Stress Differentially Regulates the Effects of Voluntary Exercise on Cell Proliferation in the Dentate Gyrus of Mice

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ABSTRACT: It has been well-established that cell proliferation and neurogenesis in the adult mouse dentate gyrus (DG) can be regulated by voluntary exercise. Recent evidence has suggested that the effects of voluntary exercise can in turn be influenced by environmental factors that regulate the amount of stress an animal is exposed to. In this study, we use bromodeoxyuridine and proliferating cell nuclear antigen immunohistochemistry to show that voluntary exercise produces a significant increase in cell proliferation in the adult mouse DG in both isolated and socially housed mice. This effect on proliferation translates into an increase in neurogenesis and neuronal branching of new neurons in the mice that exercised. Although social condition did not regulate proliferation in young adult mice, an effect of social housing could be observed in mice exposed to acute restraint stress. Surprisingly, only exercising mice housed in isolated conditions showed an increase in cellular proliferation following restraint stress, whereas socially housed, exercising mice, failed to show a significant increase in proliferation. These findings indicate that social housing may increase the effects of any stressful episodes on hippocampal neurogenesis in the mouse DG.

KEY WORDS: neurogenesis; dentate gyrus; stress; cell proliferation; mice

INTRODUCTION

Exercise has long been associated with a number of positive benefits for the body, including weight management, improving stamina, and even combating chronic diseases such as high blood pressure and cholesterol. The benefits of exercise are also apparent in the central nervous system, where exercise can protect against neurodegenerative disorders, facilitate learning and memory, and alleviate symptoms of psychiatric disorders such as depression and anxiety (Barbour et al., 2007; Cotman et al., 2007). One region of the brain that is dramatically affected by exercise is the dentate gyrus (DG) subfield of the hippocampus. The DG is known to be intimately involved in learning and memory processes and is one of the few regions of the brain that maintains the ability to produce a substantial number of new neurons well into adulthood (Cameron and McKay, 2001). Indeed, the very existence of adult neurogenesis indicates that the hippocampus may possess an innate regenerative capacity that, once understood, could be harnessed for other regenerative purposes. In corroboration of this hypothesis that exercise can benefit neuronal function, adult neurogenesis can be enhanced markedly in rats and mice if they engage in exercise (van Praag et al., 1999a,b; Farmer et al., 2004; Eadie et al., 2005). Interestingly, both forced and voluntary exercise can increase hippocampal neurogenesis (van Praag et al., 1999b; Farmer et al., 2004; Overstreet et al., 2004; Llorens-Martin et al., 2006; Redila et al., 2006; Uda et al., 2006), although a recent study conducted in rats have raised the question as to whether social and/or environmental factors might modulate the ability of exercise to enhance neurogenesis in the adult DG (Stranahan et al., 2006).

This controversy may, in part, reflect differences in the social habits between rodent species. Social preferences in rats differ from those in mice, a more common tool for biological research where genetic manipulations are required. To date, the effects of voluntary exercise on neurogenesis has been examined in group housed male mice (Fabel et al., 2003; Kitamura et al., 2003; Kronenberg et al., 2005), group housed female mice (van Praag et al., 1999a,b; Brown et al., 2003), as well as isolated mice (Holmes et al., 2004; van Praag et al., 2005). Male mice prefer to be socially housed when given preference tests; furthermore, the preference to be socially housed is stronger than a preference for more standard environmental enrichment (Van Loo et al., 2003; Van Loo et al., 2004). Although rats also show a preference towards social housing, this preference does not appear to be as strong in comparison to other types of enrichment (Patterson-Kane et al., 2001; Patterson-Kane et al., 2004). The strong social preference in mice, but not rats, suggests that exercise-induced effects on the mouse DG may differ from those in the rat DG.

