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Morphological changes of neurons involved in long-term memory in day-old chicks hatching from hypomagnetic field space

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Hatched from natural geomagnetic conditions, 1-day-old chicks were stimulated with Methyl Anthranilate (MeA) and labeled as the control group (CG). We found that the line density of dendritic spines (LDds) from the neurons in the memory-related nuclei [Intermediate Medial Hyperstriatum Ventrale (IMHV) and lobus parolfactorius (LPO)] was increased by 38.7%, and the total dendritic lengths (TLds) of individual neurons in IMHV were increased by 37.1 to 45.4%. In addition, the average LDds of individual neurons in imprinted chicks (ICs) were increased by 10.8%, and the neurons that had long dendrites were increased by 50%. In contrast, in the experimental group (EG), where chicks were hatched from hypomagnetic field, the average LDds of each neuron in IMHV and LPO of the native chick (NC) were similar to the NC of CG. After a 12 h exposure to MeA, the LDds from IMHV and LPO of the no-imprinted chick (n-IC) and IC were similar to NC but decreased by 17.4% to the n-IC and IC of CG. Furthermore, the average TLd of single neurons from the memory nuclei in NC, n-IC and IC were significantly decreased by 30.9%. However, the LPOs were not different in n-IC. These results indicate that if the natural geomagnetic environment is disrupted, the development of the dendritic spines from the neurons in the related memory nuclei is unchanged, but the hyperplasia of the dendritic spines in the neurons involved in long-term memory was ablated by MeA. Further, the development and growth of dendrites were significantly reduced.

Key words: Hypomagnetic field space, day-old chicks, memory-related nuclei (Medial Hyperstriatum Ventrale and lobus parolfactorius), neuron morphology.

INTRODUCTION

The biological effects of a hypomagnetic field space (HMFS), where the magnetic intensity was reduced below 1000 nT (1 μ T), had been studied extensively by the former Soviet Russia in the 1960 to 1970s. Their

studies focused on the effects of the HMFS on the development and growth of various organisms. However, the studies that examined the influence of HMFS on the function of the central nervous system were limited to the measurement of the critical flicker fusion rate (Dubrov, 1978). To date, studies have examined the effects of HMFS on the learning of the birdsong and on the levels of neurotransmitters in the rat brain (Jiang et al., 1998; Li

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et al., 2001), and focused on the effects of weak magnetism, where the magnetic intensity was similar to the geomagnetism on learning and memory (Lovely et al., 1992); studies on the biological effects of extremely low intensity magnetism (below 1 μ T) have not been conducted. Using a previous model for creating the HMFS (Jiang et al., 2003), we showed that long-term memory was impaired in a one-trial passive avoidance task in 1-day-old chicks that hatched from the HMFS (Wang et al., 2003), and we also found that learning and memory were significantly impaired in *Drosophila* that were continuously housed in the HMFS (Zhang et al., 2004).

These results indicate that if natural geomagnetism is abolished during the development of the embryo, the structure and function of the neurons involved in learning and memory will be altered.

Since Gibbs and Ng (1976) suggested a three-term memory model for the one-trial avoidance task of 1-day-old chicks many studies in the neuroscience and cognitive science fields have focused extensively on memory and increased our understanding of the neural mechanisms involved in the formation of short-, medium- and long-term memory.

The areas of the brain involved in the formation of memories in chicks are the intermediate medial hyperstriatum ventrale (IMHV) and the lobus parolfactorius (LPO) (Rose, 1991; Squire et al., 1993). In addition, the formation of long-term memory is important in the synthesis of α -tubulin and glycoprotein (Scholey et al., 1992, 1993; Rose, 1995), and it is closely related to morphological changes in neurons (such as the density of dendritic spines, quantity of vesicles, and the density and height of synapses (Stewart et al., 1992; Stewart and Rusakov, 1995; Rusakov et al., 1993; Rose and Stewart, 1999; Ünal et al., 2002). Long-term memory initially forms in the IMHV and is then stored in the LPO (Rose, 1991; Tiunova et al., 1998). Recent studies have shown that the N-Methyl-D-aspartate (NMDA) receptor (NMDAR) and the α -Amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA) receptor (AMPA) in the IMHV and LPO in chicks are important in the origination of memory formation and retention (Soderling and Derkach, 2000). In addition, the regulation of glutamate and the activation of NMDAR can increase Ca^{2+} concentration within cells, which affects synaptic structures (such as prolonging, shortening and collapsing the dendritic spines (Segal et al., 2000). Therefore, we examined the morphological changes in neurons from 1-day-old chicks that were hatched from a hypomagnetic field and had long-term memory impairments. This comparative study on 1-day-old chicks hatched in a natural geomagnetic field and a hypomagnetic field showed that during the development of the chick embryo, the hypomagnetic field does not affect the growth of the dendritic spines. However, in the one-trial avoidance task, hyperplasia of the dendritic spines was reduced, and the development and growth of the

dendrites were significantly inhibited.

MATERIALS AND METHODS

The compensated hypomagnetic field space comes from 3 pairs of Helmholtz coils intersecting vertically one another whose diameters are 2.01, 1.80 and 1.61 m and compensating the values of magnetic field in vertical, north and south, and east and west, respectively. The value of residual magnetic field is in 100 to 680 nT. In the hypomagnetic field space, a HJ-LC112 intelligent non-ferromagnetic incubator is arranged (Jiang et al., 2003).

The 1-day-old chicks that were hatched from the natural geomagnetic field in the control group (CG) and the hypomagnetic field in the experimental group (EG) were tested in a one-trial avoidance task using MeA (Methyl Anthranilate (Wang et al., 2003). For all groups, three naive chicks (NC), three no-imprinted chicks (n-IC) and three imprinted chicks (IC) were chosen, and the neuronal morphology was observed 12 h after being trained.

