Brief Communication

Effects of Hypomagnetic Field on Noradrenergic Activities in the Brainstem of Golden Hamster

Xu Zhang, Jun-Feng Li, Qi-Jiu Wu, Bing Li*, and Jin-Chang Jiang

State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing, China

Previous studies found that elimination of the geomagnetic field (GMF) interferes with the normal brain functions, but the underlying mechanism remains unknown. The present study examined the effects of long-term exposures to a near-zero magnetic environment on the noradrenergic activities in the brainstem of golden hamsters. Both the content of norepinephrine (NE) and the density of NE-immunopositive neurons in the tissue decreased significantly after the treatment, and the effects could be progressive with time. These variations may substantially contribute to behavioral and mood disorders reported in other studies when animals are shielded from the GMF. Bioelectromagnetics. © 2006 Wiley-Liss, Inc.

Key words: near-zero magnetic field; norepinephrine; NE content; NE-immunopositive neuron; brain function

The geomagnetic field (GMF) constitutes one of the principal characteristics of the terrestrial environment for living organisms but does not apply in outer space. It has been well established that the elimination of GMF may interfere with the normal functions of life in many aspects [Kopanev et al., 1979; Dubrov, 1989]. By means of shielding or compensation of the ambient magnetic field, a number of studies have examined the biological effects of exposures to near-zero magnetic environments, that is, hypomagnetic field or HMF. The outcomes have included, for instance, disruption of circadian activity rhythm in house sparrow [Bliss and Heppner, 1976], early developmental abnormalities in newt [Asashima et al., 1991], hypercondensation of chromatin in human fibroblasts and lymphocytes [Belyaev et al., 1997], reduction of stress-induced analgesia in mice [Del Seppia et al., 2000; Choleris et al., 2002]; however, further studies found that daily repeated exposures could induce analgesia and the effects could be altered with the presence of visible light [see Prato et al., 2005; Koziak et al., 2006], impairment of long-term memory in day-old chicks [Wang et al., 2003], and gradual amnesia in drosophila of successive generations [Zhang et al., 2004]. After long-term exposures (days or weeks), there are reports of animals exhibiting various behavioral and mood disorders, for example, inactivation, depression, mania, anxiety, and so on, indicating that the central nervous systems of the subjects might have been affected by GMF deprivation. A previous study found that the content of γ-aminobutyric acid (GABA) in cerebellum and basal ganglia of golden hamster decreased steadily after 30 days of raising in HMF [Li et al., 2001]. However, to date, little is understood regarding the neuronal mechanisms underlying the effects caused by exposures to HMF.

Norepinephrine (NE), as a major biogenic amine transmitter, is important for many complex neuromodulatory functions. In the mammalian central nervous system, noradrenergic neurons are distributed mainly in the brainstem and project diffusely to almost every major region of the brain, throughout the cerebral cortex, diencephalon, cerebellum, and spinal cord. The

Grant sponsor: Chinese Academy of Sciences; Grant number: KJCX1-09-03; Grant sponsor: National Natural Science Foundation of China; Grant number: 30400090.

*Correspondence to: Bing Li, State Key Laboratory of Brain and Cognitive Science, Institute of Biophysics, Chinese Academy of Sciences, Beijing 100101, China. E-mail: lib@sun5.ibp.ac.cn

Received for review 9 September 2005; Final revision received 8 June 2006

DOI 10.1002/bem.20290
Published online in Wiley InterScience (www.interscience.wiley.com).

noradrenergic system is involved in memory consolidation and retrieval [McGaugh and Roozendaal, 2002; Murchison et al., 2004], control of sleep and wakefulness [Espana and Scammell, 2004], modulation of behavioral state and state-dependent cognitive processes [Aston-Jones et al., 1999; Berridge and Waterhouse, 2003], and plays a primary role in mood disorders [Ressler and Nemeroff, 1999; Elhwuegi, 2004]. Therefore, it is plausible to hypothesize that some kind of abnormality in noradrenergic transmission may contribute to the biological effects of HMF on behavior and mood. Here, a preliminary attempt was performed to test this possibility by examining the content of norepinephrine and the distribution of noradrenergic neurons in the brainstem of golden hamsters which were raised in HMF.

