

## Impact of a simulated gravity load for atmospheric reentry, 10 g for 2 min, on conscious mice

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**Abstract** The Japan Aerospace Exploration Agency recently performed a mouse experiment in the International Space Station in which mice were raised for 35 days, retrieved using the Dragon spacecraft, and then harvested for analysis 2 days after splashdown. However, the impact of the retrieval procedure, which exposed mice to 5–10 g for 2 min during atmospheric reentry and splashdown, was unknown. Therefore, the purpose of this study was to examine the impact of a 10 g load for 2 min (using a gondola-type centrifuge with a 1.5-m arm installed at Gifu University) on conscious mice. Plasma corticosterone increased at 30 min after load application and recovered at 90 min. Significant Fos expression was observed in the vestibular nuclei (VeN), paraventricular hypothalamic nucleus (PVN), and central nucleus of the amygdala (CeA). Rearing behavior and food intake were suppressed. Mice with vestibular lesions demonstrated increased corticosterone and Fos expression in the PVN, but neither suppression of food intake and rearing behavior nor increased Fos expression in the VeN and CeA. These results suggest that the simulated gravity load induced a transient stress

response, hypoactivity, and a vestibular-mediated suppression of food intake.

**Keywords** Hypergravity · Atmospheric reentry · Fos · Corticosterone · Vestibular lesion

### Introduction

The Japan Aerospace Exploration Agency (JAXA) has developed a novel mouse habitat cage unit [1] to isolate the impact of microgravity on small mammals in the absence of the uncontrolled factors that exist in the International Space Station (ISS), such as lack of convection, microbial environment [2], cosmic radiation [3], and stressful conditions during launch and landing [4]. This cage is designed for installation in the Cell Biology Experiment Facility (CBEF) in the Japanese Experimental Module “Kibo” of the ISS. The CBEF has two compartments: a microgravity section and an artificial 1 g section, in which a 1 g environment is produced by a centrifuge [5]. Mice can be raised in the 1 g compartment or microgravity compartment, and the pure impact of microgravity can be examined by comparing these two groups.

Using the CBEF, JAXA performed a mouse experiment in which live mice were retrieved by transfer from the CBEF to a transportation cage unit and loaded on the Dragon spacecraft (SpaceX), which splashed down in the Long Beach offing. The mice were then harvested for detailed analysis 2 days after splashdown. JAXA is planning to conduct this type of space experiment twice a year. The retrieval procedure, which involved exposure to 5–10 g for 2 min during atmospheric reentry and splashdown, had an unknown impact on the mice used in this experiment. Accordingly, the purpose of the present study

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was to examine the impact of hypergravity, and to verify whether the effects disappeared within 24 h. Therefore, we examined Fos expression in the brain, changes in behavior, and plasma stress hormone concentration in response to 10 g for 2 min. This simulates a worst-case gravitational load for reentry and splashdown, since the actual gravity load varies depending on the reentry trajectory. In addition, we compared responses in vestibular-intact (Sham) mice and vestibular-lesioned (VL) mice to determine the role of the vestibular system in gravity load-induced responses.

## Materials and methods

Animals used in the present study were maintained in accordance with the “Guiding Principles for Care and Use of Animals in the Field of Physiological Science” of the Physiological Society of Japan. The experiments were approved by the Animal Research Committees of Gifu University (27-79) and JAXA (015-008A). Male C57BL/6J mice ( $n = 46$ ) were used for the experiment, which was performed using a centrifuge at Gifu University.

Animals were divided into two groups: VL ( $n = 16$ ) and Sham ( $n = 30$ ). Six-week-old mice were anesthetized by isoflurane (Escain, Pfizer, Tokyo, Japan) inhalation via a face mask, and the VL or sham surgery was performed using an external auditory meatus approach as described previously [5]. For the sham surgery, the tympanic membrane was removed, but the auditory ossicles were left intact. The success of VL was confirmed by observing impairment in swimming behavior [5]. At the end of the experiment, histological examination of the labyrinth was performed to ensure the absence of sensory epithelia in the saccule and utricle.

