Effects of microgravity and bone morphogenetic protein II on GFAP in rat brain

J. R. DAY,[1] A. T. FRANK,[1] J. P. O'CALLAGHAN,[2] AND B. W. DEHART[1]
[1]*Department of Biology, The Pennsylvania State University, University Park, Pennsylvania 16802; and* [2]*Health Effects Laboratory Division, Centers for Disease Control and Prevention, National Institute for Occupational Safety and Health, Morgantown, West Virginia 26505*

Day, J. R., A. T. Frank, J. P. O'Callaghan, and B. W. DeHart. Effects of microgravity and bone morphogenetic protein II on GFAP in rat brain. *J. Appl. Physiol.* 85(2): 716–722, 1998.—This study evaluated effects of bone morphogenetic protein II (BMP) on glial fibrillary acidic protein (GFAP) in the brain of female Fischer 344 rats during 14 days of spaceflight. GFAP mRNA decreased in vehicle-implanted rats flown on the space shuttle by 53 and 48% in the stratum moleculare and stratum lacunosum moleculare hippocampal subregions, respectively. GFAP mRNA was not significantly affected by BMP implantation during spaceflight. Rats returning from space exhibited a 56% increase in serum corticosterone. BMP treatment did not additively increase corticosterone elevations in microgravity but appeared to increase serum corticosterone and reduce GFAP mRNA in the stratum moleculare in control rats. These data suggest that exposure to microgravity reduces GFAP expression in hippocampal astrocytes.

hippocampus; spaceflight; stress; glucocorticoids; astrocytes; glial fibrillary acidic protein

MICROGRAVITY is the extremely weak gravitational pull that occurs while a spacecraft is in orbit around the Earth. Current spaceflight technology necessitates exposure to microgravity, which adversely affects several aspects of human and animal physiology. Some of these effects include skeletal muscle atrophy (19, 63), bone loss (43), and shifts of body fluids from the lower extremities to the upper body (37). There are shifts in endocrine homeostasis, including decreased circulating testosterone concentrations (22). Decreased levels of total catecholamines (32) and increased catecholamine output from the adrenal glands have been reported (3). Increased stroke volume and cardiac output are effects reported in the cardiovascular system (68). Changes in the central nervous system (CNS) include increased numbers of 5-hydroxytryptamine type 1 receptors in the hippocampus (36) and pineal gland (24). Attempts to study the neurodegenerative effects of microgravity on the CNS have reported ultrastructural changes, mostly confined to the somatosensory, visual, olfactory, and vestibular systems (8, 14, 15, 29, 38, 50, 51). These ultrastructural changes include loss of axon terminals in the somatosensory, visual, and olfactory cortices (14, 15), increased ribbon synaptic plasticity in hair cells of the utricular maculas (51), and increased neural adaptation in the primary afferents of the semicircular canal (8, 50). Spaceflight research has not yet determined whether microgravity causes degeneration in the hippocampus (see Fig. 1), where learning and memory might be affected.

The stratum moleculare (sm) and stratum lacunosum moleculare (slm) of the hippocampus were chosen as the focus of this study for three reasons. First, glial fibrillary acidic protein (GFAP) mRNA expression in the sm changes in response to a variety of factors, including corticosterone (Cort), lesions, aging, and stress. A recent study showed similar increases with aging in GFAP mRNA expression in the sm and slm that were attenuated by dietary restriction (35). It is reasonable to assume that these two regions would respond similarly to stress and Cort. Second, microgravity-induced changes in 5-hydroxytryptamine type 1 receptors have been reported, suggesting that the hippocampus is sensitive to microgravity (36). Third, any hippocampal degeneration induced by prolonged exposure to microgravity could produce serious cognitive impairments similar to dementia of the Alzheimer's disease type.

Immunoreactivity and mRNA expression of GFAP both serve as useful neurodegenerative markers, because increased expression corresponds to a characteristic cellular hypertrophy referred to as astrogliosis (16, 33). GFAP is an intermediate filament protein in astrocytes, and its expression increases in response to injury, neurodegenerative disease, and aging (20, 34, 39, 46). GFAP also appears to be regulated by local changes in neuronal activity. GFAP immunoreactivity increased in rat lateral geniculate nuclei after deprivation of visual information and after intraocular implantations of tetrodotoxin (5). In the chick nucleus magnocellularis, tetrodotoxin also increased GFAP expression in axon terminal projection fields (4). However, other studies have shown that increased neuronal activity associated with seizures can increase GFAP expression (60). Shifts in circulating steroid hormone concentrations can also alter GFAP expression.

Glucocorticoids are secreted by the adrenal gland, and large increases in secretion denote stress in primates, rodents, and other animals. Some hippocampal neurons are sensitive to adrenal steroids and possess high concentrations of both the type I glucocorticoid receptor and the lower affinity type II mineralocorticoid receptor (66). Substantial increases in circulating glucocorticoids selectively damage subpopulations of hippocampal pyramidal neurons of primates (53), guinea pigs (1), and rats (52) and might accelerate cognitive aging. Cort manipulations can alter astrocyte gene

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

expression in rats (30, 41, 44, 62). GFAP mRNA and protein decrease in intact and adrenalectomized adult male rats after chronic and short-term Cort administration (42, 44). Elevated cortisol levels have been reported in astronauts [one study cites an increase of 300% (59)] and suggest that spaceflight elicits a stress response (26). Thus it seems plausible that chronic Cort elevations during space missions could cause the loss of vulnerable neurons in the hippocampus. On longer space missions, this could cause cognitive impairment or dementia similar to Alzheimer's disease.