The Golgi silver stain has been widely used to study neuronal morphology (Jiang et al., 2003; Peters and Kaiserman-Abramof, 1970; Rusakov and Stewart, 1995; Sojka et al., 1995). According to the improved method, we were able to localize each neuron to the memory-related nuclei. The anesthetized chicks were perfused and fixed with physiological saline and 10% formalin for 20 min, and then the brains were removed. Following Youngren's brain atlas (Youngren and Phillips, 1978), the tissue masses (1 \times 1.5 \times 2 mm) of the right and left IMHVs and LPOs were excised, and they were then post-fixed for 1 week at room temperature. Next, the tissues were washed with DW and then immersed in 0.75% silver nitrate for 6 days. After being washed with 40% alcohol for 40 min, the tissues were dehydrated sequentially using 70, 80 and 95% alcohol.

The tissues were immersed in alcohol for approximately 2 h, dipped into a mixture of ether and alcohol (1:1) for 3 h, and infused with 4 and 8% collodion for 24 and 1 h, respectively. The tissues were indurated by 70% alcohol and sliced at 20 μ m. The slices were dehydrated sequentially using 70, 80 and 95% alcohol. After being cleared by creosote for 5 min, the slices were mixed sufficiency with oil of turpentine to dissolve the creosote. Next, the slices were placed onto slides, covered with Canada gum, and air-dried for 3 weeks.

The dried slices were detected using an inverted microscope (Olympus), and photos were taken using a micro-CCD photography system (Cold Spring Harbor Corp.). The photo managing and length measuring were analyzed using Image-pro Plus software.

Thirty neurons were selected from each nucleus, and a clear, medial segment of dendrites was chosen from the neurons. The sampling length of the dendrite (SLd) and the number of dendritic spines (Nds) were measured using a high-fold microscope lens. The line density of the dendritic spines (LDds) was calculated using the formula: LDds = Nds/SLd. Furthermore, for the nuclei related to memory, the total length of the dendrites (TLds) of the neurons that were easily identifiable was measured.

RESULTS

LDds and TLd in the control group

Figure 1 shows the neuronal morphology in the memory related nuclei of the NC, n-IC and IC from the control group. In Figure 1A-1-4, 1B-1-4 and 1C-1-4, the atramentous body and the dendritic spines of the stellate neurons can be clearly observed, which was consistent with Stewart et al. (1992). In addition, the branches from

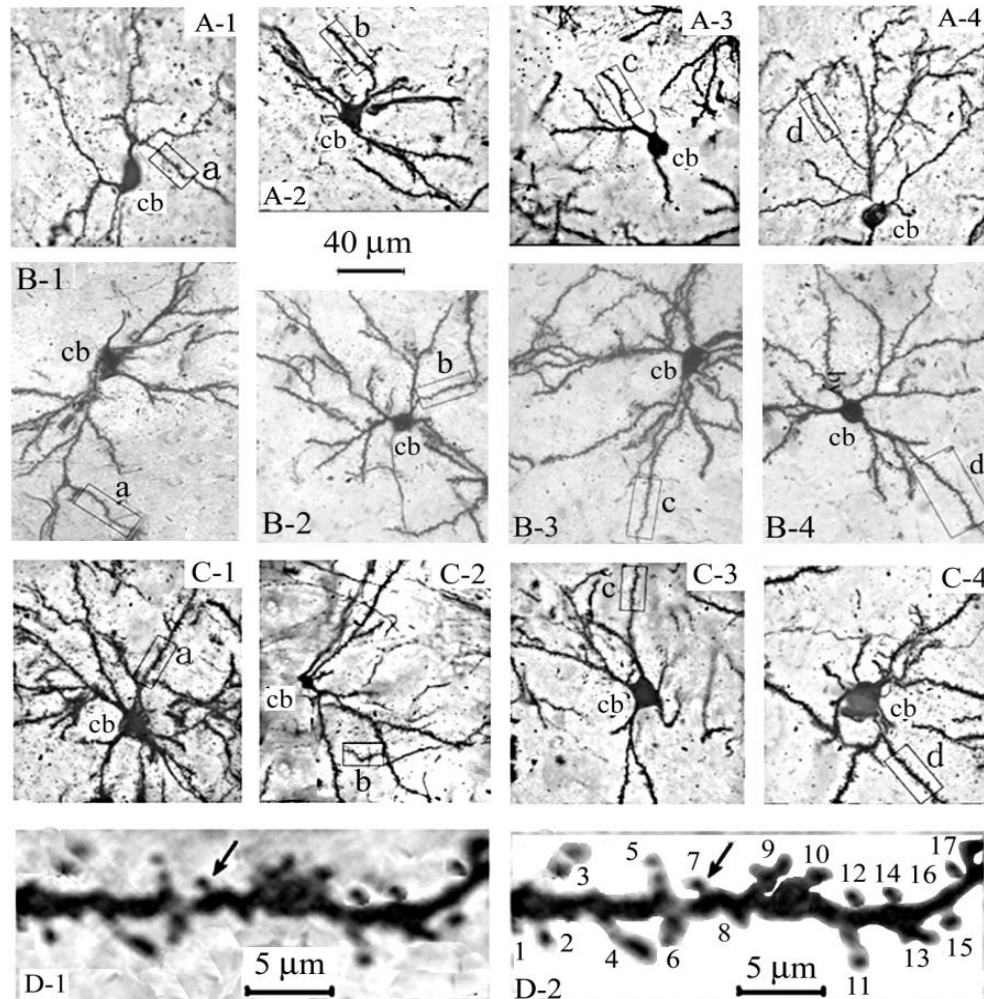


Figure 1. The morphology of neurons and dendritic spines in the memory-related nuclei of 1-day-old chicks from the control group. The neurons of the R-IMHV, R-LPO, L-IMHV and L-LPO in the NC (A-1-4), n-IC (B-1-4) and IC (C-1-4), respectively; cb: cell body; a-d: sampling segment of the dendrite. D-1 is a magnification of from A-1. D-2 shows D-1, in which the surrounding background of the dendritic spines was removed. The arrow shows a dendritic spine.

the central neurons in the memory-related nuclei in the NC were significantly less than in the n-IC and IC, and the R-IMHV of the IC was the most dense among them (Figure 1C-1). This was consistent with the TLd data (Figure 3).