For prevention of infection and pain relief, penicillin G potassium (3000 U/kg, Meiji Seika Pharma, Tokyo, Japan) and buprenorphine (3  $\mu$ g/kg, Lepetan, Otsuka, Tokyo, Japan) were administered subcutaneously prior to returning

the animals to their home cages. After recovery for 4 weeks from VL or sham surgery, the mice were moved to individual cages (width 10  $\times$  10 cm and height 8 cm) where they were maintained for the remainder of the experiment.

The individual cages were set in a rotating box, and video recording was initiated. All mice had access to food and water ad libitum, and the temperature in the cage was maintained at  $24 \pm 1$  °C with a 12:12 h light–dark cycle (light 07:00–19:00; dark 19:00–07:00). During a daily 15-min break (10:00–10:15), the cages were cleaned, water and food were refreshed, and body mass and daily food intake were measured. On the third day, mice were exposed to 10 g ( $98.0665 \text{ m/s}^2$ ) for 2 min (12:00–12:02), induced by centrifugation in the custom-made, gondola-type rotating box with a 1.5-m arm (Shimadzu, Kyoto, Japan). The centrifugation protocol included a 25-s increment phase of acceleration from 1 to 10 g, a 95-s-long 10 g plateau phase, and a 45-s decrement phase of deceleration from 10 to 1 g. The angular velocity at 10 g was  $2.57\pi \text{ rad/s}$ . For mice undergoing behavioral analysis, video monitoring and measurement of food intake were continued for one more day. To evaluate activity, the frequency of rearing behavior was counted for every hour except 10:00–11:00. At the end of the experiment, mice were deeply anesthetized by isoflurane inhalation and perfused transcardially with 20 ml of heparinized saline, followed by 40 ml of 4% paraformaldehyde in phosphate buffer. The brains were then removed and stored in 4% paraformaldehyde at 4 °C for subsequent Fos expression measurement (Sham-10 g + 1d,  $n = 6$ ; VL-10 g + 1d,  $n = 6$ ).

For stress response and Fos expression analyses, Sham and VL mice were exposed to 10 g for 2 min and anesthetized by isoflurane inhalation at 30 (Sham  $n = 6$ ; VL  $n = 5$ ), 90 (Sham  $n = 6$ ), or 180 min (Sham  $n = 6$ ) after the 10 g load, and 0.8 ml of venous blood was collected from the inferior vena cava. Blood samples were also collected from Sham ( $n = 6$ ) and VL ( $n = 5$ ) mice of the 1 g control group (i.e., not exposed to the 10 g load). Brain specimens of the 90-min group and 1 g control group were collected for Fos expression measurement (Sham-1 g, Sham-10 g + 90 min, VL-1 g, and VL-10 g + 90 min). Plasma corticosterone concentration was measured using enzyme-linked immunosorbent assays (Enzo Life Sciences, Farmingdale, NY, USA).

To examine whether the 10 g load induced compressional injuries of the liver and muscle, plasma aspartate aminotransferase (AST) and creatine kinase (CK) concentrations were measured with a chemistry analyzer (VetTest, IDEXX Laboratories, Westbrook, ME, USA), using plasma samples obtained from 1 g control mice and from Sham and VL mice 30 min after the 10 g load.

