Acta Med. Okayama, 2004
Vol. 58, No. 3, pp. 143-149

Acta Medica Okayama
http://www.lib.okayama-u.ac.jp/www/acta/

Original Article

Exposure of Mouse to High Gravitation Forces Induces Long-Term Potentiation in the Hippocampus

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The central nervous system is highly plastic and has been shown to undergo both transient and chronic adaptive changes in response to environmental influences. The purpose of this study was to investigate the effect of hypergravic field on long-term potentiation (LTP) in the mouse hippocampus. Exposure of mice to 4G fields for 48 h had no effect on input-output coupling during extracellular stimulation of Schaffer collaterals and paired pulse facilitation, suggesting that the hypergravic exposure had no detrimental effect on basal neurotransmission in the hippocampus. However, the exposure to 4G fields for 48 h significantly induced LTP compared with the control mouse hippocampus. In contrast, no significant changes of late-phase LTP (L-LTP) were found in the hippocampi of mice exposed to the hypergravic field. Exposure of mice to 4G fields for 48 h enhanced AMPA receptor phosphorylation but not cyclic AMP-responsive element binding protein (CREB) phosphorylation. These results suggest that exposure to hyperdynamic fields influences the synaptic plasticity in the hippocampus.

Key words: long-term potentiation (LTP), AMPA receptor, cyclic AMP-responsive element binding protein (CREB), plasticity, synapse

Postnatal environmental conditions such as stress or an enriched environment cause altered gene expression, morphological changes and altered synaptic plasticity in the brain [1-5]. The hippocampus is one of the brain regions most strongly affected by the environment, and the environmental conditions under which rats are reared affect hippocampal-dependent forms of learning and memory [6, 7]. For instance, exposure of rodents to stress inhibits the induction of long-term potentiation (LTP) in the hippocampus and suppresses spatial learning [8]. Exposure to an enriched environment enhances performance on behavioral paradigms of learning and memory and visual acuity [9, 10]. Moreover, the experience of pregnancy, giving birth and lactation improves learning and memory in rodents [11, 12].

The gravitational force environment is also thought to affect morphological changes and functions in the brain. Circadian rhythms, regulated by the suprachiasmatic nucleus (SCN) of the hypothalamus, are initially disrupted under a hyperdynamic field [13]. In the hippocampus, changes in the gravitational environment can cause alterations in the number of receptors for serotonin [14]. In addition, chronic exposure to hypergravity increases thyrotropin-releasing hormone (TRH) levels in the rat brainstem and cerebellum [15].

Given the evidence that an altered gravitational force affects central nervous system functioning, we have hypothesized that changes in the dynamic gravitational field may influence synaptic plasticity in the hippocampus, which appears to be a critical structure in learning and

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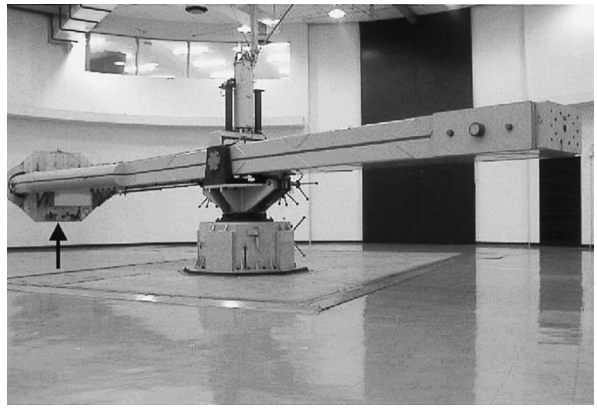
memory [16]. LTP is a form of synaptic plasticity that has been extensively studied as a putative mechanism underlying learning and memory [16]. Like memory, LTP has an early phase that is independent of protein and RNA synthesis and a late phase that is reduced by inhibitors of those processes [17, 18]. Early-phase LTP (LTP) can be induced by brief tetanization, such as one train of 100 Hz stimulation for 1 sec, and lasts ~1 h. By contrast, late-phase LTP (L-LTP) is usually induced by 3 or 4 trains of tetanization with 5–10 min between trains and lasts > 3 h [19].