Normal adult golden hamsters at ∼60 days of age were used in the study. All the protocols had been approved by the local committee of animal use for research and education. A HMF space was produced by shielding the natural GMF (∼52.2 μT in our laboratory) with a cylindrical chamber made of Permalloy and insulation material (see Fig. 1). The ambient electric field in the animal room was minimized by removing all unnecessary electrical appliances, so that the time-varying (50 Hz) magnetic field was very low (<0.01 μT outside the chamber, <0.002 μT inside). The experimental hamsters were raised in cages placed between positions b and d, where the residual magnetic field intensity was below 0.2% of the natural GMF. A wooden chamber of the identical size was used for raising control animals. The two chambers were abreast with each other in the same room, so that the hamsters inside would have been exposed equally to all the other external influences, such as temperature, humidity, and illumination.

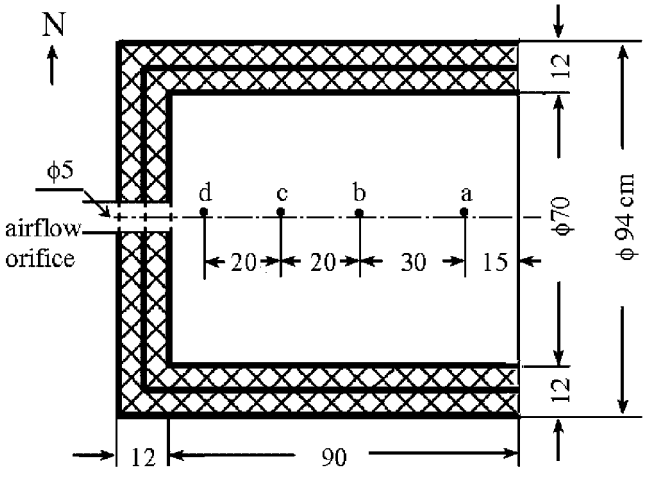

Fig. 1. Sectional drawing of the HMF space used in the present study. The natural GMF was substantially shielded in the cylindrical chamber, consisting of three laminae of Permalloy plates (1 mm thickness) and intermediate insulation fillers. The residual magnetic field intensity, measured with a fluxgate magnetometer, was 2.8, 0.1, 0.048, and 0.022 μT at positions a–d, respectively. The hamsters were held in cages placed between b and d. All dimensions in cm.

The assay of NE was conducted by a modification of the method of Miller et al. [1970]. The animals were sacrificed by decapitation. The brain was removed immediately and dissected at −20 °C to collect the lower brainstem including medulla and pons of both hemispheres. The tissue was weighed and then homogenized in cold-acidified *n*-butanol. The homogenate was centrifuged and aliquots of the supernatant were transferred to two centrifuge tubes containing benizum and hydrochloric acid. Exogenous NE was added into one tube as an internal standard. After mechanical shaking and centrifugation, the organic phase was removed and aliquots of the aqueous phase were transferred to test tubes. The fluorescence of NE was activated by oxidation reactions and measured with a spectrophotofluorometer (Hitachi, F-4500) at activation and emission wavelengths of 370 nm and 475 nm, respectively. The NE content was determined by comparing the data obtained from the two tubes.

The amount of noradrenergic neurons was examined by immunohistochemical experiments [cf. Kishimoto et al., 2000 for the procedures]. The animals were anesthetized with sodium pentobarbital (60 mg/kg, i.p.) and perfused transcardially with a fixative containing 5% glutaraldehyde and 1% sodium metabisulfite in 0.1 M cacodylate buffer. The brain was removed and postfixed in the fixative. The regions to be studied were separated and serially cut into coronal sections with a freezing microtome at a thickness of 40 μm. The sections were macerated in hydrogen

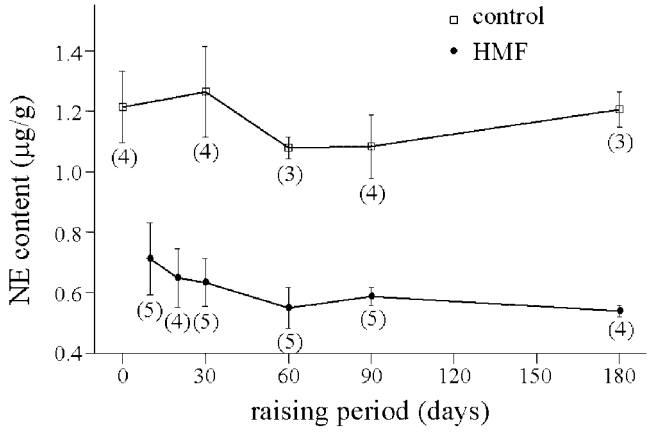

Fig. 2. Mean NE contents in lower brainstem of golden hamsters raised in HMF and control chamber. For each set of data, the number of animals is given in brackets and the standard deviation is shown with error bar.

