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# c-fos mRNA Expression in the Vestibular System following Hypergravity Stimulation in Rats

**Guang Shi Jin[1], Jae Hyo Lee[1], Jae Hee Lee[1], Moon Young Lee[1], Min Sun Kim[1], Yuan Zhe Jin[2], Jeong Hoon Song[3], and Byung Rim Park[1]**

*[1]Department of Physiology, Wonkwang University School of Medicine and Vestibulocochlear Research Center at Wonkwang University, Iks. 570-749, Korea, [2]Department of Physiology, Yanbien University College of Medicine, Jilin 133000, China, [3]Department of Plastic and Reconstructive Surgery, Wonkwang University School of Medicine, Iksan 570-749, Korea*

**Altered environmental gravity, including both hypo- and hypergravity, may result in space adaptation syndrome. To explore the characteristics of this adaptive plasticity, the expression of immediate early gene c-fos mRNA in the vestibular related tissues following an exposure to hypergravity stimulus was determined in rats. The animals were subjected to a force of 2 g (twice earth's gravity) for 1, 3, or 12 h, and were examined poststimulus at 0, 2, 6, 12, and 24 h. RT-PCR (reverse transcription polymerase chain reaction) and real-time quantitative RT-PCR were adopted to analyze temporal changes in the expression of c-fos mRNA. The hypergravity stimulus increased the expression of c-fos mRNA in the vestibular ganglion, medial vestibular nucleus, inferior vestibular nucleus, hippocampus, cerebellum, and cortex. The peak expression occurred at 0 h poststimulation in animals stimulated with hypergravity for 1 h, and at 6 h poststimulus in those stimulated for 3 h. In contrast, those stimulated for 12 h exhibited dual peaks at 0 and 12 h poststimulus. Bilateral labyrinthectomy markedly attenuated the degree of c-fos mRNA expression. Glutamate receptor antagonist also dramatically attenuated the degree of c-fos mRNA expression. These results indicate that expression of c-fos mRNA in response to hypergravity occurs in the vestibular related tissues of the central nervous system, in which peripheral vestibular receptors and glutamate receptors play an important role. The temporal pattern of c-fos mRNA expression depended on the duration of the hypergravity stimulus.**

**Key Words: Vestibular system, Hypergravity, c-fos, mRNA, Rat**

## INTRODUCTION

The vestibular, visual, and proprioceptive systems are the principal sensory systems responsible for the normal behavior of a subject with respect to spatial orientation as well as motor and autonomic regulation. The otolith organ in the vestibular system which detects gravity stimulation produces various physiological responses when a subject travels to microgravity or back to ground from the microgravity (Fujii & Patten, 1992; Correia, 1998). Weightlessness causes alterations in the pattern of inputs from the otolith organs and is accompanied by a loss of signals from visceral organs, muscle spindles, and skin of the lower body (Ross, 1994). However, the output from the semicircular canals as well as from the visual system does not change under weightlessness while the output from the otolith organs decreases. These contradictions in sensory output are responsible for the sensory conflict in the CNS during weightlessness and result in symptoms of motion sickness and spatial disorientation, which in turn decrease movement control (Thornton et al, 1987). After a few days, the CNS adapts and these symptoms disappear. Adaptation to the effect of linear as well as angular acceleration can occur, leading to disappearance of symptoms. A motion sickness response can appear again when the adapted organism returns to the normal motion environment (Dobie & May, 1994). In most astronauts, readaptation to the terrestrial environment begins immediately upon landing, proceeding rapidly for the first 10~12 h, and then continuing much more slowly for the subsequent 2~4 days until preflight stability levels are achieved (Paloski et al, 1992). It is believed that CNS adaptation is based on synaptic plasticity (Grossman et al, 2002).

Experiments with human subjects under hypogravity or hypergravity conditions have the disadvantage of not being able to cover an extended period of time. Also, it is not possible to study the molecular biological changes of the vestibular system after exposure to altered gravity to assess structural changes. Recently, animals in a centrifuge have been used to study space physiology, since it is relatively easy to perform such experiments and the results can be extrapolated to space research and used to study vestibular functioning in general. Most hypergravity research has concentrated on its effect on the morphology and function of parts of the body, such as the cardiovascular and mus-

Corresponding to: Byung Rim Park, Department of Physiology, Wonkwang University School of Medicine, Iksan 570-749, Korea. (Tel) 82-63-850-6773, (Fax) 82-63-852-6108, (E-mail) byungp@wonkwang.ac.kr

cular systems (Wunder et al, 1987; Burkovskaya & Krasnov, 1991). However, the role of neural plasticity in the CNS adaptation to altered gravity is poorly understood.