Using a high-fold lens, the dendritic spines of the sample segments (a-d) in Figure 1A-1-4, 1 B-1-4 and C-1-4 can be seen by adjusting the fine focus on the microscope. Because of the 3-dimensional structure of the dendritic spines (Rusakov and Stewart, 1995; Sojka et al., 1995), the most representative image depicting the majority of the dendritic spines was chosen.

Of the 360 SLds examined from the control group, the shortest was 24.9 μm , and the longest was 119.8 μm ; the majority was found to be from 30 to 80 μm , which accounted for 85.8% (309/360).

In Figure 1A-1-4, the lengths of the SLd were 29, 37, 37

and 33 μm , respectively, with the number of corresponding dendritic spines (Nds) in the segments being 17, 25, 22 and 21 (Figure 1D-2), and the LDds being 0.59, 0.68, 0.60 and 0.64 μm , respectively. Similarly, in Figure 1B-1-4, the SLds in a-d were 42, 40, 44 and 51 μm , respectively; the Nds were 35, 32, 37 and 40, and the LDds were 0.83, 0.80, 0.84 and 0.78 μm , respectively. In Figure 1C-1-4, the SLds in a-d were 33, 29, 30 and 34 μm , respectively; the Nds were 30, 26, 28 and 28, and the LDds were 0.91, 0.90, 0.93 and 0.82 μm , respectively. The LDds of the neurons were increased in the central memory-related nuclei in the 1-day-old chicks that were exposed to MeA (n-IC and IC) compared to the NC.

The distribution of the perpetual percentage of LDds and TLd (PFds and PFd) of the neurons from the left and the right of the IMHV and LPO of the NC, n-IC and IC from the control group is displayed in Figure 2(1) and (2).

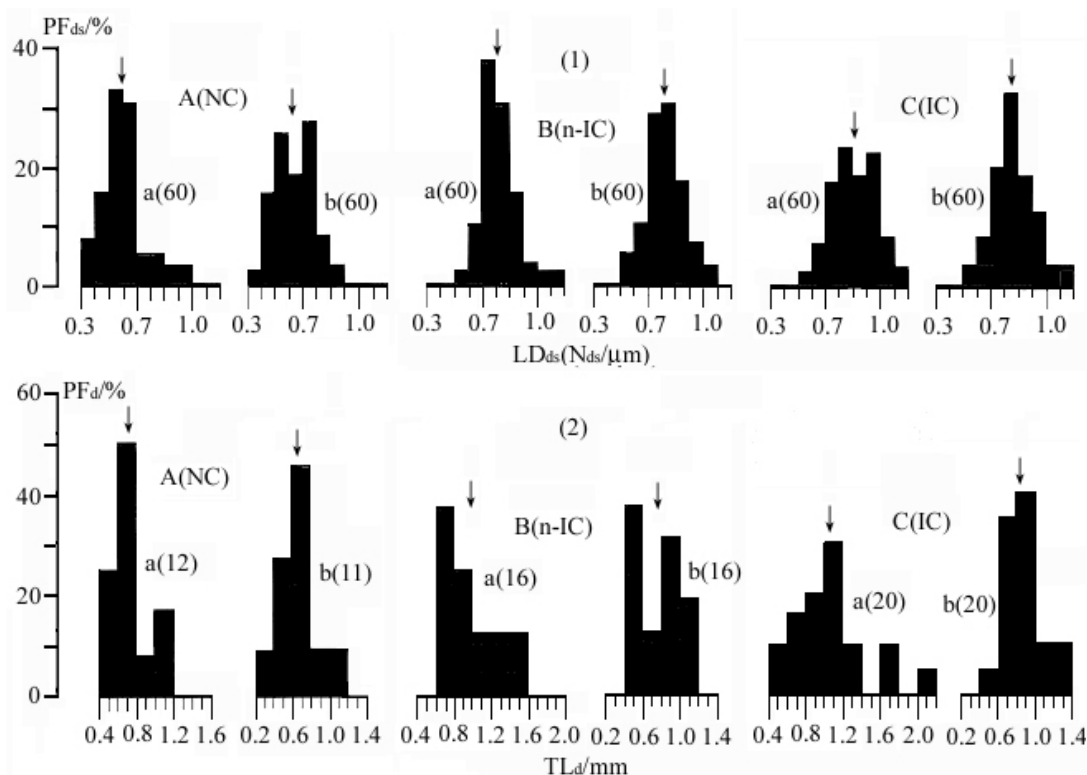


Figure 2. The distribution of LDds and TLd frequency of the percentage of the neurons in the memory-related nuclei of 1-day-old chicks from the control group (1 and 2). a, IMHV; b, LPO. The arrowheads show the mean value, and the numerals in brackets show the number of samples.

In Figure 2(1)-A-a and b, as the control group, the main distribution of the neuronal LDds frequency in the left and right side of the IMHV and LPO in the NC were 0.4 to 0.7 μm (accounting for 76.7%) and 0.4 to 0.8 μm (accounting for 85.0%), and the mean values were $0.60 \pm 0.15 \mu\text{m}$ (60) and $0.65 \pm 0.16 \mu\text{m}$ (60), respectively. There was no significant difference between the two nuclei.

Similarly, the main distribution of the neuronal LDds frequency in the left and right side of the IMHV and LPO in the n-IC were both about 0.6 to 1.0 μm , which accounted for 91.7 and 85.0%, respectively, and the mean values were $0.83 \pm 0.12 \mu\text{m}$ (60) and $0.83 \pm 0.14 \mu\text{m}$ (60), respectively. There was no significant difference between nuclei. However, compared to the NC, the mean values increased significantly by 37.9% ($t = 9.025 > t$ 0.01, $df = 118$) and 28.6% ($t = 6.945 > t$ 0.01, $df = 118$), respectively.