In addition to astrocytic GFAP, three neuron-specific proteins were chosen as potential markers for microgravity-induced neurodegeneration. Growth-associated protein-43 (GAP-43), brain-derived neurotrophic factor (BDNF), and α_1-tubulin were selected because of their established functions and known responses to hippocampal lesions. GAP-43, an axonally transported phosphoprotein, is a component of growth cone membranes and might have a function in neurite outgrowth and motility during development and regeneration. After hippocampal deafferentation by simultaneous unilateral entorhinal cortex/fimbria fornix lesion, GAP-43 mRNA increases in both the ipsi- and contralateral hilar and CA3 pyramidal neurons (54). Similarly, α_1-tubulin, which is usually elevated during development, increases in the adult hippocampus after unilateral electrolytic lesioning in the rat entorhinal cortex (47, 48, 72). BDNF, on the other hand, decreases transiently after hippocampal deafferentation (2).

The rats in this study were also treated with bone morphogenetic protein II (BMP). BMP belongs to a family of proteins that induce cartilage and bone formation in vivo (49, 71). BMP is structurally related to the transforming growth factor-β (TGF-β) family. The high-affinity TGF-β receptors that bind BMP are not confined to osteoblastic cells; they are also found on fibroblasts, kidney epithelial cells, keratinocytes, and astrocytes (27). Not surprisingly, BMP has biological functions unrelated to bone and cartilage formation. Synergistic action with tumor necrosis factor stimulates the production of nerve growth factor in fibroblasts (23). Other BMP neurotrophic activity includes differentiation of rat pheochromocytoma PC12 cells (27). BMP also induces differentiation, inhibits proliferation, and can prevent cell death in astrocyte-derived cell lines (9, 10). The present study sought to determine whether BMP would adversely affect neurons in the hippocampus in vivo. These adverse effects could be detected by an induction of astrocyte hypertrophy characteristic of degeneration and elicit a concomitant increase in GFAP. The objective of this study was to determine whether 14-day exposure to microgravity affected the expression of astrocyte-specific GFAP in the adult female rat hippocampus and whether BMP had any effect on GFAP expression.

MATERIALS AND METHODS

Rats. Twenty-four ovariectomized female (30 days after ovariectomy) Fischer 344 rats (200 ± 20 g body wt) were housed in pairs in flight hardware cages at the Kennedy Space Center at a mean temperature of 30.1°C, and food and water were provided ad libitum. With the use of a weight-matched randomization procedure, rats were divided into the following four groups: *1*) vehicle-implanted flight rats, *2*) vehicle-implanted ground controls, *3*) BMP-implanted flight rats, and *4*) BMP-implanted ground controls. Twenty hours before launch, all rats were anesthetized with ketamine (80 mg/kg body wt) and xylazine (4 mg/kg body wt), and a 15-mm incision was made parallel and immediately adjacent to the midline. Four pockets were prepared beneath the ventral panniculus carnosus and adjacent to the abdominal musculature. Two pellets, each containing 100 μg of BMP, and two placebo pellets were implanted into every experimental rat, except for control rats, which received placebo implants only. The pellets were coated with an erodible matrix so that the BMP release was initiated ~72 h after implantation (Innovative Research of America, Toledo, OH). Immediately before launch, all rats received an intraperitoneal implantation of the bone marker calcein (20 mg/kg body wt). The flight rats were flown into space (space shuttle flight PSE4, March 1994) and exposed to microgravity for 14 days. Changes in temperature, pressure, and humidity occurring during the spaceflight were recorded. Ground control rats were exposed to the same environmental conditions. All rats were euthanized by decapitation 20 s after being removed from their cages 2–4 h after touchdown. Brains were dissected from the cranium, frozen at −70°C within 3 min, and sent to the Center for Cell Research, the Pennsylvania State University.

In situ hybridization. One-half of each brain was sectioned (35 μm thick) in the parasagittal plane on a cryostat. Sections from each rat were thaw mounted onto poly-L-lysine subbed slides. A total of 96 sections were coded for blind analysis and underwent in situ hybridization (11). Briefly, sections were fixed in 4% paraformaldehyde in PBS (137 mM NaCl, 2.7 mM KCl, 4.3 mM $Na_2HPO_4 \cdot 7H_2O$, 1.4 mM KH_2PO_4, pH 7.5) for 30 min and were rinsed in PBS. Slides were treated with 0.25% acetic anhydride in 0.1 M triethanolamine and were then dehydrated in an ethanol series immediately before hybridization. Sections were hybridized to ^{35}S-labeled antisense probes for either GFAP, GAP-43, BDNF, or α_1-tubulin. Sections were incubated with probe under coverslips for 3 h in a humidified chamber at 50°C. After hybridization, sections were digested with 20 μg/ml RNase A at 37°C for 30 min and were washed at high criterion in 50% formamide, 0.5 M NaCl, 50 mM sodium phosphate, and 1% β-mercaptoethanol at 55°C for 30 min. The slides were dehydrated in an ethanol series containing 0.3 M NH_4OAc, air dried, and dipped in Kodak NTB-2 photographic emulsion. After 2 wk of exposure at 4°C, sections were developed and counterstained with cresyl violet.

Total GFAP protein ELISA. The GFAP content in the remaining brain halves was assessed by using a sandwich ELISA (56). Hippocampal, cerebellar, striatal, and cortical regions were dissected freehand and homogenized in hot (90+°C) 1% SDS for subsequent assay of total GFAP content.

Immunocytochemistry. Immunocytochemical staining was performed by using a method developed for frozen sections (13). Thaw-mounted frozen sections (35 μm thickness) were placed on a slide warmer, adjusted to the lowest setting, and dried just until the last trace of moisture evaporated. Sections were fixed in 4% paraformaldehyde in PBS for 30 min, thoroughly rinsed in three changes of PBS, and quenched with 0.3% H_2O_2 solution. After three rinses in PBS, sections were boiled in 10 mM citric acid (pH 6.0) in a standard microwave oven at 1-min intervals for 5 min. Nonspecific binding was reduced by incubating sections in 10% horse serum for 3 h. Sections were incubated for 18 h at 4°C in monoclonal anti-GFAP antibody diluted 1:400 in PBS (Boeh-

ringer Mannheim). Sections were incubated in biotinylated secondary antibody diluted 1:200 (Elite Vectastain ABC Mouse IgG Kit, Vector Laboratories) for 1 h at room temperature. The peroxidase bridge was completed by incubating the sections in an avidinbiotin-peroxidase complex solution for 30 min. Immunoreactivity was visualized with 3,3′-diaminobenzidine tetrahydrochloride (10 mg/15 ml, Sigma Chemical) and 0.024% H_2O_2 in 50 mM of Tris buffer (pH 7.6).

