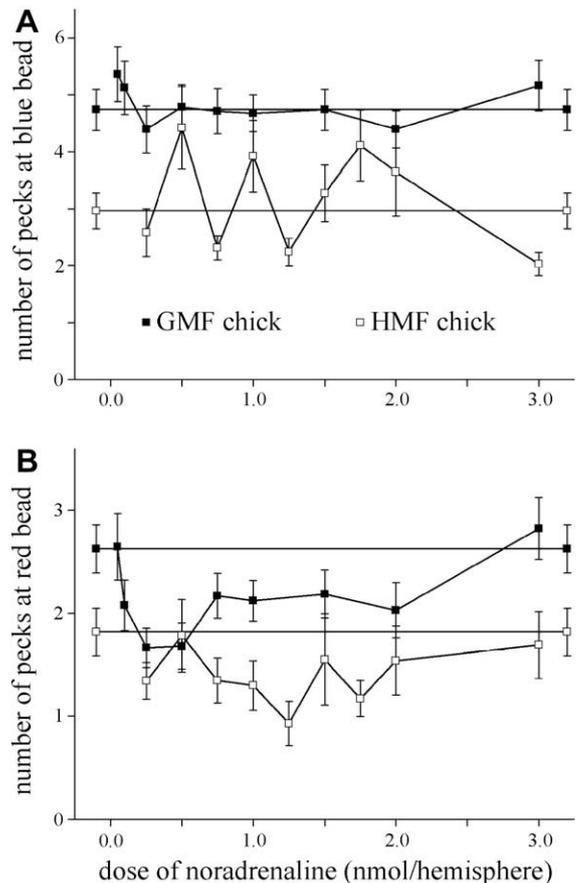


Fig. 3. Effect of exogenous noradrenaline on pecking behavior of day-old chicks. The average number (mean \pm standard error) of pecks at blue (A) or red (B) bead during the test trial is plotted against the dose of noradrenaline. The data were obtained from the same samples as in Fig. 2.

($F_{7,1020} = 1.453$, $p = 0.181$) and a moderate interaction effect ($F_{7,1020} = 2.009$, $p = 0.051$). For pecks at the red bead, there was a significant main effect for magnetic exposure ($F_{1,1020} = 18.985$, $p < 0.0001$), a moderate main effect for drug ($F_{7,1020} = 1.703$, $p = 0.104$) and a non-significant interaction effect ($F_{7,1020} = 0.870$, $p = 0.530$). Post hoc comparisons revealed a significant increase of NR at 0.25 and 0.5 nmol/hem in GMF chicks ($p < 0.005$), and at 1.25 nmol/hem in HMF chicks ($p < 0.05$), when compared to the baseline obtained from the sham-control chicks. Significant variation of NB was only found at 0.5 nmol/hem in HMF chicks ($p < 0.05$). Nevertheless, the baselines for GMF chicks were clearly higher than that for HMF chicks ($p < 0.005$ for NB, $p < 0.05$ for NR).

In summary, as shown in Fig. 3, the HMF chicks displayed a significant reduction in overall responsiveness to the beads as compared with the GMF chicks. The administration of noradrenaline did not induce any systematic change in NB in either category, though the fluctuating range appeared to be broader for HMF chicks (see Fig. 3A). In contrast, the variations of NR were obviously dose-dependent (see Fig. 3B). For GMF chicks, the NR curve was just like an up-down mirror-image reversal of the DR curve in Fig. 2A; for HMF chicks, the relationship of reversal was not as pronounced but still discernible between the DR and NR curves.

4. Discussion

The relationship between GMF and life is complicated and of great scientific interest, especially as new advances in aerospace technology make extended-duration space missions possible. There is a consensus within the scientific community that a natural GMF may be important to the normal development and functioning of living beings. Geomagnetic disturbances of solar origin can affect human physiological and psychological status (Babayev and Allahverdiyeva, 2007; Dimitrova, 2006), and the elimination of GMF may interfere with the normal activities of life in many aspects (Dubrov, 1989; Belyavskaya, 2004). However, the mechanisms involved in the action of terrestrial magnetism on biological functions are poorly understood. In the present study, as in an earlier work in this area (Wang et al., 2003), the GMF deprivation had little influence on the hatch rate and the incubation period of chicks but produced more cripples or weaklings. Despite the removal of these ‘sick’ subjects from the sample population, the one-trial passive avoidance learning paradigm was not as widely successful in HMF chicks as in GMF chicks (see the elimination rates in Table 1). These phenomena suggest that at least some processes in embryonic development were disrupted by the sustained exposure to HMF throughout the incubation period, and further indicate that the central nervous system was among the affected structures. In view of the findings obtained with golden hamster (Li et al., 2001; Zhang et al., 2007), it is plausible to hypothesize that GMF deprivation may influence the

activities of neurotransmitters in the brain, in particular the noradrenergic system which is important for modulation of behavioral state and state-dependent cognitive processes (Berridge and Waterhouse, 2003).