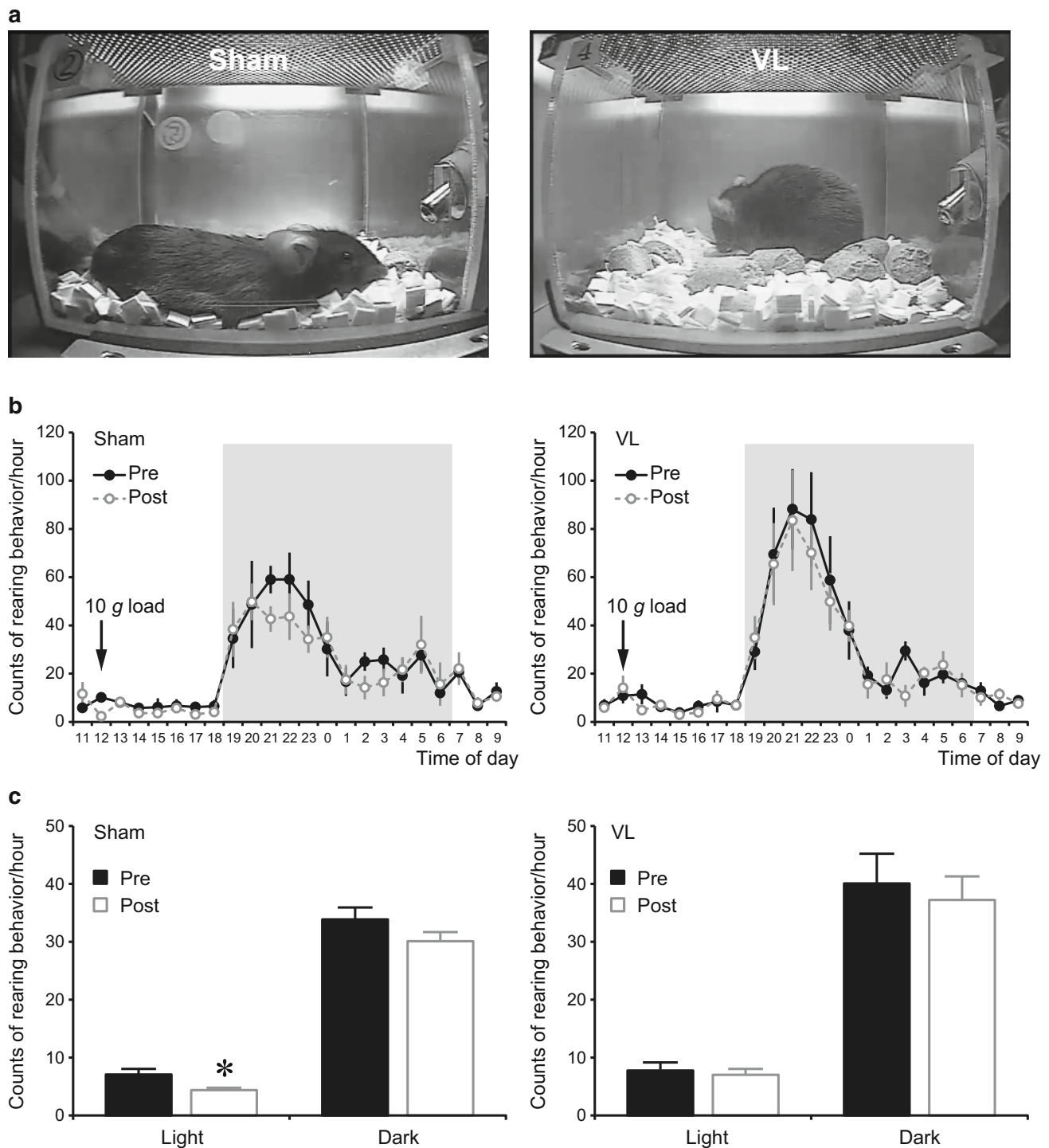
**Table 1** Effects of a 10 g load for 2 min on body mass and food intake

	Pre	Post
Body mass (g)		
Sham ( $n = 6$ )	$25.9 \pm 0.3$	$25.5 \pm 0.4$
VL ( $n = 6$ )	$24.2 \pm 0.6^\dagger$	$24.2 \pm 0.5^\dagger$
Food intake/10 g body mass (g)		
Sham ( $n = 6$ )	$1.46 \pm 0.04$	$1.20 \pm 0.09^*$
VL ( $n = 6$ )	$1.53 \pm 0.09$	$1.53 \pm 0.09^\dagger$

Values are mean  $\pm$  SEM

VL vestibular-lesioned

\*  $p < 0.05$  vs. Pre.  $^\dagger p < 0.05$  vs. Sham



**Fig. 1** **a** Posture of sham control (left) and vestibular-lesioned (VL) mice (right) at 5 min after the 10 g load. The trunk of the sham mouse was flattened, but the VL mouse demonstrated a rounded trunk shape. **b** Counts of rearing behavior recorded 1 day before (closed circle) and on the day of (open circle) the 10 g load. The 2-min 10 g load

was initiated at 12:00. **c** Averaged data for counts of rearing behavior during 7 h of light time and during 12 h of dark time. Closed bar represents data for one day before the load (Pre), and open bar represents data for the day of the load (Post). \* $p < 0.05$  vs. Pre