Serum Cort. Trunk blood samples were obtained from rats at death for Cort radioimmunoassay.

Data analysis. GFAP mRNA was quantified in the sm and slm of the hippocampus (Fig. 1). BDNF, GAP-43, and α_1-tubulin mRNAs were quantified in the pyramidal cell layer of CA3 and in polymorphic cells of the hippocampal hilus. Bright-field microscopic computer-assisted videodensitometry (Image 1, Universal Imaging) at ×1,250 magnification was employed to count the silver grains over labeled cells. Background counts were subtracted from each labeled cell count. Background measurements were taken from emulsion-coated regions of the slides where no tissue was present. Statistical evaluations were performed by paired *t*-tests.

RESULTS

GFAP mRNA expression. The rats were flown into space (space shuttle flight PSE4, March 1994) and exposed to microgravity for 14 days. All rats were euthanized by decapitation 2–4 h after touchdown. The rats flown on space shuttle flight PSE4 displayed significantly ($P < 0.05$) lower GFAP mRNA expression in the hippocampus compared with ground controls.

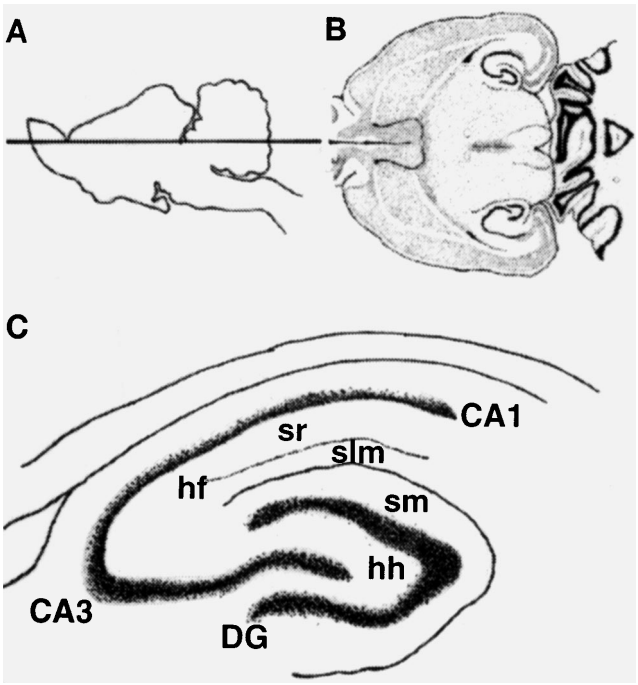

Fig. 1. *A*: parasagittal view of rat brain showing plane of section displayed in *B*. *B*: horizontal section of rat brain showing hippocampus on each posterior lateral margin of cortex just rostral to cerebellum. *C*: close-up view of hippocampus showing pyramidal neuron subregions CA1, CA3, and dentate gyrus (DG). Stratum radiatum (sr), stratum moleculare (sm), and stratum lacunosum moleculare (slm) are the major projection fields of extrinsic hippocampal afferent axons. Other structures shown are hippocampal fissure (hf) and hippocampal hilus (hh).

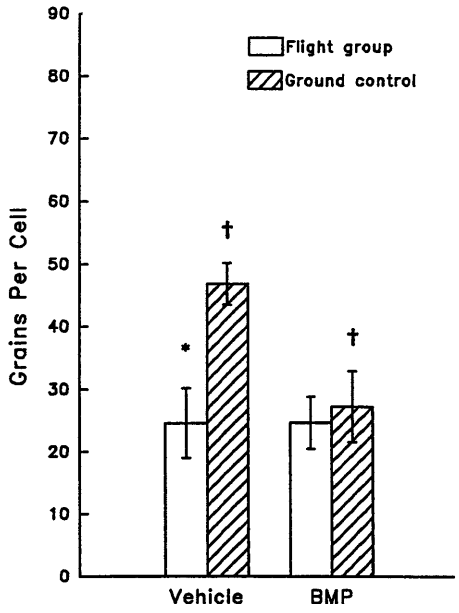

Fig. 2. Glial fibrillary acidic protein (GFAP) mRNA expression in sm of hippocampus. Values are means ± SE; $n = 6$ rats/group: vehicle-implanted flight and ground control rats, and bone morphogenetic protein II (BMP)-implanted flight and ground control rats. *Vehicle-implanted flight rats showed a significant reduction in GFAP mRNA compared with vehicle-implanted ground controls ($P = 0.0058$). †Significant difference between BMP-implanted and vehicle-implanted ground rats ($P = 0.012$).

This difference was seen in both the sm and slm of the hippocampus (Fig. 1). A comparison of vehicle-implanted control rats demonstrated that GFAP mRNA was reduced by 53% in the sm and by 48% in the slm in rats that experienced spaceflight compared with ground controls (Figs. 2 and 3). In contrast, a comparison of

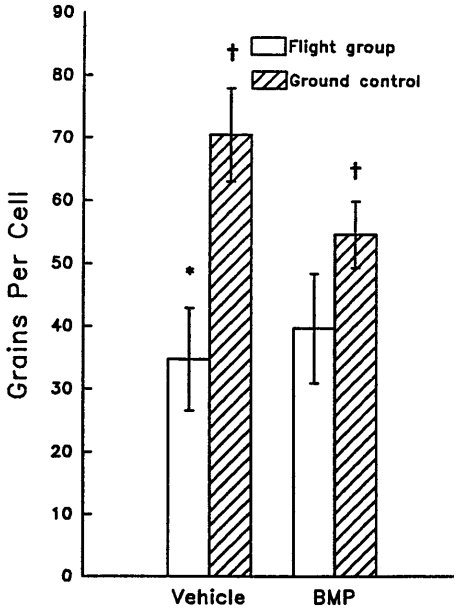

Fig. 3. GFAP mRNA expression in slm of hippocampus. Values are means ± SE; $n = 6$ rats/group. *Significant reduction in mRNA expression in vehicle-implanted flight rats compared with vehicle-implanted ground controls ($P = 0.012$). †Significant difference between BMP-implanted and vehicle-implanted ground rats ($P = 0.012$).