In addition, the main distribution of the neuronal LDds frequency in the left and right side of the IMHV and the LPO in the IC was 0.7 to 1.1 μm , which accounted for 80.0 and 81.7%, respectively, the mean values were $0.92 \pm 0.15 \mu\text{m}$ (60) and $0.87 \pm 0.15 \mu\text{m}$ (60), respectively, and there was no significant difference. However, compared to the NC, the mean values were increased significantly by 52.8% ($t = 10.432 > t$ 0.01, $df = 118$) and 33.8% ($t = 7.907 > t$ 0.01, $df = 118$), respectively. Further, the LDds

from the two sides of the IMHV in the IC increased, on average, by 10.8% ($t = 3.621 > t$ 0.01, $df = 118$) compared to the n-IC.

Therefore, the neuronal LDds frequency of the central memory-related nuclei in the 1-day-old chicks treated with MeA (n-IC and IC) increased, on average, by $38.3 \pm 10.4\%$ (4) compared to the NC.

As shown in Figure 2(2)-A-a and b, we found that in the control group, the main distribution of the TLd of the neurons from the left and right side of the IMHV and the LPO in the NC was 400 to 800 μm , which accounted for 75 and 72.5%, respectively, and the mean values were $733 \pm 170 \mu\text{m}$ (12) and $673 \pm 243 \mu\text{m}$ (11), respectively. There was no significant difference between the two nuclei. The neurons with TLd over 1000 μm accounted for 16.7 and 9.1%, respectively.

Similarly, we showed in Figure 2(2)-B-a and b that the main distribution of the TLd of the neurons from the left and right side of the IMHV and the LPO in the n-IC was 600 to 1000 μm and 400 to 1000 μm , which accounted for 62.5 and 81.3%, respectively, and the mean values were $1005 \pm 317 \mu\text{m}$ (16) and $778 \pm 203 \mu\text{m}$ (16). The TLd in the IMHV increased, on average, by 29.2% ($t = 2.409 > t$ 0.05, $df = 30$) compared to that in the LPO. The neurons with TLd over 1000 μm accounted for 37.5 and 18.8%, respectively. Moreover, the average TLd in the

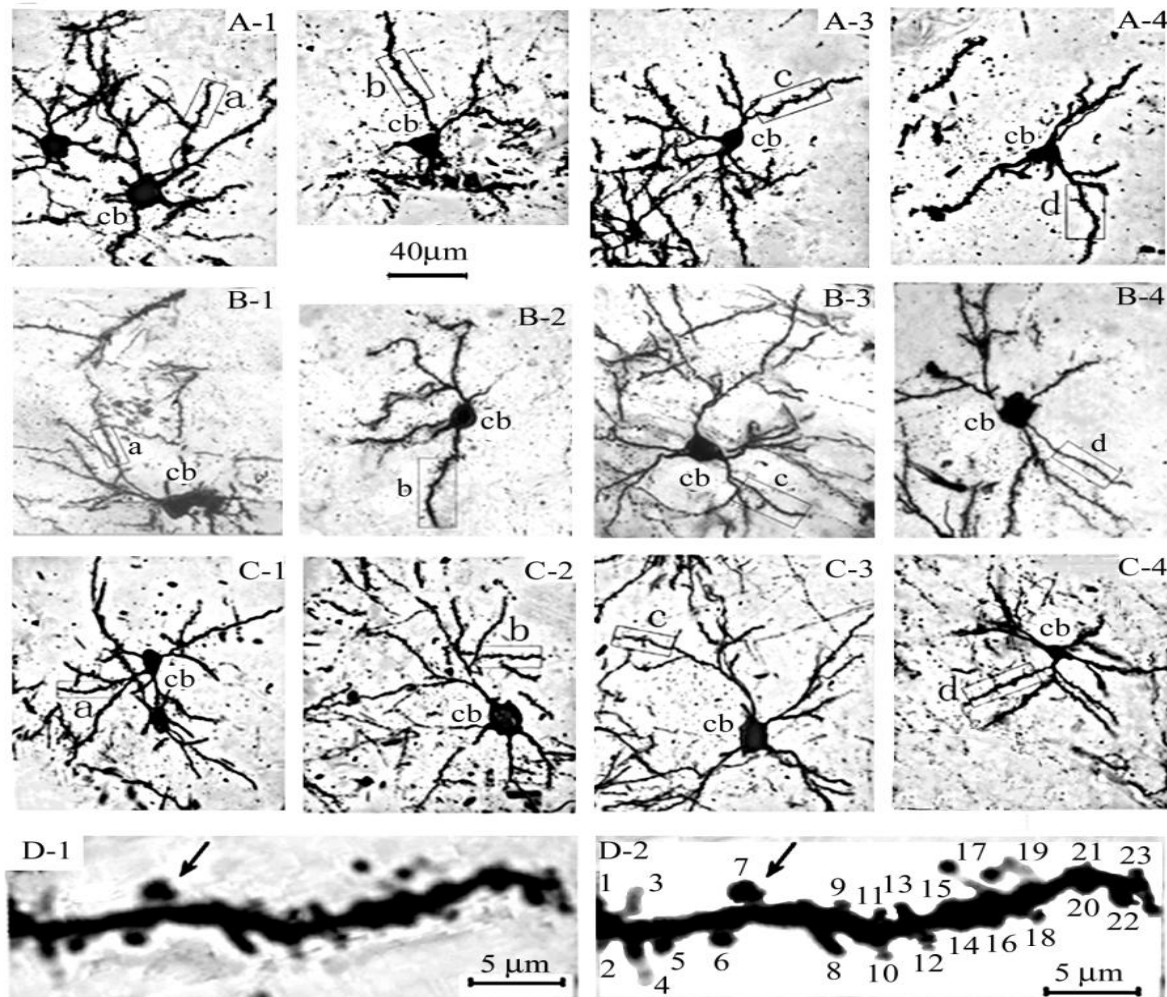


Figure 3. The morphology of the neurons and the dendritic spines in the memory-related nuclei of 1-day-old chicks in the experimental group. The neurons of the R-IMHV, R-LPO, L-IMHV and L-LPO in the NC (A-1-4), n-IC (B-1-4) and IC (C-1-4), respectively; cb, cell body; a-d, sampling segment of dendrite. D-1 shows the magnification of Figure 3A-1-a; D-2 shows D-1 with the surrounding background of the dendritic spines removed. The arrow indicates a dendritic spine.