Previous studies have shown that different signaling pathways are involved in the induction of LTP and L-LTP. In the CA1 region of the hippocampus, L-LTP is mediated by cyclic AMP-responsive element binding protein (CREB) phosphorylation, and cAMP-dependent protein kinase (PKA) and MAP kinase are involved in the phosphorylation [12, 18, 19]. In contrast, LTP is usually mediated by the phosphorylation of AMPA (α-amino-3-hydroxy-5-methyl- isoxazole-4- propionic acid) receptor GluR1 subunit by calcium/calmodulin-dependent protein kinase II (CaMKII) [20].

In the present study, we investigated the effect of hyperdynamic fields on the induction of LTP and L-LTP in the hippocampus of the mouse. Our results indicate that hyperdynamic fields induce LTP in the hippocampus through AMPA receptor phosphorylation and suggest that alterations of the gravitational environment may cause changes of the neuronal plasticity in the hippocampus.



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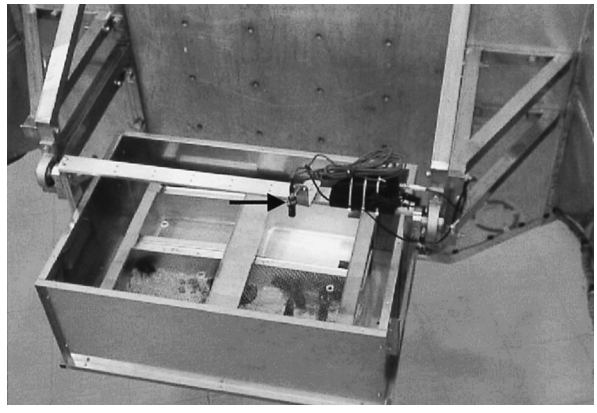

B

Fig. 1 Centrifugal acceleration test facility in NASDA. A, Whole view of the centrifuge. The mice were housed in standard mouse cages, and the cages were placed on a mounted table (arrow) at one end of the arm of the centrifuge. B, Mice on the centrifuge. The activity of mice was monitored using a CCD camera (arrow) all day.

Materials and Methods

Animals. Male C67BL6 mice (8–9 weeks old) were used for all experiments. The mice were housed on a 12-h light/dark schedule and were killed in accordance with the guidelines of the Health Sciences Division of Okayama University Graduate School of Medicine and Dentistry.

Hyperdynamic field. The mice were housed in standard mouse cages and given food and water ad libitum. The cages were placed on a mounted table at one end of the arm of a 13-meter foot diameter centrifuge (National Space Development Agency of Japan (NASDA), Fig. 1). The activity of the mice and the condition of their health were monitored using a CCD camera all day during centrifugation (Fig. 1). Four groups of mice were exposed to 2G or 4G for 24 h or 48 h. The mice were housed on a 12-h light/dark schedule at an ambient temperature of 25 ± 2 °C. As a control, mice that did not undergo centrifugation were housed in the same room as the mice being centrifuged.

Electrophysiological recording. Field excitatory postsynaptic potential (fEPSP) recording was performed as described previously [21]. Briefly, the mice were housed in a 1G field for 24 h after the centrifugation and the brain was then removed. The hippocampus was dissected, and 400-μm transverse slices were prepared. The hippocampal slices were incubated in an interface-recording chamber maintained at 28 °C for at least 1.5 h before recording and were constantly perfused with gas-saturated artificial cerebrospinal fluid (ACSF) at 1.2 ml/min. The composition of the

ACSF was as follows: NaCl, 124 mM; KCl, 4.4 mM; $CaCl_2$, 2.5 mM; $MgSO_4$, 1.3 mM; NaH_2PO_4, 1 mM; $NaHCO_3$, 26 mM; glucose, 10 mM.

To record the field EPSPs, a glass micropipette filled with ACSF (1–5 MΩ resistance) was placed in the stratum radiatum of the CA1 region, and a bipolar stimulating electrode was placed along the Schaffer collateral fibers. The intensity of the stimulation was adjusted to produce an EPSP with a slope between 35% and 50 % of the maximum. The test stimulation was delivered once per min (0.017 Hz).