Differences in housing preference between rodent species may also reflect how stressful the animals perceive the housing condition to be. Stress is a complex response that can alter a number of physiological processes, including levels of corticosterone (CORT), a hormone commonly associated with the stress response. Elevated CORT levels stimulated through...
either stress (Gould et al., 1997; Gould et al., 1998) or directly via CORT application (Wong and Herbert, 2006) can produce a dramatic decrease in cell proliferation and neurogenesis in the DG. Interestingly, voluntary exercise enhances cell proliferation and neurogenesis (van Praag et al., 1999b; van Praag et al., 2005) while also increasing CORT levels. This suggests that voluntary exercise might be protective against some deleterious effect of CORT on neurogenesis. In the current study, we examined how the effects of voluntary exercise on neurogenesis in the mouse DG are influenced by both a mild stressor (social isolation), and a more pronounced stressor (acute restraint stress).

**MATERIALS AND METHODS**

**Animals and Housing Conditions**

Male C57BL/6J mice (2-month-old, 23–25 g; Charles River, QC, Canada) were individually/group housed in standard cages in a colony maintained at 21°C. Mice were maintained on a 12 h light/dark cycle with access to food and water ad libitum. Following a 1 week acclimatization period, mice were assigned to one of two housing conditions: isolated (I) or social (S) containing three animals per cage. Following a 1 week period in these respective housing conditions, animals were assigned to one of the two groups: voluntary exercise runners (RUN) that did not contain a running wheel and sedentary control (CON) that contained a running wheel. To accommodate for the size of the running wheel, all mice were housed in large cages (46 cm × 24 cm × 20 cm). In total, this yielded four groups: isolated controls (I-CON), isolated runners (I-RUN), social controls (S-CON), and social runners (S-RUN). Group-housed mice were carefully monitored for signs of aggression throughout the experiment. If any mice displayed aggression and/or signs of injury, the cohort of animals in the cage was removed from the study; in the present study, a group of three mice were excluded. All procedures were approved by the University of British Columbia Animal Care Committee and in accordance with the Canada Council on Animal Care.

**Bromodeoxyuridine (BrdU) Administration and Tissue Preparation**

Mice were maintained in running/control housing conditions for 12 days, as outlined in Figure 1A. On Day 12, animals received a single intraperitoneal (IP) injection of bromodeoxyuridine (BrdU; 200 mg/kg), a thymidine analog that is incorporated into the DNA of cells during S-phase. Mice were removed from their cage for an average of 30 s for BrdU injection. Two hours following BrdU administration, subjects were deeply anesthetized with urethane and trunk blood samples were collected (2–3 h after the onset of the night cycle). An additional set of mice used for proliferating cell nuclear antigen (PCNA) immunohistochemistry were not administered BrdU injections. Mice were perfused transcardially with 0.9% saline, followed by 4% paraformaldehyde. Brains were postfixed in 4% paraformaldehyde for 24 h before being transferred to 30% sucrose. Twelve series of coronal sections were cut throughout the entire hippocampus using a Leica VT1000 vibratome at a thickness of 40 μm and stored in 0.1 M tris-buffered saline (TBS; 0.15 NaCl, 0.1M Tris-HCl, pH 7.5) at 4°C.

**Restraint Stress**

In restraint stress experiments, mice were placed in restraint tubes for 15 min at the time of BrdU administration, as outlined in Figure 2A. Restraint tubes were made from plastic 50 ml Falcon tubes with small holes drilled in them to facilitate gas exchange. Tissue preparation was carried out as described earlier.

**Immunohistochemistry**

A 1:12 series of brain sections were processed for immunohistochemical detection of BrdU-labeled cells as previously described (Farmer et al., 2004; Christie et al., 2005; Eadie et al., 2005; Redila et al., 2006). Briefly, free floating brain sections were first washed in 0.1 M TBS. To block endogenous peroxidase activity, tissue was immersed in 0.6% H2O2 in TBS for 30 min. Sections were then placed in 50% formamide/2× SSC (0.3 M NaCl and 0.3 M sodium citrate) at 65°C for 2 h, rinsed for 5 min in 2× SSC, then incubated in 2 N HCl at 37°C for 30 min. Sections were placed in 0.1 M boric acid (pH 8.5) for acid neutralization. The tissue was then rinsed six times in TBS for a total of 90 min and incubated in TBS++ (0.1% Triton X-100 and 3% donkey serum in 0.1 M TBS). Sections were incubated with a biotinylated monoclonal anti-mouse BrdU antibody (diluted 1:100; MAB3262B, Chemicon, Temulcula, CA) overnight at 4°C. Following antibody incubation, tissue was rinsed in TBS and an avidin-biotin-peroxidase solution (ABC Elite Kit, Vector Laboratories, Burlingame, CA) was applied for 2 h. Tissue was rinsed and treated with a peroxidase detection kit using 3,3-diaminobenzidine (DAB) as the chromogen (DAB Kit, Vector Laboratories) according to manufacturers’ directions. The sections were then mounted on 2% gelatin coated slides. A cresyl violet background stain was applied prior to samples being cover-slipped for microscopic analysis.