Brain specimens were stored overnight in 20% sucrose in PBS at 4 °C and then frozen on dry ice. Serial sections (40  $\mu$ m) were cut with a cryostat (Leica CM1850;

Leica Instruments, Wetzlar, Germany). Fos-containing cells were identified with an anti-c-Fos antibody (c-Fos Ab-5; Merck, Darmstadt, Germany) using the 3,3'-

diaminobenzidine reaction. Hypergravity-induced Fos expression was examined in the nucleus of the solitary tract (NTS), vestibular nuclei (VeN), paraventricular hypothalamic nucleus (PVN), and central nucleus of the amygdala (CeA). The numbers of Fos-positive cells were counted using ImageJ 1.47v. In each group, 18 portions of bilateral NTS, 21 portions of unilateral VeN, 26 portions of unilateral PVN, and 22 portions of unilateral CeA were examined.

All data are presented as mean  $\pm$  SEM. Recovery time from flattened trunk (posture) and time elapsed before first rearing were analyzed using unpaired *t* tests (Sham vs. VL). Body mass and food intake data (Table 1) were analyzed using repeated measures two-way analysis of variance (ANOVA), with group (Sham vs. VL) and time (Pre vs. Post) as factors. The number of rearing incidents (Fig. 1c) was analyzed using a paired *t* test (Pre vs. Post). Plasma concentrations of corticosterone, AST, and CK (Fig. 2), as well as Fos expression data (Fig. 3b), were analyzed using one-way ANOVA. If the *F* ratio indicated

statistical significance, Scheffé's multiple comparison test was used for between-group comparisons. A value of  $p < 0.05$  was considered statistically significant for post hoc tests.

## Results

Mean body mass of VL mice was significantly smaller than that of Sham mice (Table 1). However, there was no difference in daily food intake per body mass between the two groups. Gravitational load of 10 g for 2 min significantly decreased daily food intake in Sham mice but not that in VL mice. Body mass of Sham mice tended to decrease at 1 day after load application, but the difference was not statistically significant.

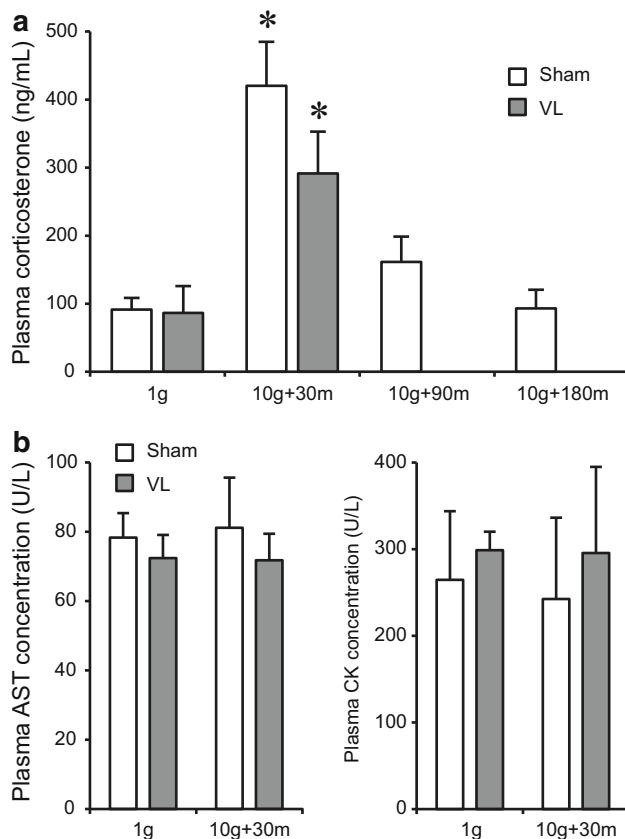
Behavioral analysis revealed that the round shape of the lumbar area was lost and the trunk flattened during the 10 g load in both sham and VL mice. The round shape recovered in VL mice soon after the end of the load ( $0.5 \pm 0.1$  min), while the recovery in Sham mice was significantly delayed ( $15.2 \pm 1.3$  min; Fig. 1a). Furthermore, the latency of rearing after the 10 g load was longer in Sham mice ( $47.0 \pm 12.3$  min) than in VL mice ( $1.8 \pm 0.9$  min).