IMHV in the n-IC increased significantly by 37.1% ($t = 2.687 > t 0.02$, $df = 26$) compared to the NC.

As shown in Figure 2(2)-C-a and b, in the control group, the main distribution of the TLd of the neurons in the left and right side of the IMHV and the LPO in the IC was 600 to 1200 μm and 600 to 1000 μm , which accounted for 65.0 and 80.0%, respectively, and the mean values were $1066 \pm 386 \mu\text{m}$ (20) and $851 \pm 196 \mu\text{m}$ (20), respectively. The average TLd in the IMHV increased by 25.3% ($t = 2.222 > t 0.05$, $df = 38$) as compared to the LPO. The neurons with TLd over 1000 μm in the IMHV and LPO accounted for 55.0 and 20%, respectively. Moreover, the average TLd in the IMHV in the IC increased significantly by 45.4% ($t = 2.721 > t 0.02$, $df = 30$) compared to the NC. In contrast, there was no significant difference between the TLd in the IMHV in the IC and the n-IC, but the neurons with TLd over 1000 μm

in the IC were 1.5 times more than in the n-IC.

LDds and TLd in the experimental group

Figure 3 shows the neuronal morphology in the memory-related nuclei isolated from the NC, n-IC and IC in the experimental group.

In Figure 3A-1-4, 3B-1-4 and 3C-1-4, the atramentous body and the numerous dendritic spines of the satellite neurons are clearly visible, which was similar to the control group? In contrast to the control group, the NC showed reduced neuronal dendritic branches in the memory-related nuclei compared to the n-IC and IC.

Using the high-magnification lens, the dendritic spines sample segments (a-d) in Figure 3A-1-4, 3B-1-4 and 3C-1-4 can be observed by adjusting the fine focus of the

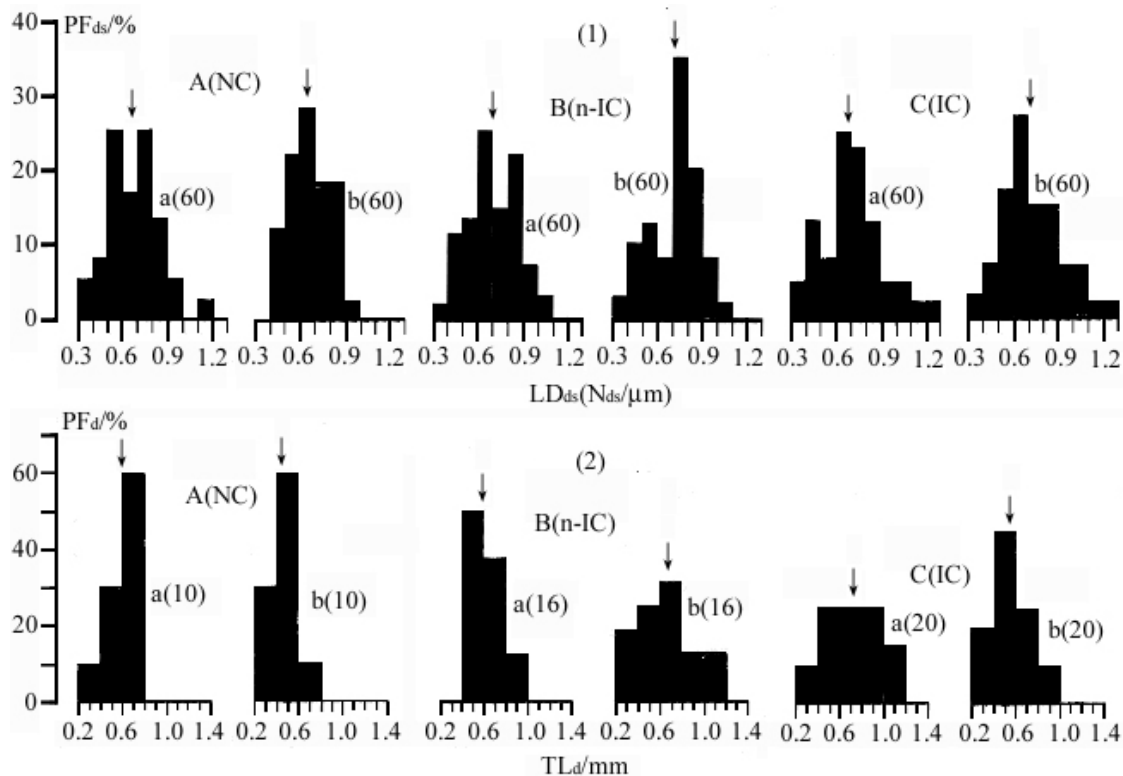


Figure 4. Distribution of the LDds and TLd frequency percentage of the neurons in the memory-related nuclei of 1-day-old chicks in the experimental group (1 and 2). a, IMHV; b, LPO. The arrowheads show the mean value, and the numerals in brackets show the number of samples.

microscope. Similar to the control group, the images are representative of the majority of visible dendritic spines. Moreover, some spines were in the developing stage (14 to 16, 21 and 22). Of the 360 SLds examined from the experimental group, the shortest was 17.7 μm , and the longest was 120.5 μm ; the majority was found to be between 30 to 70 μm , which accounted for 81.4% (293/360). In Figure 3A-1-4, the lengths of the SLd were 32, 38, 42 and 35 μm , respectively, and the Nds in the segments were 23, 24, 28 and 22 (Figure 3D-2). Therefore, the LDds were 0.72, 0.63, 0.67 and 0.63 μm , respectively. Similarly, in Figure 3B-1-4, the SLd in a-d were 25, 52, 39 and 33 μm , respectively, the Nds were 18, 35, 26 and 23, and the LDds were 0.72, 0.67, 0.67 and 0.70 μm , respectively. In Figure 3C-1-4, the SLds in a-d were 39, 33, 30 and 34 μm , respectively; the Nds were 26, 24, 20 and 24, and the LDds were 0.67, 0.73, 0.67 and 0.71 μm , respectively. Compared to the NC, the LDds was not significantly different in the central memory-related nuclei from the 1-day-old chicks that had been treated with MeA (n-IC and IC).