In general, Fos protein is more effectively induced when a novel stimulus is applied, while prolonged or repeated exposure to the stimulus usually produces rapid habituation of the Fos response (Cullinan et al, 1995; Herdegen & Leah, 1998). The basal level of the fos family is generally low in the adult brain of various mammalian species. Fos expression kinetics show an initial fast increase in c-fos mRNA level within a few minutes, followed by Fos protein translation peaking 1~3 h later and progressively declining to the basal level within 4 to 8 h. Fos can be seen as a neural activity marker with a higher spatial resolution, and Fos up-regulation in the brain is also thought to reflect activation of neural pathways involved in sensorimotor adaptation to novel environments (Morgan & Curran, 1989). Therefore, Fos expression is widely used to investigate neural plasticity in altered gravity (Kaufman et al, 1992; Duflo et al, 2000; Nakagawa et al, 2003; Pompeiano et al, 2002, 2004).

Up until now, most hypergravity research has concentrated on its effect on the morphology and function of parts of the body and the vestibular system (Wunder et al, 1987; Burkovskaya & Krasnov, 1991). A few studies have investigated the effect of hypergravity on neural plasticity for adaptation (Kaufman et al, 1992; Duflo et al, 2000; Pompeiano et al, 2002; Nakagawa et al, 2003). When the subject moves to a higher gravity environment, changes in the nervous system occur because the otolith organ and non-labyrinthine receptors are stimulated continuously under higher gravity; *i.e.*, adaptation occurs. Returning to normal gravity also causes changes in the nervous system through decreased gravity stimulation of the otolith organ, and readaptation occurs. Prolonged hypergravity stimulation for 1 week results in reduced Fos expression in the vestibular nuclei, suggesting a process of habituation (Nakagawa et al, 2003). Hypergravity stimulation induces Fos expression in both bulbar nuclei and suprabulbar structures while Fos expression is limited to suprabulbar areas by hypogravity stimulation (Duflo et al, 2000). Neural plasticity in an altered gravity environment has been studied by assessing immediate early genes just after gravity stimulation (Pompeiano et al, 2002; d'Ascanio, 2003). These studies investigated short-term adaptation and readaptation to altered gravity. In the present study, we investigated the changes in c-fos mRNA expression after hypergravity stimulation with time in the vestibular ganglion, medial and inferior vestibular nuclei, hippocampus, cerebellum, and cortex of rats. We also evaluated the role of peripheral vestibular receptors and glutamate on expression of c-fos mRNA in an altered gravity environment.

## METHODS

### *Materials*

Vestibular function was assessed in 129 male Sprague-Dawley rats weighing 200~250 g by means of an elevated body swing test to select intact labyrinthine animals (Borlongan & Sanberg, 1995). Experimental animals were divided into three groups: a control group with intact labyrinth, a bilateral labyrinthectomy group, and a group treated with CNQX. All procedures were approved by the Institutional Ethical Committee on the Experimental Use of Animals.

### *Hypergravity stimulation*

Each animal was subjected to hypergravity under 2 g conditions. Animals were placed in individual cages (25×25×25 cm) without any restrain and exposed to hypergravity on an animal centrifuge device (WKU, Korea). The turntable was rotated at a constant rate of 56 rpm, and achieved a resultant linear acceleration of 2 g acting on the animal along the back-to-abdomen axis (Takeda et al, 1996). Animals were placed in the centrifuge cage for 30 min before rotation to adapt to the cage, and rotation was performed in darkness to avoid visual stimulation. Animals were rotated for three different durations of 1, 3, and 12 h.

### *Labyrinthectomy*

Surgical labyrinthectomy was performed as described previously (Park et al, 1995). After chloral hydrate (300 mg/kg, i.p.) anesthesia, a small opening was made around the oval window using a dental burr by a ventral approach under a surgical microscope. Through this opening, the membranous labyrinth was destroyed surgically with a small, right-angled hook and removed by aspiration. Unilateral labyrinthectomy was confirmed by the appearance of spontaneous nystagmus and postural asymmetry after recovery from anesthesia, and vestibuloocular and vestibulospinal abnormalities were abolished by secondary labyrinthectomy to the opposite labyrinth. Incised skin was sutured and antibiotics were injected for 3 days. Experiments were performed 14 days after bilateral labyrinthectomy.

### *Drug administration*

Animals were treated with AMPA receptor antagonist CNQX (3 mg/kg, i.p.; Sigma, St. Louis, MO, USA) or saline as a control, just before rotation.