Rearing behavior on the day of the 10 g load was compared with that on the day before application of the load. The 10 g load somewhat suppressed counts of rearing behavior in Sham mice, as shown by a small dip at 12:00 h in Fig. 1b. Averaged counts of rearing behavior during 7 h of light time (12:00–19:00) and during 12 h of dark time (19:00–07:00) are shown in Fig. 1c. The 10 g load significantly suppressed rearing behavior during light time, but not during dark time. In VL mice, there was no suppression of counts of rearing behavior.

In Sham mice, the plasma corticosterone concentration significantly increased from  $91 \pm 17$  to  $420 \pm 65$  ng/ml at 30 min after the 10 g load (Fig. 2a). The increase was no longer observed at 90 and 180 min after the 10 g load. In VL mice, the plasma corticosterone level increased at 30 min after the 10 g load (from  $87 \pm 39$  to  $292 \pm 62$  ng/ml), and was not different from that in Sham mice.

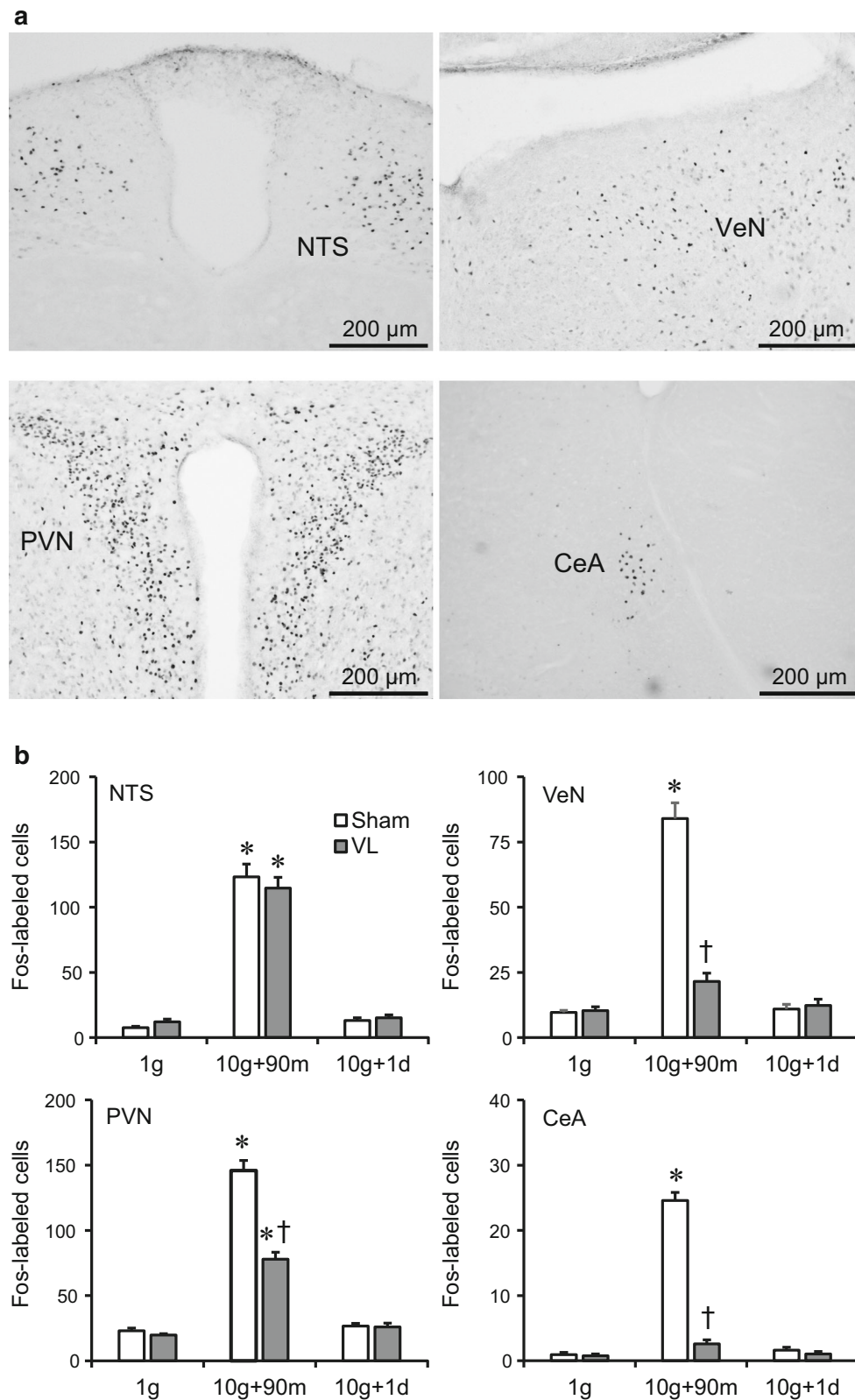
Figure 2b shows the plasma concentration of AST and CK at 1 g and 30 min after the 10 g load. The 2-min, 10 g load had no influence on these variables relative to the 1 g control group.