TABLE 1. Mean Densities and Standard Deviations (Given in Profiles/mm^2) of NE-Immunopositive Neuron in Regions A3 and A7 of the Brainstem, Obtained From Golden Hamsters Which Were Raised in HMF or Control Chamber for 60 or 180 Days

	60 days				180 days			
	HMF	Control	Difference	P (t-test)	HMF	Control	Difference	P (t-test)
A3	374.0 ± 20.4	491.6 ± 32.6	-23.9%	$<.01$	303.8 ± 15.3	460.9 ± 21.4	-34.1%	$<.001$
A7	413.2 ± 13.6	569.2 ± 32.7	-27.4%	$<.01$	312.7 ± 23.7	481.8 ± 32.1	-35.1%	$<.005$

Three animals were used for each measurement. For each animal, the density value was obtained by averaging four or five sections collected alternately from the corresponding region.

peroxide solution for 30 min to eliminate the intrinsic peroxidase and subsequently incubated in (1) the primary antibody: rabbit anti-norepinephrine serum (Gemac, Versailles, France; diluted 1:1000) for 60–72 h at 4 °C; (2) the secondary antibody: goat anti-rabbit IgG (Sigma, St. Louis, MO, USA; diluted 1:40) for 2–3 h at room temperature; (3) peroxidase anti-peroxidase complex (PAP, Sigma, diluted 1:100) for 1.5–2 h at room temperature; and (4) 0.025% 3,3′-diaminobenzidine-tetrahydrochloride-dihydrate (DAB) and 0.006% hydrogen peroxide in Tris-HCl buffer (pH 7.5) for 10–15 min at room temperature. After dehydration, the sections were mounted on gelatin-coated glass slides and observed under a microscopy (Zeiss, Zeiss, Germany; LSM-10) to count the number of NE-immunopositive neurons (profiles) and then calculate the density.

The variation of NE content as a function of days is displayed in Figure 2. In the control, the NE content fluctuated by some extent but did not show any discernible tendency of going up or down with time, and the differences were insignificant among the five sets of data (ANOVA, $F_{4,13} = 2.19$, $P > .1$). In other words, the value was roughly changeless for normal hamsters of 60–240 days old. For the exposed animals, the NE content was much lower (the Student's t-test, $P < .002$ for all possible comparisons between the control and the HMF groups), indicating that the GMF deprivation may lead to a significant reduction of norepinephrine in the brainstem of golden hamster. In addition, there were significant differences among the six HMF groups (ANOVA, $F_{5,22} = 3.41$, $P \approx .02$), though in the multiple comparisons, the probability of $P < .05$ could be reached only between 10 and 60 day groups and 10 and 180 day groups. Accordingly, the decrement of NE content might be progressive during the critical period of 0–60 days, while little effect could be observed with further exposure (over 60 days).

In light of the results of NE assay, immunohistochemical experiments were carried out on brain sections prepared from hamsters, which were raised for 60 and 180 days, respectively. The sections from regions A3 and A7 of the brainstem were taken into analysis. As shown in Table 1, in comparison with the control, the mean profile density of NE-immunopositive neurons decreased significantly (∼25%) in both regions after being exposed to HMF for 60 days and this decrement became even more pronounced (∼35%) when the exposing period was elongated to 180 days. On the other hand, the profile density was generally lower for the 180-day group than the corresponding 60-day group, nevertheless, the difference was small in the control ($P \approx .25$ for A3, $P \approx .03$ for A7) but significant in the HMF animals ($P \approx .01$ for A3, $P < .01$ for A7). No evidence was found for any region-specific effect in relation to GMF deprivation.

The variation of profile density was in accordance with the decrement of NE content in the tissue, except for some details concerning the degree and duration of change. Specifically, the profile density displayed a smaller but more sustaining reduction than the NE content, implying that both the number of NE-immunopositive neurons and the NE content in individual neurons might be affected by long-term exposure to HMF. It awaits further investigations to explore the courses and mechanisms of these effects.

Summarily, our experiments demonstrate that the noradrenergic activities in the brainstem of golden hamster were impaired by GMF deprivation, as both the NE content and the profile density of NE-immunopositive neurons in the tissue decreased significantly after the treatment. Since NE is involved in a number of important functions of the central nervous system, the abnormality in noradrenergic transmission may substantially contribute to behavioral and mood disorders in the animals exposed to HMF.