BMP-implanted flight and ground rats did not result in significant differences in GFAP mRNA expression after spaceflight. Furthermore, vehicle controls and BMP-implanted rats flown on the shuttle showed similar levels of GFAP mRNA. Within the groups not flown in space, GFAP mRNA expression in the sm was significantly ($P < 0.05$) reduced in BMP-implanted rats compared with vehicle-implanted controls (Fig. 2). This difference was not seen in the slm (Fig. 3).

GAP-43, BDNF, and α_1-tubulin mRNA expression. A comparison among the four experimental groups did not show any significant differences in either GAP-43, BDNF, or α_1-tubulin mRNA expression in any hippocampal regions (data not shown).

GFAP ELISA. GFAP immunoreactivity in the hippocampus is slightly (4–8%), but not significantly, lower in the flight rats than in ground controls (Fig. 4). BMP did not affect GFAP in ground controls. In addition, similar levels of immunoreactivity were observed in vehicle-implanted ground and vehicle-implanted flight rats. No significant differences were found among any of the groups for the cerebellar, striatal, or cortical regions (data not shown). The data from the immunocytochemically stained tissue sections confirmed the ELISA results (data not shown).

Cort. Serum Cort concentrations were elevated by 56% in vehicle-implanted flight rats compared with vehicle-implanted ground controls (Fig. 5). Cort was also significantly ($P < 0.05$) elevated in BMP-implanted ground rats compared with vehicle-implanted ground controls (Fig. 5). No significant differences in Cort were found between BMP-implanted and vehicle-implanted flight rats.

DISCUSSION

These data show that GFAP mRNA decreases by 53% in the sm and by 48% in the slm in rats flown into space on space shuttle flight PSE4. This decrease demonstrates that this 14-day space mission altered GFAP gene expression in the rat brain. The decrease in GFAP mRNA, however, was not accompanied by a significant decrease in the immunoreactivity or protein content. This suggests that the transcriptional change in GFAP gene expression was transient and might have been stress related.

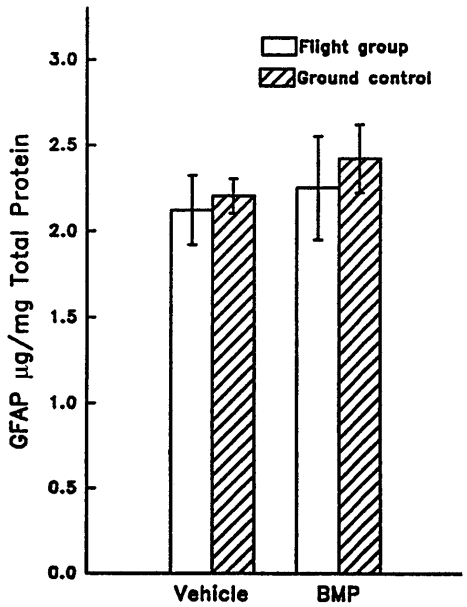

Fig. 4. Total GFAP protein in hippocampal homogenates assayed by ELISA. Values are means ± SE; $n = 6$ rats/group. There were no significant differences among any of the groups.

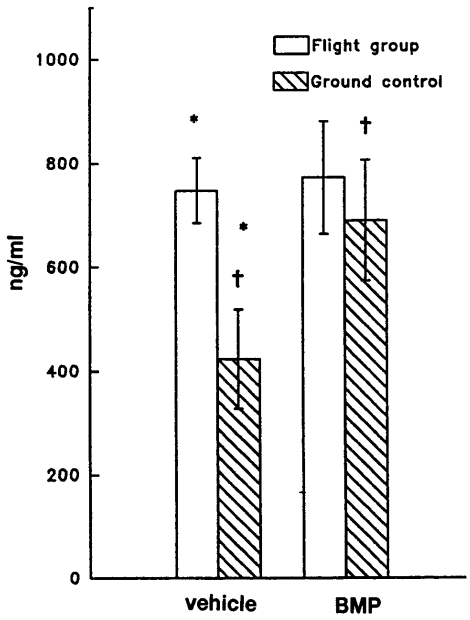

Fig. 5. Serum corticosterone concentrations in 4 experimental groups. Values are means ± SE; $n = 6$ rats/group. *Significant increase in corticosterone in vehicle-implanted flight rats compared with vehicle-implanted ground controls ($P < 0.05$). †Significant rise in serum corticosterone for BMP-implanted ground rats compared with vehicle-implanted ground controls ($P < 0.05$).

Another goal of this study was to determine whether BMP affects GFAP expression. This study found that BMP does not affect GFAP during spaceflight. BMP also does not seem to have an effect on GAP-43, BDNF, or α_1-tubulin expression. No differences were found between BMP-implanted flight and ground rats. However, among ground controls, BMP-implanted rats showed a decrease in GFAP mRNA in the sm and showed elevated serum Cort. This suggests that this TGF-β related protein can attenuate GFAP transcription under normal gravity. Because serum Cort was elevated by 56%, the decrease in GFAP might be Cort mediated. Further studies are required to determine whether the BMP regulation of GFAP mRNA is independent of Cort action. If so, BMP might be therapeutically beneficial for the intervention of neurodegenerative diseases. Otherwise, the benefits of BMP treatment that might be gained by stemming bone loss might be minimal in relation to the Cort-induced neurotoxicity.