To evaluate central activation induced by the 10 g load for 2 min, Fos expression in the NTS, VeN, PVN, and CeA was examined. Figure 3a shows representative photomicroscopic images of serial sections obtained from a Sham mouse 90 min after the 10 g load, in which clear Fos expression is evident in the NTS, VeN, PVN, and CeA. Figure 3b summarizes the counts of Fos-labeled cells. In



**Fig. 2** **a** Plasma corticosterone concentration obtained from 1 g control mice (1 g) and 10 g loaded mice at 30 (10 g + 30 min), 90 (10 g + 90 min), and 180 (10 g + 180 min) min after the load. \* $p < 0.05$  vs. 1 g. **b** Plasma aspartate aminotransferase (AST) and creatine kinase (CK) concentrations obtained from 1 g and 10 g + 30 min mice. Open bar represents Sham mice, and closed bar represents VL mice





**Fig. 3 a** Representative photomicroscopic images of serial sections of the nucleus of the solitary tract (NTS), vestibular nuclei (VeN), paraventricular hypothalamic nucleus (PVN), and central nucleus of the amygdala (CeA) obtained from a Sham-10 g + 90 min mouse.

**b** Averaged data for the numbers of Fos-labeled cells obtained from 1 g control mice (1 g) and 10 g loaded mice at 90 min (10 g + 90 min) and 1 day (10 g + 1d) after the load. \* $p < 0.05$  vs. Sham-1 g or VL-1 g. † $p < 0.05$  vs. Sham

Sham mice, the numbers of Fos-labeled cells increased significantly at 90 min (Sham-10 g + 90 min) in the NTS, VeN, PVN, and CeA compared with those in 1 g control mice (Sham-1 g). In VL mice, significant increases were found in the NTS and PVN at 90 min (VL-10 g + 90 min) compared with those in the VL-1 g mice, but not in the VeN and CeA. Furthermore, the increase in the PVN was smaller in the VL-10 g + 90 min group compared with that in the Sham-10 g + 90 min group, whereas the increase in the NTS was comparable between groups. These increases were not observed the day after the 10 g load for either the Sham-10 g + 1d or VL-10 g + 1d groups.

## Discussion

The major findings of the present study show that a gravity load similar to that of atmospheric reentry induced central Fos activation, an increase in plasma corticosterone concentration, and suppression of activity and food intake. Some of these changes were reduced or abolished by VL, and all of these changes, except for food intake, recovered within 24 h.

Previous studies from our laboratory demonstrated that a gravitational load of 2 g for 90 min induced central Fos activation in the NTS, VeN, and PVN [5, 6]; this expression pattern is associated with activation of corticotropin-releasing factor neurons in the PVN and an increase in plasma corticosterone concentration [6]. These responses were abolished by VL, suggesting that they are mediated via the vestibular system. In the present study, the 10 g load, though applied for only 2 min, also induced central Fos activation and increased plasma corticosterone concentration in Sham mice. In VL mice, the increase in Fos expression in the VeN was abolished, while increases in the NTS and PVN were preserved, although the increase in the PVN was smaller than that in Sham mice. These results suggest that 10 g load-induced central Fos activation and corticosterone release were mediated not only by the vestibular system but also by non-vestibular systems. A hypergravity-induced increase in body weight (body mass  $\times$  gravity) might mechanically stimulate or compress somatic and proprioceptive receptors in the skin, muscle, tendon, and joints, not to mention visceral sensory receptors [7–10]. Afferent input from these receptors projects to the NTS, and then to the PVN [11–13]. However, these responses are transient, as evidenced by the recovery of plasma corticosterone concentration and central Fos activation to normal levels after 90 min and 24 h, respectively.