From a separate set of mice not subjected to BrdU injection, an additional series of free-floating brain sections were processed for immunohistochemistry against the endogenous cell cycle marker PCNA, using a similar protocol to one previously described (Gil et al., 2005). Antigen retrieval was achieved by incubating brain sections in 10 mM sodium citrate buffer (in TBS, pH 6.0) at 95°C for 5 min. To completely unmask the antigens, this step was repeated twice (sections were allowed to cool for ~20 min in between). Sections were then quenched in 3% H2O2/10% methanol in 0.1 M TBS for 15 min. After preincubation with 5% normal goat serum for 1 h, the sections were incubated with a rabbit polyclonal antibody against PCNA (diluted 1:100; Santa Cruz Biotechnology, Santa Cruz, CA).
FIGURE 1. Voluntary exercise promotes cellular proliferation, irrespective of social condition. (A) Schematic drawing of the experimental paradigm and BrdU injection protocol. Exercising mice were allowed access to running wheels for 12 days. On Day 12, mice were administered BrdU and perfused 2 h later; d: day. (B–E) Representative pictures of BrdU-labeled cells in the mouse DG of isolated controls (I-CON, B), isolated runners (I-RUN, C), social controls (S-CON, D), and social runners (S-RUN, E). Scale bar: 100 μm. (F) Both isolated and social running groups (black bars) demonstrate increases in the amount of BrdU-labeled cells in the mouse DG in comparison to their sedentary controls (white bars). (G) Corticosterone levels obtained from trunk blood of animals administered BrdU shows no effects of exercise (black bars = running; white bars = sedentary controls) or social condition. (H) Labeling of the endogenous marker PCNA confirms that cellular proliferation is promoted in isolated and social running groups (black bars) in comparison to sedentary controls (white bars). (I) Corticosterone levels of animals not subjected to BrdU injection demonstrate no main effects of exercise (black bars = running; white bars = sedentary controls) or social condition. Error bars represent SEM, (*) denotes significance ($P < 0.05$) in comparison to controls (two-way ANOVA and Fisher post-hoc test).
CA) for 24 h at room temperature. The sections were then incubated with the secondary antibody biotinylated goat anti-rabbit IgG (diluted 1:200; Vector Laboratories), and visualized with an avidin-biotin-peroxidase complex system (Vectastain ABC Elite Kit, Vector Laboratories) using DAB as the chromogen (DAB Kit, Vector Laboratories). The sections were then mounted on 2% gelatin coated slides, dehydrated, and coverslipped.

To determine the rate of neurogenesis in these animals, we performed immunohistochemistry against Doublecortin (DCX), a cytoskeletal protein that is expressed exclusively in migrating immature neurons or neuroblasts (Rao and Shetty, 2004). DCX is thought to be expressed in immature hippocampal granule cells for ~3 weeks after cell division (Phillips et al., 2005). A 1:6 series of brain sections were processed for DCX immunohistochemistry as previously described (Spanswick et al., 2007). Sections were washed in 0.1 M TBS and incubated at room temperature for 16 h in goat anti-DCX polyclonal primary antibody (diluted 1:1,000; Jackson ImmunoResearch Labs, Baltimore, MD) in 0.1 TBS, 0.5% Triton-X, and 2% normal rabbit serum at 4°C. Sections were washed three times for 10 min in 0.1 M TBS, then incubated in secondary antibody biotinylated rabbit antigoat IgG (Chemicon; diluted 1:1,000) for 1 h at room temperature. Tissue was then rinsed and the bound antibodies were visualized with an avidin-biotin-peroxidase complex system using DAB as the chromogen (Vector Laboratories). The sections were then mounted on 2% gelatin coated slides, dehydrated, and coverslipped.