Analysis of emulsion-coated autoradiograms did not demonstrate significant differences in the expression of mRNA for BDNF, GAP-43, and α_1-tubulin. GAP-43 is a growth cone protein induced by nerve growth factor, and glucocorticoids can inhibit its expression (6, 18). If there were some stress- and/or microgravity-related degeneration that would induce GAP-43 expression,

the rise in serum Cort could conceivably mask it. Similarly, BDNF has been reported to decrease in response to stress; however, it is not known whether this decrease is Cort mediated (57). No clear correlations between α_1-tubulin and stress and Cort have been established. The fact that no change in mRNA was observed for these neuron-specific proteins suggests that little if any neuron loss occurred. However, without actually counting neurons, it is impossible to say that no neuron loss occurred in these animals.

The decrease in GFAP mRNA was accompanied by a 56% increase in the adrenal glucocorticoid Cort. Although no in-flight blood samples were taken, this marked rise in Cort could be evidence of a stress response associated with a recent event such as reentry or landing. This idea is supported by a previous study, which showed that urinary cortisol of the space shuttle's crew was elevated threefold during landing (59). Other studies reported stress responses to the vibration and deceleration associated with reentry (17). The elevation in Cort might account for the observed decrease in GFAP. Glucocorticoids can alter astrocytic gene expression (31, 41, 62). In particular, GFAP mRNA and protein decrease in intact and adrenalectomized adult male rats after short-term elevations in serum Cort concentrations (42, 44).

According to previous studies, the response of GFAP mRNA and protein to Cort regulation can vary. In the hippocampus of intact or adrenalectomized rats, GFAP mRNA decreases by 50% within 8–32 h after glucocorticoid administration (40, 42). In contrast, a 5-day exposure to Cort reduced GFAP protein by 20–40% in similarly treated adult male rats (45). The 50% decrease in GFAP mRNA noted in the present study is consistent with these reports.

No significant change in the protein content or immunoreactivity was observed in the present study. One possible explanation is that the time between landing and death might have been too brief to allow for significant changes in GFAP protein content. This interval might have been too short for Cort to reduce GFAP more than the 4–8% observed in this study compared with the 20–40% reported after 5 days (45). In support of this, the turnover of GFAP is estimated to span from several days to several weeks (12, 58). A previous study reported that 40% of the radioactivity incorporated into GFAP in mouse spinal cord in vivo is still present after 9 wk (12). A similar result was obtained by using cultured astrocytes, for which the half-life of GFAP was reported to be ~1 wk (7). Because GFAP mRNA always shows an earlier and larger change than GFAP protein, the small change in GFAP mRNA found in the brains of spaceflight rats would not be expected to show any corresponding change in GFAP protein. This dissociation between GFAP protein and GFAP mRNA is typically observed after trauma (25) and Cort replacement after adrenalectomy (45). Unfortunately, no blood or tissue samples were collected during space shuttle flight PSE4. Analysis of these samples would have shown the profile of Cort secretion during spaceflight. In addition to the possible effects of reentry, handling of the rats before death can cause stress. This seems unlikely, however, since the ground controls, which were handled in the same manner, did not exhibit the increase in Cort concentration.

Using human subjects, Stein and Schluter (59) reported rapid endocrine adaptation to the microgravitational field. Urinary interleukin-6 and cortisol excretions drastically increased only on the first day of spaceflight, suggesting that the initiation of spaceflight constitutes an acute rather than a chronic stress response. This acute stress response might be masking other effects of microgravity and spaceflight. These concerns have been expressed by other investigators (17, 28, 64).

In contrast to dentate granule cells, which are dependent on glucocorticoids for survival during development (21, 55), certain pyramidal cells of the hippocampus (CA1) are vulnerable to adrenal steroids in adulthood. Repeated stress and/or elevations of glucocorticoids decrease the number of apical dendritic branch points (69, 70) and cause pyramidal neuron loss in the hippocampus (53). This neuron loss is thought to result from impaired energy metabolism and might include astrocyte dysfunction or damage. Glial cells have been reported to play neurotrophic and neuroprotective roles in vitro (61, 65). Cort has been shown to inhibit glucose transport and glutamate uptake by hippocampal astrocytes (67). Thus the decrease in GFAP mRNA observed in the present study might reflect a weakened cellular energy potential secondary to neuronal excitotoxicity. This type of excitotoxic damage might take several months to develop. Clearly, there was no evidence of excitotoxicity-induced gliosis in this short spaceflight study. Studies of long-term spaceflight are needed to determine whether excitotoxicity is a consequence of microgravity.

In summary, the decrease in GFAP mRNA suggests that spaceflight and/or exposure to microgravity can alter gene expression in hippocampal astrocytes. This decrease might be mediated by stress-induced elevations in circulating glucocorticoids. BMP treatment does not alter the gene expression of the markers employed in this study. However, under conditions of normal gravity, BMP treatment might increase serum Cort and indirectly attenuate GFAP mRNA. The results of this study also demonstrate the need for in-flight data collection to elucidate the specific effects of microgravity on neurodegeneration.

Physiological modifications observed during space missions reflect responses to a variety of factors besides reduced gravity. The rats in this study were also subjected to changes in electromagnetic fields, background radiation, vibration, and altered day-night cycles (38). Attempts have been made to simulate microgravity in the laboratory. Hindlimb suspension, water submersion, and head-down tilting experiments are commonly used. The validity of these types of studies is limited because they tend to focus only on one aspect of the microgravitational field such as hindlimb muscle atrophy. Hence, to study the specific effects of space-

flight and microgravity on mammalian CNS, it is necessary to collect in-flight data.

This study was funded by grants from the American Federation for Aging Research in conjunction with the Glenn Foundation and from the National Science Foundation (IBN-9511869) (to J. R. Day).

We thank the National Aeronautics and Space Administration and Dr. William W. Willfinger from the Center for Cell Research at The Pennsylvania State University for providing us with the tissues. We also thank Elizabeth H. Boykin for technical assistance, Dr. William Kramer from the Center for Sports Medicine at The Pennsylvania State University for providing us with the serum corticosterone data, and Asha Patel for original artwork.

Address for reprint requests: J. R. Day, Dept. of Biology and Gerontology Center, 208 Erwin Mueller Laboratory, The Pennsylvania State Univ., University Park, PA 16802 (E-mail jrd6@psu.edu).