Vestibular-mediated nausea and vomiting are common symptoms of motion sickness, prompting diagnosis in

humans [14, 15]. However, judgment of motion sickness in mice and rats is difficult because they lack the vomiting reflex [16]; kaolin consumption and other behaviors indicative of nausea have been used to assess motion sickness in such animals [17–19]. Furthermore, hypergravity-induced Fos expression in the CeA is implicated in emetic and nausea responses [20]. Systemic and intracerebroventricular injections of emetic agents induce Fos expression in the CeA [17, 21], and hypergravity-induced pica behavior, an index of motion sickness, is suppressed by bilateral lesion of the amygdala [22]. Fos activation in the CeA and behavioral changes observed in Sham mice, i.e., flattened trunk and reduced rearing, suggest that Sham mice suffered motion sickness after the gravity load. In contrast, these same changes were not observed in VL mice. Thus, the suppressed food intake in the sham mice might be due to motion sickness.

In addition to motion sickness, hypergravity may also cause compressional injuries of organs. The liver and skeletal muscle, in particular, may be susceptible to compressional stress. A 9 g load for 1 h induced significant increases in AST and CK, suggesting compressional organ injury [23]. However, the 2-min 10 g load had no influence on plasma AST and CK concentrations, suggesting that 2 min is too short a time to induce compressional injuries.

In conclusion, a gravity load simulating that of atmospheric reentry induced stress responses and motion sickness in conscious mice. However, these responses were transient and recovered within 24 h.

**Author Contributions** H.M., D.S., M.S., and S.T. were involved in the conception and design of the experiments. H.M. and A.Y. performed the experiments and were involved in collection, analysis, and interpretation of the data. H.M. wrote the manuscript and prepared all figures. All authors reviewed the manuscript and approved the final version. All persons designated as authors qualify for authorship, and all those who qualify for authorship are listed.

## Compliance with ethical standards

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**Conflict of interest** The authors have no conflicts of interest to declare.