Western blotting analysis. For Western blotting analysis, the mice were housed in a 1G field for 24 h after the centrifugation, and the hippocampus was then removed. The hippocampus was homogenized in homogenize buffer containing 1% SDS. Western blotting analysis was carried out as described previously [22]. Blots were probed with primary antibodies against phospho-CREB (1:200 dilution, anti-phospho-CREB, Upstate, Charlottesville, VA, USA), total-CREB (1:500 dilution, anti-CREB, Upstate), phospho-GluR1 (1:200 dilution, anti-phospho-GluR1(S831), Upstate), and total-GluR1 (1:1000 dilution, anti-GluR1, Upstate). HRP-conjugated secondary antibodies were used to visualize the bands using a commercial ECL detection kit (Amersham Biosciences).

Statistical analysis. Data are shown as the mean (± S.D.). Data were analyzed using either Student's *t*-test to compare 2 conditions or ANOVA followed by planned comparisons of multiple conditions, and $P < 0.05$ was considered to be significant.

Results

To clarify whether exposure of mice to a hyperdynamic field affects the basal synaptic function, we examined the input-output coupling during extracellular stimulation of Schaffer collaterals in the hippocampal slices of mice exposed to 1G or 4G for 48 h. No significant changes were found in the fEPSP slope responses to the presynaptic stimulation in slices in 4G exposed mice compared with those in 1G-exposed mice (Fig. 2A). We also examined the effect of a 4G hyperdynamic field on paired-pulse facilitation (PPF) in the hippocampus was also examined. PPF is a transient form of presynaptic plasticity in which the second of 2 closely-spaced stimuli elicits enhanced transmitter release owing to residual calcium in the presynaptic terminals after the first stimulus [23]. PPF was not altered in the slices of 4G-exposed mice relative to control mice (Fig. 2B; 1G, 25 msec interval; 1.42 ± 0.05, 50 msec; 1.54 ± 0.1, 75 msec; 1.41 ± 0.06, 100 msec; 1.28 ± 0.03, n = 5; 4G, 25 msec; 1.38 ± 0.06, 50 msec; 1.5 ± 0.07, 75 msec; 1.43 ± 0.02, 100 msec; 1.25 ± 0.04, n = 6). These results suggest that exposure to a 4G hyperdynamic field does not affect basal neurotransmission presynaptically or postsynaptically.