Received 20 January 1998; accepted in final form 6 April 1998.

REFERENCES

1. **Aus der Muhlen, K., and H. Ockenfels.** Morphologische veraenderungen im deincephalon und telencephalon nach storungen des regelkreises adenohypophyse-nebennierenrinde III. Ergebnisse beim meerschweinchen nach verabreichung von cortison und hydrocortisone. *Z. Zellforsch. Mikrosk. Anat.* 93: 126–138, 1969.
2. **Beck, K. D., F. Lamballe, R. Klein, M. Barbacid, P. E. Schauwecker, T. H. McNeill, C. E. Finch, F. Hefti, and J. R. Day.** Induction of noncatalytic TrkB neurotrophin receptors during axonal sprouting in the adult hippocampus. *J. Neurosci.* 13: 4001–4014, 1993.
3. **Berry, C. A.** Summary of medical experience in the Apollo 7 through 11 manned spaceflights. *Aerospace Med.* 41: 500–519, 1970.
4. **Canady, K. S., R. L. Hyson, and E. W. Rubel.** The astrocytic response to afferent activity blockade in chick nucleus magnocellularis is independent of synaptic activation, age, and neuronal survival. *J. Neurosci.* 14: 5973–5985, 1994.
5. **Canady, K. S., J. F. Olavarria, and E. W. Rubel.** Reduced retinal activity increases GFAP immunoreactivity in rat lateral geniculate nucleus. *Brain Res.* 663: 206–214, 1994.
6. **Chao, H. M., and B. S. McEwen.** Glucocorticoids and the expression of mRNAs for neurotrophins, their receptors and GAP-43 in the rat hippocampus. *Brain Res. Mol. Brain Res.* 26: 271–276, 1994.
7. **Chiu, F., and J. Goldman.** Synthesis and turnover of cytoskeletal proteins in cultured astrocytes. *J. Neurochem.* 42: 166–174, 1984.
8. **Correia, M. J., A. A. Perachio, J. D. Dickman, I. B. Kozlovskaya, M. G. Sirota, S. B. Yakushin, and I. N. Beloozerova.** Changes in monkey horizontal semicircular canal afferent responses after spaceflight. *J. Appl. Physiol.* 73, *Suppl.*: 112S–120S, 1992.
9. **D'Alessandro, J. S., and E. A. Wang.** Bone morphogenetic proteins inhibit proliferation, induce reversible differentiation and prevent cell death in astrocyte lineage cells. *Growth Factors* 11: 45–52, 1994.
10. **D'Alessandro, J. S., J. Yetz-Aldape, and E. A. Wang.** Bone morphogenetic proteins induce differentiation in astrocyte lineage cells. *Growth Factors* 11: 53–69, 1994.
11. **Day, J. R., N. J. Laping, T. H. McNeill, S. S. Schreiber, G. Pasinetti, and C. E. Finch.** Castration enhances expression of GFAP and SGP-2 in the intact and lesion-altered hippocampus of the adult male rat. *Mol. Endocrinol.* 4: 1995–2002, 1990.
12. **DeArmond, S. J., Y. L. Lee, H. A. Kretzschmar, and L. F. Eng.** Turnover of glial filaments in mouse spinal cord. *J. Neurochem.* 47: 1749–1753, 1986.
13. **DeHart, B. W., R. K. Kan, and J. R. Day.** Microwave superheating enhances immunocytochemistry in the freshly frozen rat brain. *Neuroreport* 7: 2691–2694, 1996.
14. **D'yachkova, L. N.** Ultrastructural characteristics of plastic changes in the brain cortex of rats exposed to space flight. *Physiologist* 34: S185–S186, 1991.
15. **D'yachkova, L. N., and V. N. Sluch.** Ultrastructure of the cerebral cortex of white rats exposed to a 2-week space flight (Abstract). *USSR Space Life Sci. Dig.* 31: 67, 1991.
16. **Eng, L. F.** Glial fibrillary acidic protein (GFAP): the major protein of glial intermediate filaments in differentiated astrocytes. *J. Neuroimmunol.* 8: 203–214, 1985.
17. **Fareh, J., J. M. Cottet-Emard, J. M. Pequignot, G. Jahns, J. Meylor, M. Viso, D. Vassaux, G. Gauquelin, and C. Gharib.** Norepinephrine content in discrete brain areas and neurophysiological vasopressin in rats after a 9-d spaceflight (SLS-1). *Aviat. Space Environ. Med.* 64: 507–511, 1993.
18. **Federoff, H. J., E. Grabczyk, and M. C. Fishman.** Dual regulation of GAP-43 gene expression by nerve growth factor and glucocorticoids. *J. Biol. Chem.* 263: 19290–19295, 1988.
19. **Fuchs, H. S.** Man in weightlessness: physiological problems, clinical aspects, prevention and protection. *Riv. Med. Aeronaut. Spaz.* 43: 332–346, 1980.
20. **Goss, J. R., and D. G. Morgan.** Enhanced glial fibrillary acidic protein RNA response to fornix transection in aged mice. *J. Neurochem.* 64: 1351–1360, 1995.
21. **Gould, E., C. S. Woolley, and B. S. McEwen.** Short-term glucocorticoid manipulations affect neuronal morphology and survival in the adult dentate gyrus. *Neuroscience* 37: 367–375, 1990.
22. **Grindeland, R. E., I. A. Popova, M. Vasques, and S. B. Arnaud.** Cosmos 1887 mission overview: effects of microgravity on rat body and adrenal weights and plasma constituents. *FASEB J.* 4: 105–109, 1990.
23. **Hattorl, A., M. Tsujimoto, K. Hayashi, and M. Kohno.** Bone morphogenetic protein-2 is markedly synergistic with tumor necrosis factor in stimulating the production of nerve growth factor in fibroblasts. *Biochem. Mol. Biol. Int.* 38: 1095–1101, 1996.
24. **Holley, D. C., M. R. Soliman, F. Kaddis, C. L. Markley, and I. Krasnov.** Pineal physiology in microgravity: relation to rat gonadal function aboard Cosmos 1887. *Aviat. Space Environ. Med.* 62: 953–958, 1991.
25. **Hozumi, I., D. A. Aquino, and W. T. Norton.** Biochemical and immunocytochemical changes in GFAP after stab wounds. *Brain Res.* 524: 64–71, 1990.
26. **Huntoon, C. L., P. C. Johnson, and N. M. Cintron.** Hematology, immunology, endocrinoloy and biochemistry. In: *Space Medicine and Physiology*, edited by A. E. Nicogossian, C. L. Huntoon, and S. L. Pool. Philadelphia, PA: Lea & Febiger, 1989, p. 222–239.
27. **Iwasaki, S., A. Hattori, M. Sato, M. Tsujimoto, and M. Kohno.** Characterization of the bone morphogenetic protein-2 as a neurotrophic factor. Induction of neuronal differentiation of PC12 cells in the absence of mitogen-activated protein kinase activation. *J. Biol. Chem.* 271: 17360–17365, 1996.
28. **Kvetnansky, R., J. Culman, L. V. Serova, R. A. Tigranian, T. Torda, and L. Macho.** Catecholamines and their enzymes in discrete brain areas of rats after space flight on biosatellites cosmos. *Acta Astronaut.* 10: 295–300, 1983.
29. **Lackner, J. R., and A. Graybiel.** Variations in gravitoinertial force level affect the gain of the vestibulo-ocular reflex: implications for the etiology of space motion sickness. *Aviat. Space Environ. Med.* 52: 154–158, 1981.
30. **Landfield, P. W., J. C. Waymire, and G. Lynch.** Hippocampal aging and adrenocorticoids: quantitative correlations. *Science* 202: 1098–1102, 1978.
31. **Laping, N. J., B. Teter, N. R. Nichols, I. Rozovsky, and C. E. Finch.** Glial fibrillary acidic protein: regulation by hormones, cytokines, and growth factors. *Brain Pathol.* 4: 259–275, 1994.
32. **Lelkes, P. I., E. M. Ramos, D. M. Chick, J. Liu, and B. R. Unsworth.** Microgravity decreases tyrosine hydroxylase expression in rat adrenals. *FASEB J.* 8: 1177–1182, 1994.
33. **Lindsay, R. M.** Reactive gliosis. In: *Astrocytes: Cell Biology and Pathology of Astrocytes*, edited by S. Fedoroff and A. Vernadakis. Orlando, FL: Academic, 1986, vol. 3, p. 231–262.
34. **Linnemann, D., and T. Skarsfelt.** Regional changes in expression of NCAM, GFAP, and S100 in aging rat brain. *Neurobiol. Aging* 15: 651–655, 1994.
35. **Major, D. E., J. P. Kesslak, C. W. Cotman, C. E. Finch, and J. R. Day.** Life-long dietary restriction attenuates age-related