### *Immunohistochemistry*

Animals were deeply anesthetized with urethane (1 g/kg), transcardially perfused, fixed with 4% paraformaldehyde dissolved in 0.1 M phosphate buffer (PB), and decapitated. Brains were removed, post-fixed, rinsed in PB, and immersed in a 30% sucrose solution for 1 to 2 days at 4°C. Brains were sectioned (40 mm) on a freezing microtome (Leica, Solms, Germany), incubated for 30 min with 6% hydrogen peroxide, rinsed twice for 10 min in 0.1 M phosphate buffered saline (PBS) containing 5% milk powder, and incubated with 0.8% Triton X-100 dissolved in 0.1 M PBS containing 0.5% bovine serum albumin (PBS-BSA). After a brief wash, tissue was incubated overnight at room temperature with anti-c-Fos polyclonal antibody (Ab-5; 1 : 1,000 dilution; Oncogene, Boston, MA, USA). The next day, the tissue sections were rinsed with PBS-BSA and incubated with a biotinylated secondary antibody (goat anti-rabbit; DAKO, Carpinteria, CA, USA) using an ABC Elite Kit (Vector, Milwaukee, WI, USA). Neurons with immunopositive nuclei were visualized by incubating the tissue with 0.05% diaminobenzidine HCl (DAB) and 0.003% hydrogen peroxide. After the DAB reaction, the tissue was rinsed with 0.1 M PB, mounted on gel-coated slides, air-dried, dehydrated, mounted on coverslips with

Permount (Fisher, Pittsburgh, PA, USA), and analyzed under bright-field microscopy. For quantification, c-Fos-positive neurons in the vestibular nuclear complex were counted using a digital image analysis system (Image Pro Plus; Media Cybernetics, Silver Spring, MD, USA).

### *RNA isolation and RT-PCR*

Total RNA was isolated from the vestibular ganglion, medial vestibular nucleus, inferior vestibular nucleus, hippocampus, flocculonodular lobe of the cerebellum (vestibular cerebellum), and parietoinsular vestibular cortex using Trizol$^{R}$ Reagent (Invitrogen, Carlsbad, CA, USA), following the manufacturer's instructions. Reverse transcription of the RNA was performed using Accupower RT PreMix (Bioneer, Daejeon, Korea) with Oligo (dT)-18 primer. One microgram of RNA and 10 pmol primers were preincubated at 70°C for 5 min. The total reaction volume was 20 $\mu$l. The cDNA synthesis was performed at 42°C for 60 min, followed by RT inactivation at 80°C for 15 min. Thereafter, the cDNA (1 $\mu$l) was amplified using Accupower RT PreMix (Bioneer).

The primers used for cDNA amplification and PCR conditions were as follows:

GAPDH: forward 5'-GTGATGCCTGGTGCTGAGTATGTC-3'
reverse 5'-CAGTCTTCTGAGTGGCAGTGATG-3'
c-fos: forward 5'-CACTGACTGAGCTGGTGCAT-3'
reverse 5'-CAATACACTCCATGCGGTTG-3'

Amplification consisted of an initial denaturation at 94°C for 5 min and 30 (GAPDH) and 33 (c-fos) cycles for detection of mRNA decay; each cycle consisted of 30 s denaturation at 94°C, 30 s annealing at 62°C, 30 s extension at 72°C, and a final amplification at 72°C for 7 min. The expected PCR products were 450 bp (GAPDH) and 241 bp (c-fos). PCR products were resolved on 1.6% agarose gel (Promega, Madison, WI, USA) and stained with ethidium bromide. Amplification gels were visualized and photographed under UV light, and analyzed by computerized densitometry scanning of the images using an AlphaEaseRFC Imaging System (Alpha Innotech, San Leandro, CA, USA). Relative changes in c-fos mRNA expression were calculated by dividing the level of expression following hypergravity stimulation by the level of expression before stimulation (as a control)$\times$ 100.