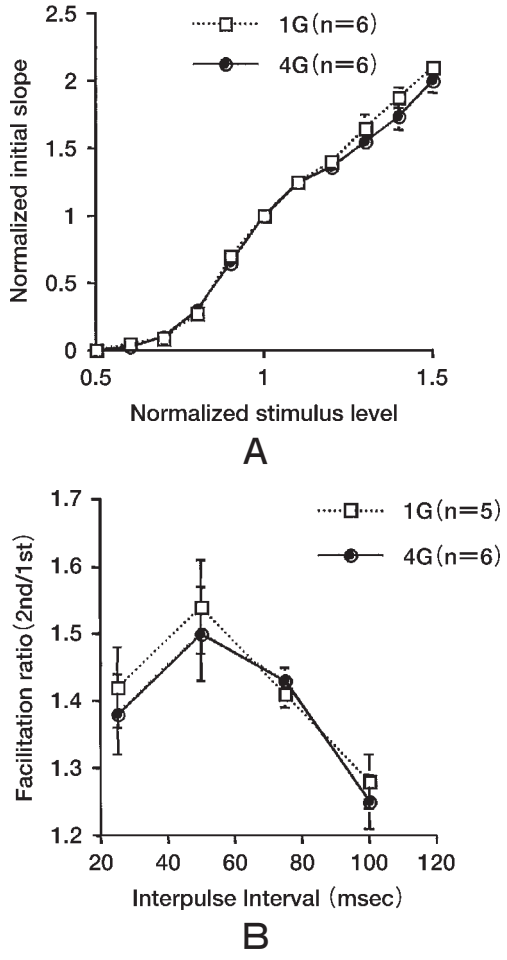

Fig. 2 The effect of a hypergravity field on basal neurotransmission in the hippocampus. **A**, Comparison of baseline i/o relationships between hippocampal slices of mice to 1G versus 4G for 48 h. No differences in mean initial slope were significant at any stimulus level examined (all, $P > 0.05$). **B**, The effect of 4G hypergravity for 48 h on paired-pulse facilitation (PPF). PPF was induced by two pulses of stimulation separated by intervals of 25, 50, 75 and 100 msec. Data show the ratios of the second EPSP slopes to the first EPSP slopes. Exposure to 4G did not affect PPF.

We next examined the effect of a hyperdynamic field on LTP in the hippocampus. The mice were exposed to 2G or 4G hypergravity for 24 h or 48 h. The hippocampus was then removed after the respective centrifugation, and the slices were subjected to electrophysiological measurements. Exposure to 4G for 48 h significantly induced LTP expression. The fEPSP slope 1 h after tetanus stimulation (100 Hz, 1s) in the mice exposed to 4G for 48 h was greater than that in control mice (Fig. 3A and B, 1G, 138 ± 7.1, $n = 6$; 4G, 170 ± 5.0, $n = 6$, $P < 0.01$), whereas exposure to 4G for 24 h did not significantly induce LTP (Fig. 3B, 4G, 150 ± 11.5, $n = 6$, $P > 0.05$ *v.s.* 1G). Moreover, exposure to 2G for 24 h or 48 h had no effect on the induction of LTP compared with the LTP in control mice (Fig. 3B, 2G for 24 h, 133 ± 11.6, $n = 6$; 2G for 48 h, 137 ± 6.8, $n = 6$).

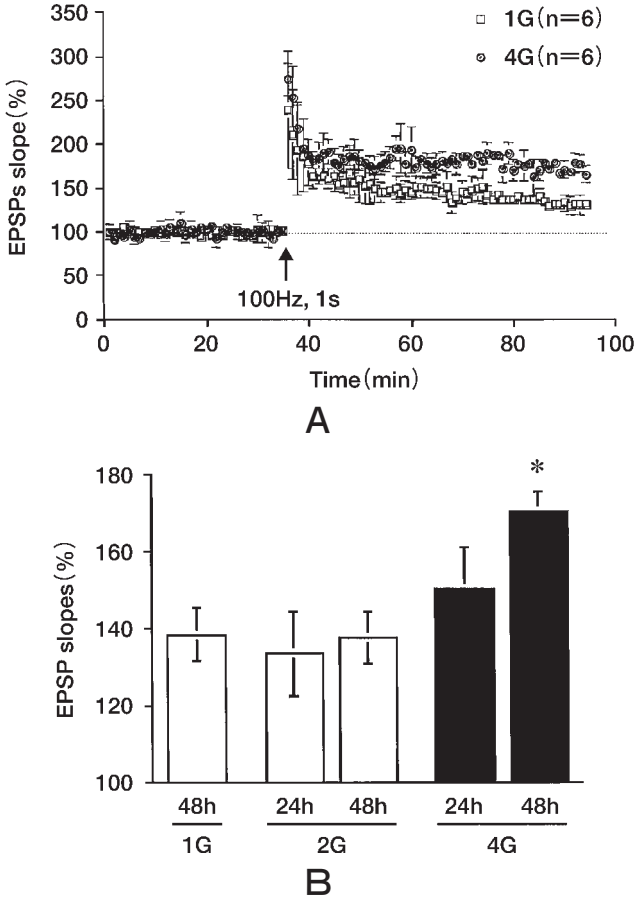

Fig. 3 The effect of a hypergravity field on LTP induction in the mouse hippocampus. **A**, Induction of LTP by exposure to 4G. The hippocampal slices of mice exposed to 4G or 1G for 48 h were stimulated by 1 train of 100 Hz for 1 sec to induce LTP after recording stable EPSP slopes (arrow). **B**, Comparison of EPSP slopes 1 h after tetanus stimulation among slices exposed to 2G or 4G for 24 h or 48 h and control slices. Exposure to 4G for 48 h with one train of tetanus significantly induced LTP. Data are given as mean (± S.D.). $*P < 0.01$.