increases in hippocampal glial fibrillary acidic protein mRNA. *Neurobiol. Aging* 18: 523–526, 1997.
36. **Miller, J. D., B. A. McMillen, M. M. McConnaughey, H. L. Williams, and C. A. Fuller.** Effects of microgravity on brain neurotransmitter receptors. *Eur. J. Pharmacol.* 161: 165–171, 1989.
37. **Moore, T. P., and W. E. Thornton.** Space shuttle inflight and postflight fluid shifts measured by leg volume changes. *Aviat. Space Environ. Med.* 58: A91–A96, 1987.
38. **Newberg, A. B.** Changes in the central nervous system and their clinical correlates during long-term spaceflight. *Aviat. Space Environ. Med.* 65: 562–572, 1994.
39. **Nichols, N. R., J. R. Day, N. J. Laping, S. A. Johnson, and C. E. Finch.** GFAP mRNA increases with age in rat and human brain. *Neurobiol. Aging* 14: 421–429, 1993.
40. **Nichols, N. R., J. N. Masters, and C. E. Finch.** Changes in gene expression in hippocampus in response to glucocorticoids and stress. *Brain Res. Bull.* 24: 659–662, 1990.
41. **Nichols, N. R., J. N. Masters, P. C. May, J. de Vellis, and C. E. Finch.** Corticosterone-induced responses in rat brain RNA are also evoked in hippocampus by acute vibratory stress. *Neuroendocrinology* 49: 40–46, 1989.
42. **Nichols, N. R., H. H. Osterburg, J. N. Masters, S. L. Millar, and C. E. Finch.** Messenger RNA for glial fibrillary acidic protein is decreased in rat brain following acute and chronic corticosterone treatment. *Brain Res. Mol. Brain Res.* 7: 1–7, 1990.
43. **Nicogossian, A. E., C. L. Huntoon, and S. Pool.** *Space Physiology and Medicine* (2nd ed.). Philadelphia, PA: Lea & Febiger, 1989.
44. **O'Callaghan, J. P., R. E. Brinton, and B. S. McEwen.** Glucocorticoids regulate the concentration of glial fibrillary acidic protein throughout the brain. *Brain Res.* 494: 159–161, 1989.
45. **O'Callaghan, J. P., R. E. Brinton, and B. S. McEwen.** Glucocorticoids regulate the synthesis of glial fibrillary acidic protein in intact and adrenalectomized rats but do not affect its expression following brain injury. *J. Neurochem.* 57: 860–869, 1991.
46. **O'Callaghan, J. P., and D. B. Miller.** The concentration of glial fibrillary acidic protein increases with age in the mouse and rat brain. *Neurobiol. Aging* 12: 171–174, 1991.
47. **Poirier, J., D. Dea, A. Baccichet, and S. Gauthier.** Modulation of gamma-actin and alpha 1-tubulin expression by corticosterone during neuronal plasticity in the hippocampus. *Brain Res. Mol. Brain Res.* 15: 263–268, 1992.
48. **Poirier, J., M. Hess, P. C. May, and C. E. Finch.** Cloning of hippocampal poly (A) RNA sequences that increase after entorhinal cortex lesion in adult rat. *Brain Res. Mol. Brain Res.* 9: 191–195, 1991.
49. **Reddi, A. H., and C. B. Huggins.** Biochemical sequences in the transformation of normal fibroblasts in adolescent rats. *Proc. Natl. Acad. Sci. USA* 69: 1601–1605, 1972.
50. **Reschke, M. F., and D. E. Parker.** Effects of prolonged weightlessness on self-motion perception and eye movements evoked by roll and pitch. *Aviat. Space Environ. Med.* 58: A153–A158, 1987.
51. **Ross, M. D.** A spaceflight study of synaptic plasticity in adult rat vestibular maculas. *Acta Otolaryngol. Suppl. (Stockh.)* 516: 1–14, 1994.
52. **Sapolsky, R. M., L. C. Krey, and B. S. McEwen.** Prolonged glucocorticoid exposure reduces hippocampal neuron number: implications for aging. *J. Neurosci.* 5: 1222–1227, 1985.
53. **Sapolsky, R. M.** *Stress, the Aging Brain, and the Mechanisms of Neuron Death*. Cambridge, MA: MIT Press, 1992.
54. **Schauwecker, P. E., H. W. Cheng, R. M. Serquinia, N. Mori, and T. H. McNeill.** Lesion-induced sprouting of commissural/associational axons and induction of GAP-43 mRNA in hilar and CA3 pyramidal neurons in the hippocampus are diminished in aged rats. *J. Neurosci.* 15: 2462–2470, 1995.