### *Real-time quantitative RT-PCR*

For some experiments, the expression levels of c-fos mRNA were evaluated by real-time RT-PCR. PCR amplification was performed using the DNA Engine Opticon for continuous fluorescence detection system (MJ Research, Waltham, MA, USA) in a total volume of 20 $\mu$l and gene specific primers using a DyNAmo SYBR Green qPCR kit (MJ Research). Each PCR was performed in triplicate under the following conditions: 94°C for 30 s, 62°C for 30 s, 72°C for 30 s, plate reading (detection of fluorescent product) for 40 cycles, followed by 7 min extension at 72°C. Melting curve analysis was carried out to characterize the double-stranded DNA product by slowly raising the temperature (0.2°C/s) from 65 to 95°C with fluorescence data collected at 0.2°C-intervals. The levels of c-fos mRNA normalized for GAPDH were expressed as fold changes relative to untreated controls. The fold change in gene expression was calculated using the following equation: fold change = $2^{-\Delta\Delta CT}$, where $\Delta\Delta C_T = (C_{T,Target} - C_{T,GAPDH})_{time\ \chi} - (C_{T,Target} - C_{T,GAPDH})_{time\ 0}$, where time $\chi$ is any time point and time 0 represents the 1$\times$ expression of the target gene of untreated cells (normalized to GAPDH) (Livak & Schmittgen, 2001). The statistical significance of differences was assessed using StatView 4.0 (Abacus Concepts, USA).

## RESULTS

### *Expression of c-Fos protein following hypergravit stimulation*

Hypergravity stimulation by centrifugal rotation may stimulate the unilateral vestibular system depending mainly on the location of the rostro-caudal axis of the body in the centrifuge cage. Expression of c-Fos protein was observed in the vestibular nuclear complex following hypergravity stimulation for 3 h, and this confirmed the laterality of centrifugal rotation in bilateral peripheral vestibular receptors. In control animals without any novel stimulus, little c-Fos protein was expressed in the vestibular nuclear complex. However, hypergravity stimulation produced marked expression of c-Fos protein in the medial, inferior, and superior vestibular nuclei, especially in the inferior vestibular nucleus, which showed the highest expression. Expression of c-Fos protein was symmetrical between both bilateral vestibular nuclei. These data suggest that centrifugal rotation for hypergravity stimulation excludes asymmetrical stimulation in the bilateral peripheral vestibular receptors (Fig. 1).

### *Expression of c-fos mRNA following hypergravity sti mulation in intact labyrinthine animals*

Temporal changes in expression of c-fos mRNA following hypergravity stimulation for 1, 3, and 12 h were measured in the vestibular ganglion, medial and inferior vestibular nuclei, hippocampus, vestibular cerebellum, and vestibular cortex. Animals subjected to hypergravity stimulation for 1 h showed that c-fos mRNA increased as much as 318 to 751% compared to the basal level of GAPDH in all areas immediately after stimulation. The medial and inferior vestibular nuclei showed the highest increment in expression of c-fos mRNA, at 674 and 751%, respectively, and the vestibular ganglion showed the lowest increment at 318%.

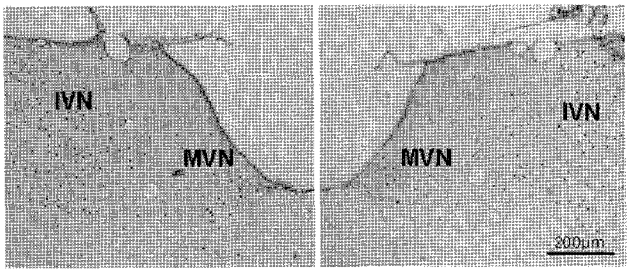


**Fig. 1.** Expression of c-Fos protein in bilateral vestibular nuclei immediately after hypergravity stimulation for 3 h. Symmetry of c-Fos protein expression between bilateral inferior vestibular nuclei was observed following centrifugal rotation, which indicates that the centrifugal stimulation used in this study did not produce asymmetrical stimulation of the bilateral peripheral vestibular receptors. MVN, medial vestibular nucleus; IVN, inferior vestibular nucleus.

The level of c-fos mRNA decreased gradually with time 2 h following stimulation (Fig. 3A).

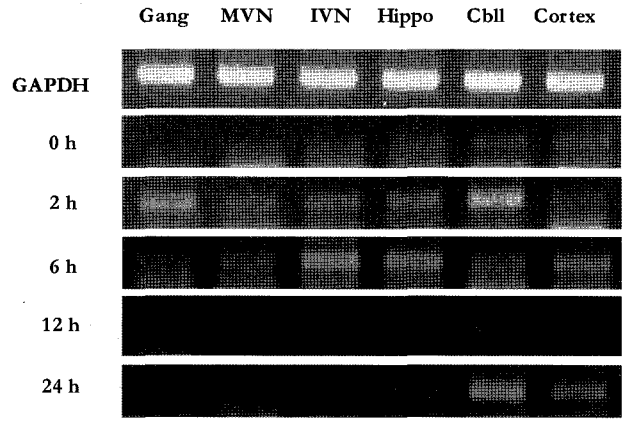


**Fig. 2.** Expression of c-fos mRNA in the vestibular ganglion (Gang), medial vestibular nucleus (MVN), inferior vestibular nucleus (IVN), hippocampus (Hippo), vestibular cerebellum (Cbll), and vestibular cortex (Cortex) following 2 g stimulation for 3 h.