ACKNOWLEDGMENTS

The authors are thankful for the technical support of Qian Wang and Xi-Wu Zhao.

REFERENCES

Asashima M, Shimada K, Pfeiffer CJ. 1991. Magnetic shielding induces early developmental abnormalities in the newt, *Cynops pyrrhogaster*. Bioelectromagnetics 12:215–224.

Aston-Jones G, Rajkowski J, Cohen J. 1999. Role of locus coeruleus in attention and behavioral flexibility. Biol Psychiatry 46:1309–1320.

Belyaev IY, Alipov YD, Harms-Ringdahl M. 1997. Effects of zero magnetic field on the conformation of chromatin in human cells. Biochim Biophys Acta 1336:465–473.

Berridge CW, Waterhouse BD. 2003. The locus coeruleus-noradrenergic system: Modulation of behavioral state and state-dependent cognitive processes. Brain Res Rev 42:33–84.

Bliss VL, Heppner FH. 1976. Circadian activity rhythm influenced by near zero magnetic field. Nature 261:411–412.

Choleris E, Del Seppia C, Thomas AW, Luschi P, Ghione G, Moran GR, Prato FS. 2002. Shielding, but not zeroing of the ambient magnetic field reduces stress-induced analgesia in mice. Proc R Soc Lond B 269:193–201.

Del Seppia C, Luschi P, Ghione S, Crosio E, Choleris E, Papi F. 2000. Exposure to a hypogeomagnetic field or to oscillating magnetic fields similarly reduce stress-induced analgesia in C57 male mice. Life Sci 66:1299–1306.

Dubrov AP. 1989. The geomagnetic field and life: Geomagnetobiology. New York: Plenum. (translated from Russian by Sinclair FL)

Elhwuegi AS. 2004. Central monoamines and their role in major depression. Prog Neuro-Psychopharmacol Biol Psychiatry 28:435–451.

Espana RA, Scammell TE. 2004. Sleep neurobiology for the clinician. Sleep 27:811–820.

Kishimoto Y, Geffard M, Arai R. 2000. Catecholamine degradation by monoamine oxidase in locus coeruleus neurons of the rat. An immunohistochemical study. Brain Res 859:373–377.

Kopanev VI, Efimenko GD, Shakula AV. 1979. Biological effect of a hypogeomagnetic environment on an organism. Biol Bull Acad Sci USSR 6:289–298.

Koziak AM, Desjardins D, Keenliside LD, Thomas AW, Prato FS. 2006. Light alters nociceptive effects of magnetic field shielding. Bioelectromagnetics 27:10–15.

Li J-F, Wu Q-J, Wang Q, Jiang J-C, Jin H-Q, Lin Y-F. 2001. Effect of magnetic free field space (MFFS) on GABA, glycine and taurine of cortex, cerebellum and basilar nucleus in hamster. Prog Biochem Biophys 28:358–361.

McGaugh JL, Roozendaal B. 2002. Role of adrenal stress hormones in forming lasting memories in the brain. Curr Opin Neurobiol 12:205–210.

Miller FP, Cox RH Jr, Snodgrass WR, Maickel RP. 1970. Comparative effects of *p*-chloroamphetamine and *p*-chloro-*N*-methylamphetamine on rat brain norepinephrine, serotonin and 5-hydroxyindole-3-acetic acid. Biochem Pharmacol 19:435–442.

Murchison CF, Zhang X-Y, Zhang W-P, Ouyang M, Lee A, Thomas SA. 2004. A distinct role for norepinephrine in memory retrieval. Cell 117:131–143.

Prato FS, Robertson JA, Desjardins D, Hensel J, Thomas AW. 2005. Daily repeated magnetic field shielding induces analgesia in CD-1 mice. Bioelectromagnetics 26:109–117.

Ressler KJ, Nemeroff CB. 1999. Role of norepinephrine in the pathophysiology and treatment of mood disorders. Biol Psychiatry 46:1219–1233.

Wang X-B, Xu M-L, Li B, Li D-F, Jiang J-C. 2003. Long-term memory was impaired in one-trial passive avoidance task of day-old chicks hatching from hypomagnetic field space. Chinese Sci Bull 48:2454–2457.

Zhang B, Lu H-M, Xi W, Zhou X-J, Xu S-Y, Zhang K, Jiang J-C, Li Y, Guo A-K. 2004. Exposure to hypomagnetic field space for multiple generations causes amnesia in *Drosophila melanogaster*. Neurosci Lett 371:190–195.