The PFds and PFd of the neurons from the left and right side of the IMHV and the LPO of the NC, n-IC and IC in the experimental group are displayed in Figure 4(1) and (2).

In Figure 4, the main distribution of the neuronal LDds

in the left and right side of the IMHV and the LPO in the NC of the experimental group was 0.5 to 0.9 μm (accounting for 80.0%) and 0.4 to 0.9 μm (accounting for 98.3%), and the mean values were $0.67 \pm 0.16 \mu\text{m}$ (60) and $0.66 \pm 0.12 \mu\text{m}$ (60), respectively. There was no significant difference between the two nuclei, which was similar to the NC in the control group.

Similarly, the main distribution of the neuronal LDds in the left and right sides of the IMHV and the LPO in n-IC was 0.40.9 μm , which accounted for 86.7%, and the mean values were $0.70 \pm 0.17 \mu\text{m}$ (60) and $0.72 \pm 0.16 \mu\text{m}$ (60), respectively. In addition, there was no significant difference between nuclei, and there was also no significant increase compared to the NC.

In addition, the main distribution of the neuronal LDds in the left and right side of the IMHV and the LPO in the IC were 0.4 to 0.9 μm (accounting for 83.3%) and 0.5 to 0.9 μm (accounting for 73.3%), and the mean values were $0.70 \pm 0.20 \mu\text{m}$ (60) and $0.72 \pm 0.20 \mu\text{m}$ (60), respectively. Further, there was no significant difference between nuclei. There were no significant different in the mean values between the NC and the n-IC.

In Figure 4(2)-A-a and b, we found that in the experimental group, the main distribution of the TLd of the neurons in the left and right side of the IMHV and the LPO in the NC ranged from 400 to 800 μm (accounting

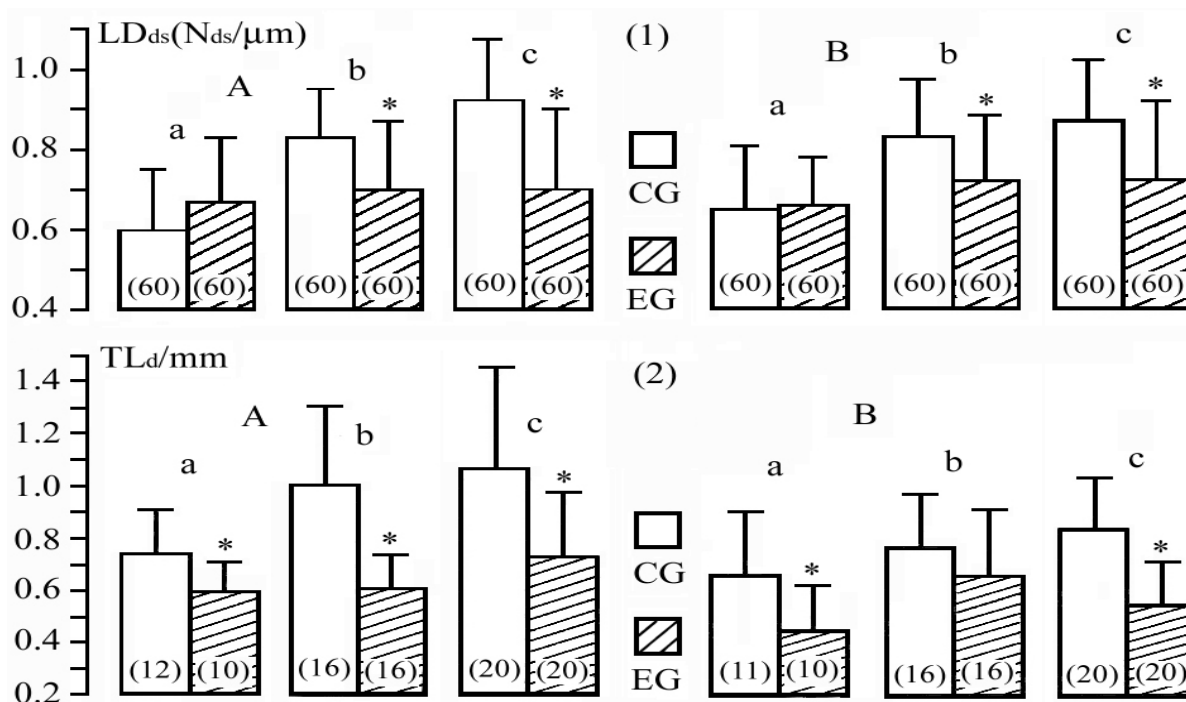


Figure 5. Comparison of the morphological features of the neurons in the memory-related nuclei between the experimental and control groups. (1), LDds, (2), TLd; A and B indicate the left and right side of the IMHV and LPO. a, b and c, NC, n-IC, and IC. The numerals in brackets represent the number of samples. *Not significantly different ($P < 0.05$).

for 90%) and 200 to 600 μm (accounting for 90%), respectively, and the mean values were $603 \pm 110 \mu\text{m}$ (10) and $462 \pm 168 \mu\text{m}$ (10), respectively. The average TLd in the IMHV was increased significantly by 30.5% ($t = 2.222 > t 0.05$, $df = 18$) compared to the LPO, but the neurons with TLd over 1000 μm were not found.