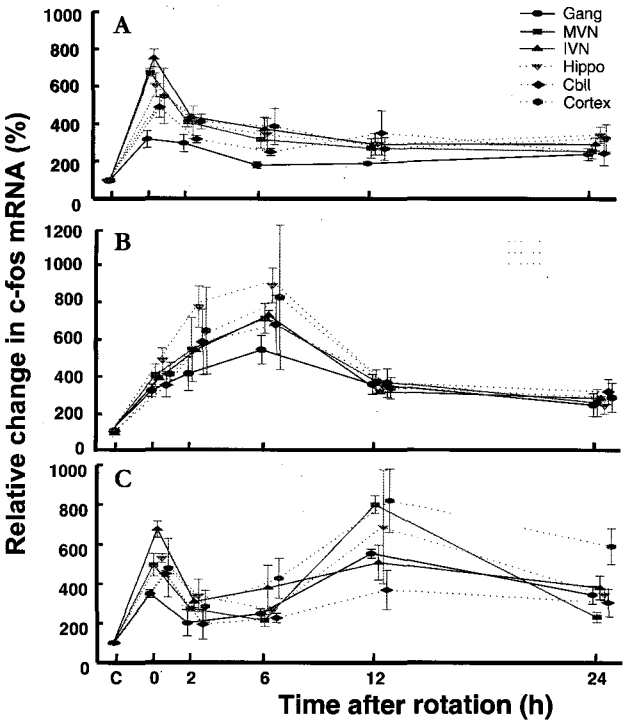


**Fig. 3.** Temporal changes in the relative quantity of c-fos mRNA expression in the vestibular ganglion (Gang), medial vestibular nucleus (MVN), inferior vestibular nucleus (IVN), hippocampus (Hippo), vestibular cerebellum (Cbll), and vestibular cortex (Cortex) following 2 g stimulation for 1 h (A), 3 h (B), and 12 h (C). Relative change in c-fos mRNA expression was calculated as the level of expression following hypergravity stimulation divided by the level of expression before stimulation as control $\times 100$. In the abscissa, c depicts the control before stimulation, and 0 h depicts immediately after hypergravity stimulation. Number of animals at each time point was five. Values are means $\pm$ SD.

Animals subjected to hypergravity stimulation for 3 h showed increased c-fos mRNA expression in all areas measured immediately after stimulation and peaked at 6 h poststimulation by as much as 526 to 905%. The hippocampus showed the highest increase with 905% and the vestibular ganglion showed the lowest increase with 526%. The level of c-fos mRNA decreased gradually with time 6 h after stimulation; however, the level of c-fos mRNA at 24 h poststimulation was still higher than that in the controls (Fig. 2, 3B). To further quantify the level of expression, c-fos mRNA in the inferior vestibular nucleus was assessed by real-time PCR using SYBR green dye. The relative value of c-fos mRNA, as normalized to internal control GAPDH, was well matched with the results of RT-PCR (Fig. 4A).

Animals subjected to hypergravity stimulation for 12 h showed two peaks in expression of c-fos mRNA immediately after and 12 h poststimulation. Immediately after stimulation, the level of c-fos mRNA expression in all areas measured was 348~675%, and expression was highest in the inferior vestibular nucleus at 675% and lowest in the vestibular ganglion at 369%. The second peak at 12 h poststimulation showed the highest expression of c-fos mRNA in the vestibular cortex at 820% and in the medial vestibular nucleus at 800%, and the lowest expression in the vestibular cerebellum at 369% (Fig. 3C).

### *Effects of bilateral labyrinthectomy or CNQX on expression of c-fos mRNA following hypergravity stimulation*

Bilateral labyrinthectomized animals with hypergravity stimulation for 3 h showed a marked reduction in c-fos mRNA expression until 6 h poststimulation compared to intact labyrinthine animals, and the level of c-fos mRNA returned to control levels 12 h poststimulation. The vestibular cortex showed the highest expression of 405% and the vestibular ganglion showed the lowest expression of 124% 6 h poststimulation. The vestibular ganglion and the medial and inferior vestibular nuclei showed the lowest expression from immediately after stimulation to 24 h poststimulation. In contrast, the vestibular cortex and ves-