**Quantification**

All morphological analyses were performed using coded slides with the experimenter kept blind as to the animal experimental group. The number of BrdU- and PCNA-immunopositive cells in the entire hippocampus was assessed by manually counting all positive cells in the granular cell layer (GCL) of the DG in both the left and right hemispheres using an Olympus BX50 microscope with a 100× objective lens. Cells within the subgranular zone, defined as the area within two cell bodies (~20 μm) of the inner edge of the GCL, were combined with the GCL for quantification. Split nuclei cells that had not finished cytokinesis were counted as one cell for a conservative estimate. The distance between the sections was ~440 μm. A modified stereological approach, which has been shown to

**FIGURE 2.** Acute restraint stress selectively modulates proliferation in exercising mice. (A) Schematic drawing of the experimental paradigm and BrdU injection protocol was similar to those used previously, except that a 15 min restraint stress period was introduced immediately after BrdU administration. Mice were perfused 2 h after BrdU administration; d, day. (B) BrdU-labeled cells in mice subjected to 15 min of restraint stress. Isolated runners subjected to acute stress still show a significant increase in cellular proliferation in comparison to their sedentary controls. This effect is not observed in social runners. (C) Comparison of BrdU-labeled cells in nonstressed mice from Figure 1 (white bars) and those subjected to restraint stress (black bars). Decreases in cellular proliferation are observed in all restraint groups; however, stressed social runners are more susceptible to restraint stress. (D) Levels of corticosterone in mice following 15 min restraint stress show no main effects of exercise (black bars = running; white bars = sedentary controls) or social condition. Error bars represent SEM (* denotes significance (P < 0.05) in comparison to controls (two-way ANOVA and Fisher post-hoc test).
demonstrate similar results to other stereological methods in the DG, was employed to estimate the total number of BrdU-positive cells/hippocampus as previously described (Nixon and Crews, 2004; Eadie et al., 2005; Kempermann, 2006).

DCX-immunopositive cells were also counted manually using the same equipment and procedures. Cells were counted only if individual membranes could be observed at 100×, thus resulting in a conservative estimate in animals with many DCX-immunopositive cells. Thirty cells per animal were chosen quasi-randomly for measures of nodes. Nodes were defined as the branch points of the immature dendrites. Neurons were not counted if dendrites were broken or if they were obscured by a neighboring cell body. Images were captured with a Cool-Snap CCD camera and processed using Image-Pro Plus (Media Cybernetics, Bethesda, MD).

**Serum CORT**

Total CORT levels (bound plus free) were measured by radioimmunoassay (RIA). A tritiated CORT RIA kit (MP Biomedicals, Solon, OH), was used to determine plasma CORT levels using an adapted protocol previously described (Weinberg and Bezo, 1987; Gabriel et al., 2002). Dextran-coated charcoal was used to absorb and precipitate free steroids after incubation. Samples were analyzed using a Scintisafe Econo2 analysis kit (Fisher Scientific, Ottawa, Canada).

**Statistical Analysis**

For all studies, data are presented as mean ± standard error of the mean (SEM). Differences between mean values of experimental groups were compared using two-way analysis of variance (ANOVA), followed by Fisher post hoc tests as appropriate. Differences were considered significant when \( P < 0.05 \).