## References

- Shimbo M, Kudo T, Hamada M, Jeon H, Imamura Y, Asano K, Okada R, Tsunakawa Y, Mizuno S, Yagami K, Ishikawa C, Li H, Shiga T, Ishida J, Hamada J, Murata K, Ishimaru T, Hashimoto M, Fukamizu A, Yamane M, Ikawa M, Morita H, Shinohara M, Asahara H, Akiyama T, Akiyama N, Sasanuma H, Yoshida N, Zhou R, Wang YY, Ito T, Kokubu Y, Noguchi TA, Ishimine H, Kurisaki A, Shiba D, Mizuno H, Shirakawa M, Ito N, Takeda S, Takahashi S (2016) Ground-based assessment of JAXA mouse habitat cage unit by mouse phenotypic studies. *Exp Anim* 65:175–187
- Vesper SJ, Wong W, Kuo CM, Pierson DL (2008) Mold species in dust from the International Space Station identified and quantified by mold-specific quantitative PCR. *Res Microbiol* 159:432–435
- Hallil A, Brown M, Akatov Y, Arkhangelsky V, Chernykh I, Mitrikas V, Petrov V, Shurshakov V, Tomi L, Kartsev I, Lyagushin V (2010) MOSFET dosimetry mission inside the ISS as part of the Matroshka-R experiment. *Radiat Prot Dosim* 138:295–309
- Stepaniak PC, Hamilton GC, Olson JE, Gilmore SM, Stizza DM, Beck B (2007) Physiologic effects of simulated+ Gx orbital reentry in primate models of hemorrhagic shock. *Aviat Space Environ Med* 78:A14–A25
- Morita H, Obata K, Abe C, Shiba D, Shirakawa M, Kudo T, Takahashi S (2015) Feasibility of a short-arm centrifuge for mouse hypergravity experiments. *PLoS One* 10:e0133981
- Abe C, Ueta Y, Morita H (2013) Exposure to hypergravity during the preweaning but not postweaning period reduces vestibular-related stress responses in rats. *J Appl Physiol* 115:1082–1087
- D'Amelio F, Wu LC, Fox RA, Daunton NG, Corcoran ML, Polyakov I (1998) Hypergravity exposure decreases gamma-aminobutyric acid immunoreactivity in axon terminals contacting pyramidal cells in the rat somatosensory cortex: a quantitative immunocytochemical image analysis. *J Neurosci Res* 53:135–142
- Miyoshi T, Nozaki D, Sekiguchi H, Kimura T, Sato T, Komeda T, Nakazawa K, Yano H (2003) Somatosensory graviception inhibits soleus H-reflex during erect posture in humans as revealed by parabolic flight experiment. *Exp Brain Res* 150:109–113
- Sand DP, Girgenrath M, Bock O, Pongratz H (2003) Production of isometric forces during sustained acceleration. *Aviat Space Environ Med* 74:633–637
- Yates BJ, Bronstein AM (2005) The effects of vestibular system lesions on autonomic regulation: observations, mechanisms, and clinical implications. *J Vestib Res* 15:119–129
- Ganchrow D, Ganchrow JR, Cicchini V, Bartel DL, Kaufman D, Girard D, Whitehead MC (2014) Nucleus of the solitary tract in the C57BL/6 J mouse: subnuclear parcellation, chorda tympani nerve projections, and brainstem connections. *J Comp Neurol* 522:1565–1596
- Micheline LC, Stern JE (2009) Exercise-induced neuronal plasticity in central autonomic networks: role in cardiovascular control. *Exp Physiol* 94:947–960
- Plotsky PM, Kjaer A, Sutton SW, Sawchenko PE, Vale W (1991) Central activin administration modulates corticotropin-releasing hormone and adrenocorticotropin secretion. *Endocrinology* 128:2520–2525
- Lackner JR (2014) Motion sickness: more than nausea and vomiting. *Exp Brain Res* 232:2493–2510
- Schmal F (2013) Neuronal mechanisms and the treatment of motion sickness. *Pharmacology* 91:229–241
- Horn CC, Kimball BA, Wang H, Kaus J, Dienel S, Nagy A, Gathright GR, Yates BJ, Andrews PL (2013) Why can't rodents vomit? A comparative behavioral, anatomical, and physiological study. *PLoS One* 8:e60537
- Lu Z, Yeung CK, Lin G, Yew DT, Andrews PL, Rudd JA (2017) Insights into the central pathways involved in the emetic and behavioural responses to exendin-4 in the ferret. *Auton Neurosci* 202:122–135
- Santucci D, Corazzi G, Francia N, Antonelli A, Aloe L, Alleva E (2000) Neurobehavioural effects of hypergravity conditions in the adult mouse. *NeuroReport* 11:3353–3356
- Uno A, Takeda N, Horii A, Sakata Y, Yamatodani A, Kubo T (2000) Effects of amygdala or hippocampus lesion on hypergravity-induced motion sickness in rats. *Acta Otolaryngol* 120:860–865
- Nakagawa A, Uno A, Horii A, Kitahara T, Kawamoto M, Uno Y, Fukushima M, Nishiike S, Takeda N, Kubo T (2003) Fos induction in the amygdala by vestibular information during hypergravity stimulation. *Brain Res* 986:114–123
- De Jonghe BC, Horn CC (2009) Chemotherapy agent cisplatin induces 48-h Fos expression in the brain of a vomiting species, the house musk shrew (*Suncus murinus*). *Am J Physiol Regul Integr Comp Physiol* 296:R902–R911
- Uno A, Takeda N, Kitahara T, Sakata Y, Yamatodani A, Kubo T (2000) Effects of vestibular cerebellum lesion on motion sickness in rats. *Acta Otolaryngol* 120:386–389
- Lee SG, Lee CG, Wu HM, Oh CS, Chung SW, Kim SG (2015) A load of mice to hypergravity causes AMPK $\alpha$  repression with liver injury, which is overcome by preconditioning loads via Nrf2. *Sci Rep* 5:15643