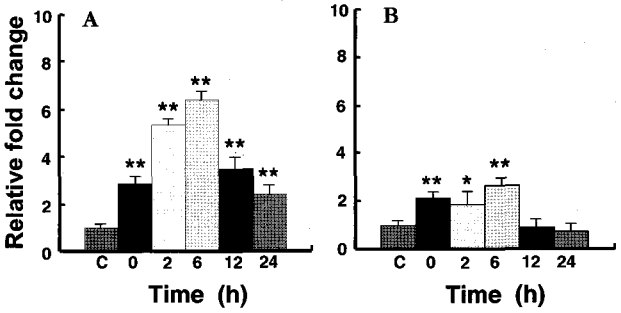


**Fig. 4.** Relative fold changes in c-fos mRNA expression in the inferior vestibular nucleus following 2 g stimulation for 3 h in intact labyrinthine animals (A) and bilateral labyrinthectomized animals (B). Real-time quantitative RT-PCR was performed to confirm the results of RT-PCR. Data were represented as the relative fold change in c-fos mRNA as normalized to GAPDH. In the abscissa, c depicts the control before stimulation, and 0 h depicts immediately after hypergravity stimulation. Number of animals at each time point was five. Values are means $\pm$ SD. *Significant difference from control (C) (*$p<0.05$, **$p<0.01$).

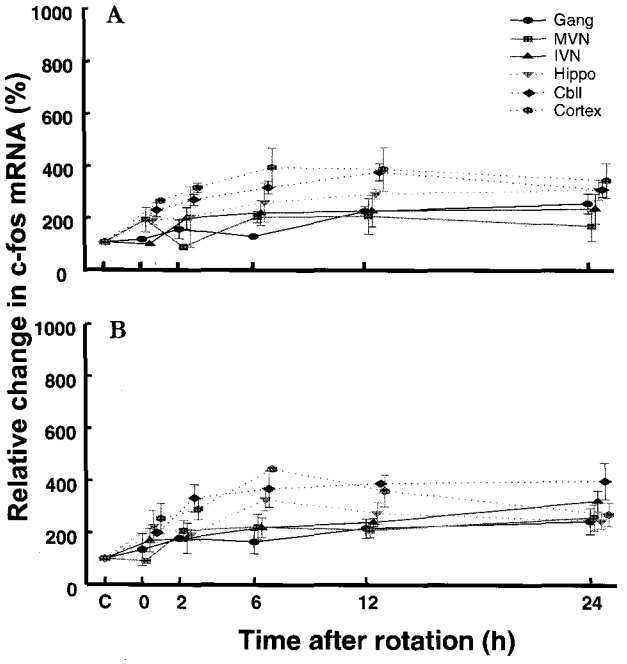


**Fig. 5.** Temporal changes in the relative quantity of c-fos mRNA expression in the vestibular ganglion (Gang), medial vestibular nucleus (MVN), inferior vestibular nucleus (IVN), hippocampus (Hippo), vestibular cerebellum (Cbll), and vestibular cortex (Cortex) following 2 g stimulation for 3 h in bilateral labyrinthectomized animals (A) and CNQX treated animals (B). Relative change in c-fos mRNA expression was calculated as the level of expression following hypergravity stimulation divided by the level of expression before stimulation as control $\times 100$. In the abscissa, c depicts the control before stimulation, and 0 h depicts immediately after hypergravity stimulation. Number of animals at each time point was five. Values are means $\pm$ SD.

tibular cerebellum showed the highest expression. The level of c-fos mRNA expression from immediately after stimulation to 24 h poststimulation was 112~259% in the vestibular ganglion, 82~207% in the medial vestibular nucleus, and 93~238% in the inferior vestibular nucleus (Fig. 5A). To further quantify the level of expression, c-fos mRNA in the inferior vestibular nucleus was assessed by real-time PCR using SYBR green dye. The relative amounts of c-fos mRNA, as normalized to internal control GAPDH, were well matched with the results of RT-PCR (Fig. 4B). Bilateral labyrinthectomy also markedly decreased c-fos mRNA expression in the vestibular related systems in animals with hypergravity stimulation for 1 or 12 h (data not shown).

Expression of c-fos mRNA in the vestibular ganglion, medial and inferior vestibular nuclei, hippocampus, vestibular cortex, and vestibular cerebellum of CNQX-treated animals was observed to investigate the effect of glutamate on c-fos mRNA expression following hypergravity stimulation for 3 h. Pretreatment with CNQX dramatically decreased expression of c-fos mRNA until 6 h poststimulation in all areas measured compared to intact labyrinthine animals, and the level of c-fos mRNA expression returned to control levels 12 h poststimulation. At 6 h poststimulation, the lowest expression of c-fos mRNA was in the vestibular ganglion at 165% and the highest expression in the vestibular cortex at 446%. The pattern of c-fos mRNA expression after hypergravity stimulation in CNQX-treated animals was similar to that in bilateral labyrinthectomized animals. For example, the vestibular ganglion and medial and inferior vestibular nuclei showed the lowest expression but the vestibular cerebellum and vestibular cortex showed relatively higher expression (Fig. 5B). Treatment with CNQX also markedly decreased c-fos mRNA expression in the vestibular related systems in animals with hypergravity stimulation for 1 or 12 h (data not shown).