**RESULTS**

**Voluntary Exercise Increases Cellular Proliferation in Socially and Individually Housed Mice**

We initially compared the rates of cellular proliferation between individually or socially housed mice with and without access to a running wheel. Both groups of mice were injected once with the thymidine analog BrdU (200 mg/kg; IP) and then sacrificed 2 h later (Fig. 1A). As shown in Figure 1F, social housing did not increase the number of BrdU-positive (BrdU+) cells compared to individually housed mice (S-CON: 1,156 ± 146.3, \( n = 5 \); I-CON: 1,227 ± 69.05, \( n = 10 \)). Free access to a running wheel increased the number of BrdU+ cells in both social and individual housing conditions compared to their respective nonrunning controls (Fig. 1F). These differences were apparent in both individually housed mice (I-RUN: 1,876 ± 146.5, \( n = 13 \) vs. I-CON: 1,227 ± 69.05, \( n = 10 \)) and socially housed (S-RUN: 2,235 ± 411.1, \( n = 4 \) vs. S-CON: 1,156 ± 146.3, \( n = 5 \)) housed mice. No significant differences were observed between I-CON and S-CON mice (\( P = 0.77 \)), indicating that socialization did not have an additive effect on hippocampal cell proliferation in exercising mice. To examine the effect of an intermediate housing condition, a separate group of animals was housed in a social environment for 1 week followed by 1 week of isolation prior to access to a running wheel. However, we did not observe any significant differences between this group and the I-RUN and S-RUN groups (data not shown).

We also sampled levels of CORT, as an index of adrenal-cortical activity, to investigate if the two housing conditions differentially affected this system. An ANOVA revealed that there was not a significant effect for either exercise or social condition on CORT levels (\( P > 0.05 \)). However, there was a trend for mice in social housing to show higher corticosteroid levels (\( P = 0.06 \), irrespective of whether or not they were given free access to a running wheel (Fig. 1G).

Although BrdU has been well-established as a proliferation marker in the DG, there are always concerns when using an exogenous marker in an exercising paradigm. Exercise increases cerebral blood volume and blood flow to the DG (Pereira et al., 2007), and it is possible that this might increase the ability of BrdU to access this region of the brain, resulting in increased staining in animals that exercise. To resolve this issue, we replicated the above experiment using PCNA, an endogenous marker for cells engaged in mitotic activity, in a separate group of mice that were not submitted to BrdU injections. This experiment also indicated that cell proliferation was not significantly different in either the individually housed (1,706 ± 52.65) or socially housed (1,665 ± 125.3) mice (Fig. 1H). Also, in agreement with the results obtained with BrdU, quantitative analysis of PCNA-labeled cells demonstrated a significant increase in cellular proliferation (\( F_{(1, 18)} = 12.0, P = 0.003 \)) in mice that exercised, irrespective of their social housing condition (I-CON: 1,706 ± 52.65, \( n = 4 \); I-RUN: 2,427 ± 243.6, \( n = 7 \); S-CON: 1,665 ± 125.3, \( n = 5 \); S-RUN: 2,403 ± 231.8, \( n = 6 \)). In addition, there were also no significant differences in CORT levels between any of these groups (Fig. 1J). Thus, these results further confirm that voluntary exercise, but not social condition, increases cellular proliferation in the mouse DG.

**Social Housing Condition Differentially Affects Cell Proliferation in Exercising Mice Subjected to Short-Term Restraint Stress**

Activation of the adrenal-cortical axis leads to an increase in the levels of corticosteroids, which can in turn be deleterious to neurogenic activity (Cameron and Gould, 1994). Because previous studies have proposed that exercise may ameliorate this effect (Adlard and Cotman, 2004; Duman, 2005), we investigated whether voluntary exercise can prevent stress-induced reductions in neurogenesis. In these experiments, mice from the different groups were placed in a restraint tube for 15 min during which time they received an injection of BrdU (200
Mice were sacrificed 2 h after the BrdU injection. Mice from the stressed I-RUN group showed a significant increase in the number of BrdU cells \( (F(1, 22) = 57.13, P < 0.01; 1,467 \pm 158.9, n = 6) \), compared to subjects in the stressed I-CON group \( (787.6 \pm 100.7, n = 6; \text{Fig. 2B}) \). In contrast, no difference was observed in the stressed socially housed subjects \( (\text{stressed S-CON: } 859.8 \pm 111.7, n = 8; \text{stressed S-RUN: } 778.7 \pm 162.0, n = 7) \). There was a main effect of social condition \( (F(1, 22) = 7.56, P = 0.01) \) as well as an interaction between exercise and social condition \( (F(1, 22) = 11.5, P = 0.003) \) reflected in the fact that stressed I-RUN mice continued to show a significant increase in cell proliferation, despite exposure to the stressor. It should be noted however, that in comparison to subjects not subjected to restraint stress, mice in all four stress conditions did show decreases in cell proliferation \( (\text{Fig. 2C}) \). Interestingly, the most pronounced reduction in the number of BrdU+ cells following acute stress was observed in the stressed S-RUN subjects. An ANOVA on CORT levels, taken at the time of perfusion from the four groups, did not show any differences \( (P > 0.05; \text{Fig. 2D}) \).