In previous behavioral experiments with strongly reinforced training (100% MeA), we compared the memory retention level of day-old chicks incubated in the HMF space or in the natural GMF (Wang et al., 2003). The GMF chicks in this study exhibited three stages of memory processing, in agreement with the time course reported in the literature (Gibbs and Ng, 1979). For the HMF chicks, the short- and intermediate-term memory was close to the normal level, but the long-term memory fluctuated dramatically with time and the mean level was lower than that of the GMF chicks. In other words, the long-term memory became labile and vulnerable in the HMF chicks. This detrimental effect was further demonstrated in the present study. As shown in Fig. 2, the administration of 0.1–0.5 nmol/hem noradrenaline could lead to fairly good memory retention in GMF chicks but had little to no effect on HMF chicks. However, the long-term memory of HMF chicks could be promoted to the normal level by injecting a relatively larger amount of exogenous noradrenaline (1.0–1.75 nmol/hem) into IMHV. These results support the hypothesis that the noradrenergic system in chick brain might have been affected by the prolonged exposure to HMF and, consequently, the endogenous noradrenaline involved in memory consolidation became lower than the normal level.

Since the memory retention level was determined by the numbers of pecks at blue and red beads during the test trial (NB and NR), further analysis of the original data would be helpful for understanding the results (see Fig. 3). The main effect for magnetic environment was highly significant on both NB and NR, indicating that the HMF chicks were, overall, less responsive to the beads than the GMF chicks. When noradrenaline was administered, for both categories of chicks, NB fluctuated around the corresponding baseline while NR decreased in a dose-dependent manner (roughly opposite to the increase of DR). In other words, the effect of noradrenaline was indistinct on pecks at the blue bead but more or less suppressive on pecks at the red bead. Together with the higher elimination rate and the broader fluctuating range of NB, the decreased numbers of pecks in the HMF chicks imply that the pecking behavior, and perhaps even the general activity level and stability of the animals, could be impaired by the GMF deprivation. The results of drug administration show that the reduction in overall responsiveness could not be repaired by the injection of exogenous noradrenaline. However, when the pecking behavior was associated with a discrimination task requiring visual conditioning memory, the involvement of noradrenaline appeared to be critical in determining the responses to the red bead and the memory retention level. Moreover, some kind of separate mechanism might contribute to the modulation of overall responsiveness to the beads.

Noradrenaline has long been known as critical for memory formation, consolidation and retrieval, especially, the

modulation and storage of memory (Gibbs, 2008; Gibbs and Summers, 2002; McGaugh and Roozendaal, 2002; Murchison et al., 2004). Previous study has found that, in the brainstem of golden hamster, both the content of noradrenaline and the density of noradrenergic neurons decrease significantly after prolonged exposure to HMF (Zhang et al., 2007). Presumably, analogous effects may occur on the chicks incubated in HMF and lead to a reduction of endogenous noradrenaline in IMHV and other memory-related nuclei. There is also the possibility that the elimination of GMF affects the processes involved in synaptic transmission (e.g. transmitter synthesis, release and binding to receptors, etc.) or the distribution of adrenoceptors. It awaits further investigation to explore the still open questions.

The time course is possibly a key factor for discussing the influence of HMF exposure on brain functions. The research works in our laboratory, either in golden hamster (Li et al., 2001; Zhang et al., 2007) or in day-old chick (Wang et al., 2003; and the present study), have been focused on the long-term effects of GMF deprivation. In a relevant study, prolonged hypomagnetic condition resulted in gradual amnesia in drosophila of successive generations (Zhang et al., 2004). On the other hand, it was shown that short-term geomagnetic shielding (for a few hours) reduced stress-induced analgesia in mice (Choleris et al., 2002; Del Seppia et al., 2000) but daily repeated shielding induced analgesia (Prato et al., 2005). Recent studies reported that exposure to a magnetic field simulating the one encountered by the International Space Station orbiting around the Earth (a short-term exposure to a slowly varying magnetic field) enhanced autonomic response to emotional stimuli (Del Seppia et al., 2006) but had little effect on attentional performance (Del Seppia et al., 2009). Taken together, the conditions of short-term exposure may show a lack of distinct influence on cognitive functions; however, the biological effects of hypomagnetic environment deserve more deepgoing studies, especially the aspects of long-term exposure that could be of particular relevance for space missions.

Besides the role in learning and memory, noradrenaline is involved in a number of important neuromodulatory functions of the central nervous system. Disturbance to noradrenergic transmission is implicated in many neurodegenerative diseases and cognitive neurodevelopmental disorders (Berridge and Waterhouse, 2003; Elhwuegi, 2004; Ressler and Nemeroff, 1999). It is conceivable that the potential noradrenergic changes under HMF exposure may lead to behavioral and mood disorders and interfere with cognitive performance, which could be an important issue in the context of space medicine. Moreover, it should be noted that HMF may act on some other neurobiological systems as well, for example, a series of studies have demonstrated that magnetic shielding could alter opioid-mediated behavior in mice (Choleris et al., 2002; Del Seppia et al., 2000; Prato et al., 2005). Considering the multiplicity of the nature, the effects of GMF deprivation are probably

related to many different factors and their interactions in living organisms, though so far little is understood regarding these events.

In summary, the findings of the present study are consistent with the hypothesis that prolonged exposure to HMF may induce disorders in the noradrenergic system in the brain and these disorders may substantially contribute to functional abnormalities in the animals being exposed. In addition, our results indicate a potentiality of counteracting the ill-effect of GMF deprivation with appropriate pharmacological manipulation.

Acknowledgements

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