Next, we determined the effect of hypergravity on L-LTP in the hippocampus (Fig. 4). The hippocampal slices in mice exposed to 4G for 48 h were stimulated by 3 trains of tetanization at 5-min intervals. We examined the enhancement of fEPSP in the slices 3 h after the tetanus stimulation and observed no difference in L-LTP induction in the hippocampal slices between 1G-exposed mice (165 ± 3, $n = 6$) and 4G-exposed mice (162 ± 7.6, $n = 6$).

To elucidate the mechanism of the hyperdynamic field-dependent improvement of LTP, we examined the phosphorylations of AMPA receptor GluR1 subunit and CREB in the hippocampi of mice exposed to 4G for 48 h using the respective phospho-specific antibodies. Exposure to 2G hypergravity for 24 h or 48 h had no effect on the phosphorylation of AMPA receptor compared with that in control mice. In contrast, exposure to 4G for 24 h slightly increased the phosphorylation, and a marked increase of phosphorylation was observed in the hippocampi of mice exposed to 4G for 48 h (Fig. 5). In contrast, exposure to 2G or 4G hypergravity did not affect the expression of phospho-CREB in the hippocampus (Fig. 5).

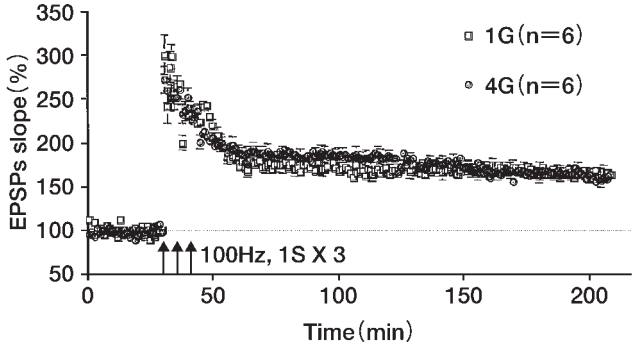

Fig. 4 The effect of exposure to 4G hypergravity for 48 h on the L-LTP in the hippocampus. The hippocampal slices of mice exposed to 4G for 48 h were stimulated by three trains of tetanus (100 Hz, 1s) at 5-min intervals, and EPSP slopes were recorded for 3 h after the tetanus stimulation. No influence was observed in the hippocampal slices of mice exposed to 4G for 48 h ($P > 0.05$ *vs.* 1G control slices).

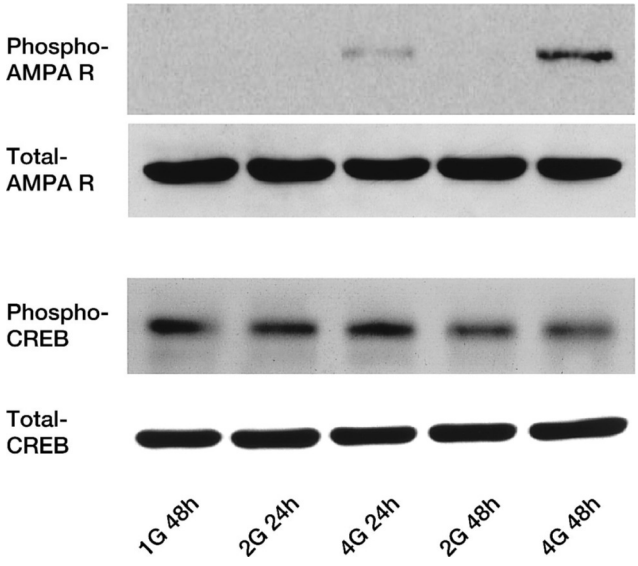

Fig. 5 Comparison of the expressions of phospho-AMPA receptor and phospho-CREB in the hippocampi of mice exposed to various conditions of hypergravity. The mice were housed in a 1G field for 24 h after centrifugation, and the hippocampus was then removed. The hippocampus was homogenized in homogenized buffer containing 1% SDS. Western blotting analysis was performed using anti-phospho-AMPA receptor GluR1 subunit (serine 831) antibody, total AMPA receptor GluR1 subunit antibody, anti-phospho-CREB antibody or total CREB antibody.