**Social Isolation Has No Effect on the Production of New Neurons in Response to Voluntary Exercise**

To investigate if the observed increase in cellular proliferation translates into an increase in the production of new neurons, we quantified the number of cells immunopositive for the immature neuronal marker DCX. DCX-immunopositive neurons were prevalent throughout the DG of mice from all groups \( (\text{Fig. 3A–D}) \). Both individually housed and socially housed subjects showed equitable neuronal numbers in control groups (black bars) show significantly more DCX-labeled cells than their sedentary counterparts (white bars). \( (\text{E}) \) Both isolated and social running subjects show an increase in DCX-labeled cells following exercise \( (\text{Fig. 3E}) \). \( (\text{F}) \) Neurons of exercising subjects (black bars) have more nodes per cell, indicating an increase in dendritic branching, in comparison to sedentary counterparts (white bars). Error bars represent SEM, (*) denotes significance \( (P < 0.05) \) in comparison to controls \( (\text{two-way ANOVA and Fisher post hoc test}) \).
Discussion

This study provides evidence that voluntary exercise increases cell proliferation and neurogenesis in the mouse DG irrespective of social condition. This is in contrast to recent reports suggesting that voluntary exercise does not increase neurogenesis in isolated rats (Stranahan et al., 2006). The reason for these conflicting results is currently unclear. It is possible that variations in rodent species may account for some of these differences (as experiments performed by Stranahan et al. (2006) were conducted in rats and the present study was conducted in mice). Both environmental and physiological factors have been demonstrated to have differential effects on adult neurogenesis, depending on the type of rodent. Estrogen, for example, has been shown to stimulate adult neurogenesis in rats, but not in mice (Mazzucco et al., 2006; Lagace et al., 2007). It has been observed, however, that in some conditions voluntary exercise can reliably increase cell proliferation in rats housed in isolation (Christie et al., 2005; Eadie et al., 2005; Stranahan et al., 2006). Alternatively, as stress can be a regulatory factor for neurogenesis, differences in experimental design or animal care management practices could account for the observed discrepancies. For example, in order to facilitate administration of BrdU, Stranahan et al. (2006) restrained rats for 60–90 s. Given that this group employed a multiple injection experiment protocol of 10 BrdU IP injections, this equates to 10 consecutive days of acute restraint. Thus, it may be possible that stress just prior to BrdU administration could modify proliferating cell numbers.

We did not find any differences in CORT levels between mice with access to running wheels and the nonexercising control animals. This is in accordance with other research showing that CORT is only increased significantly immediately prior to the onset of physical activity (Droste et al., 2003; Stranahan et al., 2006). It was also recently shown that neither isolation nor variations in group size significantly affects CORT levels of male mice (Hunt and Hamblé, 2006). Our study did not demonstrate statistically significant main effects of exercise or housing condition on CORT levels; however, a trend for socially housed mice to have increased levels of CORT was apparent. Aggression among mice may lead to elevated CORT levels as male mice, like rats, commonly display aggressive behavior when housed together. Male mice, however, are least aggressive when housed in groups of three animals per cage, in comparison to groups of five and eight (Van Loo et al., 2001). Furthermore, in this study, socially housed males were monitored carefully for signs of aggression throughout the experiment. Thus, we believe that aggression-induced stress was not a major cause for the observed trend. This is consistent with our results showing that no effects of voluntary exercise or social conditions were observed in mice that were used for PCNA immunohistochemistry rather than being subjected to the BrdU injection protocol (a potentially stressful procedure).