## DISCUSSION

Experiments with human subjects under altered gravity conditions, such as in space or during a centrifuge run, cannot investigate the mechanism of adaptation to altered gravity due to the limited period of stimulation (Bles & de Graaf, 1993). However, animal research allows us to study the effect of sustained altered gravity forces on functioning of the vestibular system and the role of the vestibular system in the genesis of neural plasticity. Recently, experiments on animals in centrifuges have increased because research in space is both expensive and difficult. Centrifugal experiments are relatively easy to perform and the results can be extrapolated to space research and used to study vestibular function in general (Sondag et al, 1995).

Hypergravity stimulation by the centrifuge used in this study produced a force of 2 g. The gravito-inertial force produced by the centrifuge induced otolith (mainly saccule) stimulation, which was confirmed by marked expression of c-Fos protein in the inferior and medial vestibular nuclei following centrifugal rotation. Symmetric expression of c-Fos protein between both sides of the vestibular nuclear complex represents symmetric bilateral stimulation of peripheral vestibular receptors, even though the location of the head is different in each animal during rotation.

Alteration of gravity induces changes in physiological function as well as posture (Martin, 1980; Wunder et al, 1987; Burkovskaya & Krasnov, 1991; Takeda et al, 1996; Jaekel et al, 1997; Yates et al, 2003), and induces changes in gene expression in the CNS (Marshburn et al, 1997; Duflo et al, 2000; Nakagawa et al, 2003; Pompeiano et al, 2002, 2004). Up-regulation of c-fos mRNA in the brain is thought to reflect activation of neural pathways involved in sensorimotor adaptation to novel environments, such as learning and memory, via long-term potentiation. Temporal expression of c-fos mRNA following hypergravity stimulation represents adaptation to hypergravity. Hypergravity stimulation for 1 h dramatically induced c-fos mRNA expression in the vestibular ganglion, medial and inferior vestibular nuclei, hippocampus, vestibular cerebellum, and vestibular cortex immediately after stimulation. Therefore, peak expression immediately after stimulation may be caused by continuous inputs from the otolith organs and an adaptive response of the CNS to hypergravity. Hypergravity stimulation for 3 h gradually increased expression of c-fos mRNA and produced peak expression of c-fos mRNA at 6 h poststimulation, which may also indicate adaptation to hypergravity. In the 12-h rotation group, the occurrence of two peaks of c-fos mRNA expression may be considered as both expression of Fos protein and Fos related antigen (FRA) proteins in the vestibular related tissues following hypergravity stimulation (Pompeiano et al, 2002). Expression of Fos protein lasts for a few hours, but FRA proteins lasts for longer periods from 12~24 h up to days (Nestler

et al, 1999). Therefore, the first peak expression may indicate Fos protein with short-term change in brain plasticity and the second peak expression may indicate FRA proteins with long-term change in the vestibular nuclei and related structures following hypergravity stimulation. However, prolonged exposure to hypergravity for 60 days from birth (Duflo et al, 2000) or prolonged exposure for 2 weeks in adult rats (Nakagawa et al, 2003) did not induce expression of c-Fos protein in the CNS, which suggests that the neural circuit for adaptation was already present in the CNS during hypergravity stimulation. The discrepancy between our study and others may be attributable to the difference in duration of exposure to hypergravity.