55. **Sloviter, R. S., G. Valiquette, G. M. Abrams, E. C. Ronk, A. L. Sollas, L. A. Paul, and S. Neubort.** Selective loss of hippocampal granule cells in the mature rat brain after adrenalectomy. *Science* 243: 535–538, 1989.
56. **Smith, P. K., R. I. Krohn, G. T. Hermanson, A. K. Mallia, F. H. Gartner, M. D. Provenzano, E. K. Fujimoto, N. M. Goeke, B. J. Olson, and D. C. Klenk.** Measurement of protein using bicinchoninic acid. *Anal. Biochem.* 150: 76–85, 1985.
57. **Smith, M. A., S. Makino, R. Kvetnansky, and R. M. Post.** Stress and glucocorticoids affect the expression of brain-derived neurotrophic factor and neurotrophin-3 mRNAs in the hippocampus. *J. Neurosci.* 15: 1768–1777, 1995.
58. **Smith, M. E., V. Perret, and L. F. Eng.** Metabolic studies in vitro of the CNS cytoskeletal proteins: synthesis and degradation. *Neurochem. Res.* 9: 1493–1507, 1984.
59. **Stein, T. P., and M. D. Schluter.** Excretion of IL-6 by astronauts during spaceflight. *Am. J. Physiol.* 266 (*Endocrinol. Metab.* 29): E448–E452, 1994.
60. **Steward, O., E. R. Torre, R. Tomasulo, and E. Lothman.** Seizures and the regulation of astroglial gene expression. The dentate gyrus and its role in seizures. *Epilepsy Res.* 7: 197–209, 1992.
61. **Sugiyama, K., A. Brunori, and M. L. Mayer.** Glial uptake of excitatory amino acids influences neuronal survival in cultures of mouse hippocampus. *Neuroscience* 32: 779–791, 1989.
62. **Tardy, M., B. Rolland, C. Fages, and M. Caldani.** Astroglial cells: glucocorticoid target cells in the brain. *Clin. Neuropharmacol.* 7: 296–302, 1984.
63. **Thornton, W. E., and J. A. Rummel.** Muscular deconditioning and its prevention in space flight. In: *Biomedical Results from Skylab*, edited by R. S. Johnston and L. F. Dietlein. Washington, DC: US Government Printing Office, 1977. (NASA SP-377)
64. **Tigranyan, R. A., L. Macho, R. Kvetnansky, S. Nemeth, and N. F. Kalita.** Stress in space flight: metabolic aspects. *Physiologist* 23: S45–S50, 1980.
65. **Vibulsreth, S., F. Hefti, M. D. Ginsberg, W. D. Dietrich, and R. Busto.** Astrocytes protect cultured neurons from degeneration induced by anoxia. *Brain Res.* 422: 303–311, 1987.
66. **Vielkind, U., A. Walencewicz, J. M. Levine, and M. C. Bohn.** Type II glucocorticoid receptors are expressed in oligodendrocytes and astrocytes. *J. Neurosci. Res.* 27: 360–373, 1990.
67. **Virgin, C. E., Jr., T. P. Ha, D. R. Packan, G. C. Tombaugh, S. H. Yang, H. C. Horner, and R. M. Sapolsky.** Glucocorticoids inhibit glucose transport and glutamate uptake in hippocampal astrocytes: implications for glucocorticoid neurotoxicity. *J. Neurochem.* 57: 1422–1428, 1991.
68. **Vorobyov, E. I., O. G. Gazenko, A. M. Genin, and A. D. Egorov.** Medical results of Salyut-6 manned space flights. *Aviat. Space Environ. Med.* 54: S31–S40, 1983.
69. **Watanabe, Y., E. Gould, and B. S. McEwen.** Stress induces atrophy of apical dendrites of hippocampal CA3 pyramidal neurons. *Brain Res.* 588: 341–345, 1992.
70. **Woolley, C. S., E. Gould, and B. S. McEwen.** Exposure to excess glucocorticoids alters dendritic morphology of adult hippocampal pyramidal neurons. *Brain Res.* 531: 225–231, 1990.
71. **Wozney, J. M., V. Rosen, A. J. Celeste, L. M. Mitsock, M. J. Whitters, R. W. Kriz, M. H. Rodney, and E. A. Wang.** Novel regulators of bone formation: molecular clones and activities. *Science* 242: 1528–1534, 1988.
72. **Zarow, C., and C. E. Finch.** Limited responses of neuronal mRNAs to unilateral lesions of the rat entorhinal cortex. *Neurosci. Lett.* 185: 87–90, 1995.