Stressful situations can result in the near immediate downregulation of cell proliferation in the hippocampus by influencing levels of glucocorticoid (Falconer and Galea, 2003; Namestková et al., 2005; Chen et al., 2006). Indeed, subjecting mice to 10 min restraint stress following a BrdU injection can decrease the number of cell proliferation (Kim et al., 2005). Our results confirm that mice introduced to short-term restraint stress demonstrate an overall decrease in the amount of proliferating cells labeled with BrdU. Even under stressful conditions however, an increase in cell proliferation was observed in running mice housed in isolated conditions in comparison to nonrunning controls. Unexpectedly, under the same stressful conditions, exercise was unable to produce an increase in cellular proliferation in socially housed mice. Although the mechanisms underlying this result are unclear, one possibility could be an enhancement of social stress immediately following restraint. Immediately after the 15 min restraint paradigm, mice are returned to their cage where they remain until the time they are perfused. In isolated conditions, individual mice may be able to partially recover from the restraint stress during this period of time. In contrast, socially housed mice may not be able to recover from the induced stress as they are housed with their equally-stressed cage mates. Stressed animals housed together may produce a “social stress” which could affect the levels of cellular proliferation in these social animals.

Short-term restraint stress did not differentially alter CORT levels of mice in the different social and exercise conditions. Several studies have investigated how voluntary exercise and stress may interact. Some studies have reported that the levels of CORT in running mice increase in response to restraint stress (Droste et al., 2003), whereas others have found that voluntary exercise was associated with increased adrenal sensitivity to restraint stress (Fediuc et al., 2006). One study showed an exaggerated increase in CORT immediately following a 2 h

Hippocampus
restraint in exercising mice; this effect was not seen, however, within 10 h of the restraint period (Adlard and Cotman, 2004). Our data supports the theory that exercise and social housing for short periods of time do not significantly influence CORT levels in stressed mice.

We also found that voluntary exercise, but not social condition, also stimulates the production of new DCX-positive neurons, and these neurons show more branch points, or nodes. The effects of exercise were quite profound; after only 12 days with free access to a running wheel, a two-fold increase in the number of new neurons was observed. This estimate can be considered conservative as DCX quantification was only conducted on cells that had distinguishable membranes. The branching of neurons was also increased through exercise. This finding is particularly striking when one considers that after cell division, primary dendrites extend from neurons to molecular layer of the DG after ~1.5 weeks (Aimone et al., 2006), approximately the amount of time subjects have been exercising. One hypothesis is that exercise can independently upregulate factors that promote dendritic outgrowth and arborization during neuronal development, in addition to increasing the number of new neurons produced. This conclusion is in accordance with recent Golgi-Cox analyses of neuronal morphology in exercising animals (Redila and Christie, 2006; Stranahan et al., 2007).

Neurogenesis has often been correlated with performance on the Morris Water Maze, a task that is believed to test for hippocampal dependent spatial learning (van Praag et al., 1999a; Cao et al., 2004; Snyder et al., 2005; Olson et al., 2006). Interestingly, male mice that have been housed in isolation for long periods of time showed no deficits in water maze learning when compared with aged-matched, socially housed animals (Voikar et al., 2005). Similarly, we were unable to observe differences in the structure of hippocampi of subjects housed in different social conditions in this study, indicating that social housing alone is not likely sufficient to induce long-term changes in the hippocampus that would benefit learning performance.

Our study has several important implications. We have demonstrated that social condition does not regulate the exercise-induced promotion of cellular proliferation and neurogenesis in mice. We have also shown that the branching of new neurons is also upregulated by exercise, but not by social condition. Taken together, this suggests that voluntary exercise can promote cellular proliferation, neurogenesis, and dendritic outgrowth in new neurons, irrespective of social condition. Finally, we have shown that restraint stress can produce differential effects on running-induced proliferation depending on social condition. This last point reveals a cautionary reminder that should be considered during the design of neurogenesis studies using mice, and that attention should be given to the level of stress that is experienced by these subjects.

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