Similarly, as shown in Figure 4(2)-B-a and b, we demonstrated that the main distribution of the TLd of the neurons in the left and right side of the IMHV and the LPO in the n-IC of the experimental group was 400 to 800 μm (accounting for 85.7%) and 200 to 600 μm (accounting for 75%), respectively, and the mean values were $607 \pm 132 \mu\text{m}$ (16) and $670 \pm 253 \mu\text{m}$ (16). Further, there were no significant differences between the TLd in the IMHV and the LPO. Moreover, the TLd in the IMHV in the n-IC was not significantly different than the NC, whereas the average TLd in the LPO was significantly increased by 45.0% ($t = 2.295 > t 0.05$, $df = 24$) as compared to the NC, and the neurons with TLd over 1000 μm accounted for 12.5%.

In Figure 4(2)-C-a and b, the main distribution of the TLd of the neurons in the left and right side of the IMHV and the LPO in the IC was 400 to 1000 μm (accounting for 75%) and 200 to 800 μm (accounting for 90%), respectively, and the mean values were $734 \pm 253 \mu\text{m}$ (20) and $560 \pm 175 \mu\text{m}$ (20). In the IMHV in the NC, the neurons with TLd over 800 μm were not found, but 40% were found in the IC. The mean value of the TLd in the IC

increased by 21.7% compared to the NC. The number of neurons with TLd over 800 μm were two times more than in the n-IC and were significantly increased by approximately 20.9% ($t = 1.816 > t 0.07$, $df = 34$).

Comparison between experimental and control groups

The comparison between the LDds and TLd of the neurons found in the memory-related nuclei in the NC, n-IC and IC in the experimental and control groups are demonstrated in Figure 5.

In Figure 5(1)-A and B, the LDds of the neurons in the IMHV and the LPO in the NC were not significantly different between groups; but both the n-IC and IC were decreased significantly by $17.4 \pm 4.4\%$ (4).

In contrast, in the control group, the deviating rates of coefficients ($C = \text{SD}/\text{mean value} \times 100\%$) of the LDds of the neurons in the IMHV and the LPO in the NC were 25.0 and 24.6%, 14.5 and 16.9% in the n-IC, and 16.3 and 17.2% in the IC, respectively. The deviation coefficients of the LDds in the memory-related nuclei were $24.8 \pm 0.3\%$ (2) and $16.2 \pm 1.2\%$ (4) in the n-IC and IC as the control of the NC, respectively. These data showed that the deviating coefficients of the MeA-stimulated chicks were significantly decreased by 34.7% ($t = 9.248 > t 0.05$, $df = 4$). In the experimental group, the

deviating coefficients of the LDds in the IMHV and the LPO in the NC, n-IC, and IC were 23.9 and 18.2, 24.3 and 22.2%, and 28.5 and 27.8%, respectively. The deviating coefficients of the LDds in the NC were $21.1 \pm 4.0\%$ (2) and were $25.7 \pm 3.0\%$ (4) in the IC. There was no significant difference between the groups. Further, compared to the control group, the deviating coefficients of the LDds in the MeA-stimulated chicks in the experimental group were increased significantly by 53.0% ($t = 5.510 > t_{0.05, df = 6}$).

In Figure 5(2)-A and B, compared to the control group, the TLd of the neurons in the LPO in the n-IC was not significantly different, but all other TLds from each side of the IMHV in the NC, n-IC and IC and the LPO in the NC and IC were significantly decreased by $30.9 \pm 7.9\%$ (5).

DISCUSSION

In this study, we showed that the majority of the 1-day-old chicks that were hatched from a natural geomagnetic field (control group) were able to form long-term memories in a one-trial avoidance task using MeA (Wang et al., 2003). After 12 h of being stimulated with MeA, both the LDds and TLd of each neuron from the two sides tested from the IMHV and the LPO in the n-IC and IC were significantly increased as compared to the NC, and the increase in both left and right sides was similar, which was consistent with previous studies¹⁴. These data indicate that by stimulating the neurons in the memory-related nuclei with MeA, the dendrites and spines would undergo hyperplasia, which would allow more synaptic connections to be modulated. These changes would constitute the structural foundation of memory function in the 1-day-old chicks (Stewart and Rusakov, 1995; Barber et al., 1999). Moreover, the deviating coefficients of the LDds in the stimulated chicks were significantly decreased by 35.1% compared to the NC, and this indicates that the dendritic spines that were stimulated by MeA showed reduced “synchronous” hyperplasia. Our results are consistent with a previous study that observed the same effect in the density of dendritic spines from neurons in the memory-related nuclei isolated from 7-day-old chicks subjected to auditory stimulation (Braun et al., 1999). For the few chicks that did not show long-term memory (n-IC), the LDds of the neurons from the two sides of the IMHV were significantly decreased by 10.5% compared to the IC, and the number of neurons with TLd higher than 1000 μm was also decreased 1.5 fold. This indicates that although adding MeA may accelerate the rapid hyperplasia that is found in the dendrites and spines of neurons in the memory-related nuclei, the nerve network was insufficient to form long-term memory in some subjects, and these effects may be related to other changes in the synaptic structure or in the deficiency to synthesize substances that are important for creating memories (Scholey et al., 1992; Rose, 1995; Soderling

and Derkach, 2000; Nguyen, 2002). Rose (1991) and Tiunova et al. (1998) have suggested that the memory flow would be L-IMHV \rightarrow R-IMHV \rightarrow LPO. Therefore, the long-term memory would be formed first in the IMHV and then be transferred to the LPO to be stored. Our results illustrated that the LDds and TLd of the neurons from both sides of the IMHV were increased significantly compared to the LPO, which indicates that after a 12 h treatment with MeA, the long-term memory has not yet been stored in the LPO. In addition, the formation of the long-term memory that was stimulated by MeA begins by 60 min and can be detected as late as 24 h (Gao and Kuang, 1996). Further, the morphological changes in the neurons are still observed after 48 h (Rose and Stewart, 1999).