The vestibular ganglion, which receives direct inputs from the peripheral vestibular receptors, shows peak expression of c-fos mRNA at 0 h poststimulus after hypergravity stimulation for 1 h, at 3 h poststimulus after hpergravity stimulation for 3 h, and at 12 h poststimulus after hypergravity stimulation for 12 h. These findings are concordant with the electrophysiological observations by Boyle et al (2001). They reported that the magnitude of response to an applied translation was on average three times greater than for controls in afferent vestibular nerve supplying the utricule of toadfishes within the first day after reentry from space flight. Bilateral labyrinthectomy markedly decreased expression of c-fos mRNA in the vestibular ganglion until 6 h poststimulus following hypergravity stimulation for 3 h compared to intact labyrinthine animals. Considering that expression of c-fos mRNA induced by stress in brain tissue peaked at 30 min poststimulation and disappeared within 2 h (Herdegen & Leah, 1998) and stress produced a small amount of c-fos mRNA expression in the medial and superior vestibular nuclei (Cullinan et al, 1995), down regulation of expression of c-fos mRNA in the vestibular ganglion after hypergravity stimulation in bilateral labyrinthectomized animals may exclude the effect of stress induced by hypergravity stimulation. The pattern of c-fos mRNA expression in the medial and inferior vestibular nuclei receiving afferent inputs mainly from the otolith organ was similar to that of the vestibular ganglion. These data indicate that peripheral vestibular receptors rather than non-labyrinthine graviceptors are closely related to perception of altered gravity. However, several experiments have indicated that perception of body position is derived in part from non-labyrinthine graviceptors (Mittelstaedt, 1996). For example, graviceptors located in the viscera near the last rib, perhaps associated with the kidney, may contribute to sensing of body position during centrifugation in humans. Also, a wide variety of non-labyrinthine sensory inputs from neck and limb muscles, as well as the skin and viscera, have been shown to influence firing of vestibular nucleus neurons (Jian et al, 2002). Hypergravity causes alterations in the pattern of inputs from otolith organs, which are accompanied by an increase in signals from non-labyrinthine sensory organs.

In this study, changes in c-fos mRNA expression in the lateral vestibular nuclei could not observed following hypergravity stimulation, which was corresponded to the previous data obtained from the lateral vestibular nuclei during the space flight at the reentry (Pompeiano et al, 2002). This could be explained by that Fos expression can be induced only by excitatory, but not inhibitory pathways (Gillespie et al, 1999). So, the lack of c-fos mRNA expression observed in the lateral vestibular nuclei following hypergravity stimulation could be attributed to parallel activation by the gravity force of the cerebellar anterior vermis, whose Purkinje cells are known to exert a GABAergic inhibitory influence on the lateral vestibular nuclei (Pompeiano et al, 2002).

It is well-known that NMDA receptors produce long-term neuroplasticity, including long-term potentiation in several forebrain structures, but also in the long-term depression which affects the cerebellar cortex (Bading et al, 1995; Azuma et al, 1996). In this study, the parallel fibers acting on Purkinje cells utilize AMPA-kainate type glutamate receptors, rather than NMDA receptors (Ito & Karachot, 1990). Blockade of AMPA-kainate receptors dramatically decreased c-fos mRNA expression in the vestibular ganglion, vestibular nuclei, hippocampus, vestibular cerebellum, and vestibular cortex until 6 h after hypergravity stimulation. The data suggest that glutamate receptors have an important role in expression of c-fos mRNA to altered gravity.

The hippocampus is implicated in spatial navigation processes (Smith, 1997; Stackman et al, 2002). The pattern of c-fos mRNA expression in the hippocampus following hypergravity stimulation was similar to that in the vestibular ganglion and vestibular nuclei of bilateral labyrinthectomized animals as well as intact labyrinth animals. Electrophysiological recordings from hippocampal neurons indicate that spatial orientation is decreased in bilateral labyrinthectomized animals (Russell et al, 2003). The flocculonodular lobe of the cerebellum following hypergravity stimulation showed a similar pattern of c-fos mRNA expression to that of the vestibular ganglion and vestibular nuclei. However, the flocculonodular lobe in bilateral labyrinthectomized animals showed more expression of c-fos mRNA than the vestibular ganglion and vestibular nuclei because the cerebellum receives afferents from the non-labyrinthine inputs, including muscle spindles and proprioceptors (Grusser & Kroller, 1979), as well as vestibular inputs. The data suggest that the cerebellum may modulate neuroplasticity induced by non-labyrinthine graviceptors. The parietoinsular vestibular cortex (PIVC) receives inputs from the vestibular system and controls vestibular reflexes (Akbarian et al, 1994; Guldin & Grusser, 1998; Park et al, 2004). PIVC following hypergravity stimulation in bilateral labyrinthectomized animals showed a similar pattern of c-fos mRNA expression to the cerebellum, which suggests that PIVC also receives afferents from the non-labyrinthine inputs, including various somatosensory signals (Brandt & Dieterich, 1999) and vestibular inputs. On the basis of the above results, hypergravity stimulation may activate a wide area of brain tissue as well as vestibular related brain tissues.

In summary, altered gravity induces expression of c-fos mRNA in the vestibular related tissues, and there is a temporal relationship between duration of hypergravity stimulation and peak time of c-fos mRNA expression. The peripheral vestibular receptors and glutamate have an important role in expression of c-fos mRNA in the vestibular related tissues to altered gravity.

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