Discussion

The present study has revealed the following 3 important findings. First, exposure of mice to high gravitation forces induced long-term potentiation (LTP) in the hippocampus. Second, exposure of mice to 4G hypergravity increased the phosphorylation of the AMPA receptor GluR1 subunit in the hippocampus. Third, a hypergravity field had no effect on basal neurotransmission or late-phase LTP (L-LTP).

The environmental conditions under which mice are reared are known to affect learning and memory performance [1]. Environmental enrichment or restriction can affect hippocampal-dependent behaviors such as spatial memory [5]. The influence of stress and stress-related mediators on learning and memory is also well known [3]. Hippocampal-dependent functions are sensitive to stress and adrenal hormones, possibly due to the pronounced concentration of glucocorticoid receptors in the hippocampus [24, 25]. In the present study, we showed that a hypergravity environment induced LTP in the hippocampus. LTP is a form of synaptic plasticity that has been extensively studied as a putative mechanism underlying learning and memory [16]. Although we did not examine the effect of the hypergravity field on spatial memory in the present study, hypergravity may induce spatial memory in mice.

Alterations of the nervous system in response to changes in gravitational fields have been examined in relatively few areas of the nervous system. The best-studied systems are the sensory and motor systems, which are the systems most directly influenced by gravity. When rodents are exposed to 2G fields, motor neurons in the spinal cord show increases in volume and metabolic activity and peri-neuronal glial cells in number, while these same characteristics are reduced in microgravity [26, 27]. Similarly, afferent proprioceptive and vestibular systems have been shown to respond directly to gravitational forces. For instance, hypergravity alters the expression of glutamate receptors in the rat peripheral and central vestibular systems [28]. In the present study, we showed that exposure to hypergravity changed the synaptic plasticity in the mouse hippocampus, which is not part of the sensory or motor systems. The expression of mechanosensor, which senses gravity, has not been reported in the hippocampus. Therefore, in contrast to the sensory and motor systems, which demonstrate specific adaptive responses to an altered gravitational environment, the neurons in the hippocampus may be affected indirectly.

Hormones have been implicated as factors mediating some of the neurological and behavioural consequences of raising animals in different environments [2]. In response to environmental changes, the hypothalamic paraventricular nucleus (PVN) of the hypothalamus is known to function as a key site in the activation of the hypothalamo-pituitary-adrenal (HPA) axis by providing the hypophysiotropic neuropeptides corticotropin-releasing hormone (CRH) and arginine vasopressin (AVP) to stimulate adrenocorticotropin (ACTH) secretion from the anterior pituitary gland [29]. Environmental enrichment decreases the chronic stress response, ultimately attenuating the release of the potentially neurotoxic glucocorticoid corticosterone [30], and the hormone suppresses LTP in the hippocampus [31]. Moreover, oxytocin is also synthesized in the PVN, and its expression and secretion increase during motherhood [32]. The hormone improves long-lasting spatial memory and L-LTP in the motherhood hippocampus [12].

These results suggest that a hypergravity field may alter the expression and secretion of some hormones, resulting in the induction of synaptic plasticity in conditions such as LTP in the hippocampus.

In the present study, we demonstrated the intracellular signal cascade of hypergravity-induced LTP. Exposure to hypergravity caused an increase of the phosphorylation of the AMPA receptor GluR1 subunit but not CREB phosphorylation. It is thought that CaMKII is involved in the phosphorylation of the GluR1 subunit, and that Ca^{2+} entry from the NMDA receptor activates the kinase activity. These results suggest that exposure to hypergravity may induce the function of NMDA receptor.

In conclusion, the exposure of mice to hypergravity induced LTP and AMPA receptor phosphorylation. These results suggest that changes in the gravity field influence the synaptic plasticity in the hippocampus.

Acknowledgements. This study was supported by a grant from the Ground-based Research Announcement for Space Utilization, promoted by the Japan Space Forum.

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