When we examined 1-day-old chicks that hatched from the HMFS (experimental group), we found that the chicks retained short- and medium-term memory in the one-trial avoidance task, but long-term memory was impaired (Wang et al., 2003). In the experimental group, the LDds of the neurons from both the left and right sides of the IMHV and the LPO in the NC, n-IC and IC were similar to that of the NC of the control group, whereas the NC of the control group was decreased significantly by 17.4% compared to the n-IC and IC of the control group. With the exception of two sides in the LPO, all of the TLds (IMHV and LPO in n-IC and IC, and LPO in NC) were decreased significantly by 30.9%. Further, in the experimental group, the deviating coefficients of the LDds in the chicks stimulated with MeA did not show any significant difference compared to the NC and were significantly increased by 53.0% compared to the stimulated chicks in the control group. These results indicate that although the deprivation of geomagnetism does not effect the normal development of the dendritic spines of the neurons from the memory-related nuclei, the hyperplasia caused by MeA was inhibited, and the “synchronous” hyperplasia was lost. In addition, the development and growth of the neuronal length were significantly inhibited. Therefore, the neural network involved in long-term memory was not able to be established, and this may be the cause of the impairment to the formation of long-term memory. Additionally, the impairment may be related to other factors, such as changes in the synaptic structure or in the deficiency of molecules involved in memory (Ünal et al., 2002; Soderling and Derkach, 2000; Nguyen, 2002). It is well-known that the NMDAR and the AMPAR in the IMHV and the LPO in 1-day-old chicks play important roles in forming and retaining new memories. The NMDAR is a voltage- and transmitting-gated diploid channel and can increase the intracellular Ca^{2+} level by regulating glutamate levels (Nguyen, 2002). It has been well established that Ca^{2+} is important in long-term potentiation (LTP) induction, and it is difficult to induce LTP in the absence of Ca^{2+} . In contrast, abundant Ca^{2+} can directly induce LTP and reduce the threshold for inducing LTP,

and LTP is the basis of learning and memory (Xu, 2000). In addition, in γ -GABAergic synapses, moderately increasing Ca^{2+} leads to the growth of dendritic spines, but an excessive increase in the Ca^{2+} levels causes the spines to form and collapse rapidly (Segal et al., 2000). The GABA-A and GABA-B receptors have a secondary role in the induction of LTP and are important in storing memories (Weng et al., 1996). Hence, the impairment of memory formation in the 1-day-old chicks that were hatched from the HMFS may not only inhibit hyperplasia of neuronal spines following stimulation with the bitter compound, MeA, but may also cause changes in membrane structure and disrupt the normal function of channels, which would result in the inhibition of LTP. This would predict that the loss of normal geomagnetism during the development of chicken embryos may affect the normal development of membrane structure and channels of the neurons in the IMHV and the LPO. The impairment of long-term memory in 1-day-old chicks using the one-trial avoidance task may be recovered. In *Drosophila* that were housed continuously in the HMFS, the ability to learn and remember was seriously impaired, but by removing the flies from the HMFS and housing them in conditions of natural geomagnetism for six generations, the ability to form long-term memories was restored (Zhang et al., 2004). In addition, the memory of chicks that hatched from the HMFS did not show any deficiency in long-term memory after being bred in normal space.

Some of the chicks that were hatched from the HMFS displayed long-term memory by exhibiting the avoidance reaction, but the density of dendritic spines in the neurons from the memory-related nuclei was similar to the chicks not showing an avoidance reaction after being stimulated with MeA. In addition, the TLd in the IMHV was increased. The lack of difference between the 2 groups may be explained by the length of the observation time because previous studies have shown that the number of dendritic spines was decreased gradually from the original peak after being trained (Stewart and Rusakov, 1995). In rats, the density of spines increased at 3 h after being trained, reached the maximum density at 6 h, and then decreased to basal levels (Ünal et al., 2002). Therefore, it is important to measure the density of dendritic spines at different time points after training. In contrast, long-term memory may not be dependent on the changes in the synaptic structure, and it is possible that some other neural-chemistry processes, such as the synthesis of α -tubulin and glycoprotein, compensated for the loss of function so that the long-term memory could be formed.

In rats, the LDd of pyramid cells in the cortex was $0.60 \pm 0.21 \mu\text{m}$ (in 42 dendrite segments, $50 \mu\text{m}$ per segment), and the deviation coefficients were 35.0% (Peter and Kaiserman-Abramof, 1970). In the control group of another study that examined 7-day-old chicks, the LDds of large neurons from the dorsocaudal neostriatum were

0.47, 0.86 and 0.97 to $1.00 \mu\text{m}$, and the deviation coefficients were 21.3, 14.0 and 10.2 to 13.0% ($n = 5$). In the experimental group that was imprinted by stimulating with sound, the LDds were 0.19, 0.54 and 0.69 to $0.76 \mu\text{m}$, and the deviation coefficients were 21.5, 13.0 and 2.7 to 5.8% (Braun et al., 1999). From these data, we can conclude that our results for the LDds are reliable. By depriving chicks of the geomagnetic field space that is normally used by all animals throughout evolution, the magnetism balance in their bodies may be eliminated, thereby affecting the motion and function of electrical particles. Previous data had indicated that the manual weak magnetic field could influence the conformation, structure and holistic behavior of the central nervous system (Li, 1994). A prolonged exposure to the HMFS could not only inhibit the growth and development of many different types of plants (Dubrov, 1978), arouse gene mutation related to the albinism of hairs and significantly reduce the content of some neurotransmitters (Li et al., 1999, 2001), but could also lead to a significant alteration in the formation of chromosomes in human lymphocytes and fibroblasts (Belyaev et al., 1997). In this vein, deprivation of geomagnetism during the development of chicken embryos could negatively affect brain function. Therefore, our study may not only offer some evidence for the effect of geomagnetic deprivation on the activity of humans in outer space (ether biology) but may also suggest a possible relationship between geomagnetism and the evolution of species.

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