Neurogenesis and Helplessness Are Mediated by Controllability in Males But Not in Females

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Background: Numerous studies have implicated neurogenesis in the hippocampus in animal models of depression, especially those related to controllability and learned helplessness. Here, we tested the hypothesis that uncontrollable but not controllable stress would reduce cell proliferation in the hippocampus of male and female rats and would relate to the expression of helplessness behavior.

Methods: To manipulate controllability, groups of male and female rats were trained in one session (acute stress) or over seven sessions (repeated stress) to escape a footshock, whereas yoked control subjects could not escape but were exposed to the same amount of stress. Cell proliferation was assessed with immunohistochemistry of bromodeoxyuridine (BrdU) and immunofluorescence of BrdU and neuronal nuclei (NeuN). Separate groups were exposed to either controllable or uncontrollable stress, and their ability to learn to escape during training on a more difficult task was used as a behavioral measure of helplessness.

Results: Acute stress reduced cell proliferation in males but did not affect proliferation in the female hippocampus. When animals were given the opportunity to learn to control the stress over seven days, males produced more cells than the yoked males without control. Repeated training with controllable stress did not influence proliferation in females. Under all conditions, males were more likely than females to express helplessness behavior, even males that were not previously stressed.

Conclusions: The modulation of neurogenesis by controllability was evident in males but not in females, as was the expression of helplessness behavior, despite the fact that men are less likely than women to experience depression.

Key Words: Controllability, dentate gyrus, depression, learned helplessness, neurogenesis, sex differences, stress

Recently, it has been proposed that neurogenesis in the adult brain might play a role in the etiology of major depression (Dranovsky and Hen 2006; Jacobs et al. 2000; Malberg et al. 2000). This idea stems from three lines of evidence. The first is that the production of new neurons in the hippocampus is reduced by stressful experiences, including predator odors, social dominance, maternal deprivation, and mild shocks (Kosorovitskiy and Gould 2004; Malberg and Duman 2003; Mirescu et al. 2004; Tamapat et al. 2001). The second line of evidence indicates that chronic treatment with most types of antidepressant drugs, including Prozac (fluoxetine), increases proliferation in the hippocampus (Chen et al. 2000; Madsen et al. 2000; Malberg et al. 2000). Moreover, preventing neurogenesis prevents the ameliorating effects of Prozac on aspects of stress-related behavior (Santarelli et al. 2003). The third line of evidence indicates that the volume of the hippocampus is reduced in depressed patients (Neumeister et al. 2005). From these lines of evidence, it is suggested that stressful life experience decreases neurogenesis, which ultimately decreases the volume of the hippocampus. The decrease in volume contributes to the symptoms of unipolar depression; the prevention of that decrease with antidepressant drugs explains their efficacy (Malberg et al. 2000).

Learned helplessness is one of the most common animal models of depression (Seligman 1975). With it, animals are exposed to either controllable or uncontrollable stressful events and then later tested on a new task in which all animals are given the opportunity to control the stressor, usually by escape (Overmier and Seligman 1967; Seligman and Maier 1967). Most often, those animals that experienced uncontrollable stressful events do not display the escape response or are retarded in their response. This response has been equated with a sense of “giving up” experienced by humans with major depression. Animals that are initially trained to control the stressor can learn during training on the new task, despite the fact that both groups were exposed to similar amounts of stressful stimuli. Thus, establishing “control” over the stress can ameliorate some of the performance deficits that occur in response to stressful life events (Seligman 1975).

A few studies have used the learned helplessness paradigm to evaluate the putative relationship between depression and neurogenesis. Malberg and Duman (2003) reported that exposure to uncontrollable stress reduces neurogenesis in the hippocampus and induces helplessness behavior, both of which were reversed by treatment with Prozac. In contrast, Vollmayr et al. (2003) reported that exposure to uncontrollable stress reduced neurogenesis but the reduction was not associated with helplessness behavior. Recently Bland et al. (2006) found that animals with control produced more cells in their hippocampus than those without control. Most studies relating neurogenesis to depression have focused exclusively on males, despite robust gender differences in the incidence of the disease in humans (Burt and Stein 2002; Kessler et al. 1993; Kornstein et al. 2000; Martenyi et al. 2001; Staley et al. 2005). Here, we evaluated the effects of controllable versus uncontrollable acute and repeated stress on hippocampal neurogenesis and learned helplessness behavior in males and females.

Methods and Materials

General Procedures

Subjects and Estrous Cycle Determination. Experiments were approved by the Rutgers University Animal Care and Facilities Committee and are in compliance with the rules and

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regulations specified by the Public Health Service policy on Humane Care and Use of Laboratory Animals and the Guide for the Care and Use of Laboratory Animals. Adult (2–3 months) male (300–450 g) and female (250–350 g) Sprague Dawley rats were individually housed with ad libitum access to food and water and maintained on a 12-hour light/dark cycle. Stages of estrus were determined in female rats with daily vaginal smears, as described (Leuner et al. 2004b). Female rats without a 4–5-day cycle including proestrus, estrus, and diestrus 1 and 2 were excluded.

**Experiment 1**

Acute Stress and Neurogenesis in Male Versus Female Hippocampus. The effect of one session (30 trials) of either controllable or uncontrollable shock on cell proliferation in the dentate gyrus was examined. Rats were injected once with bromodeoxyuridine (BrdU; 200 mg/kg), which incorporates DNA of dividing cells during the S-phase of the cell cycle (Miller and Nowakowski 1988; Cameron and McKay 2001). Males were yoked in pairs (n = 9) and trained 30 min later in an operant conditioning task known as fixed-ratio 1 (FR1) (Shors et al. 1989). Females were yoked together according to stage of estrus (n of pairs = 11) and trained similarly. Groups of naive control subjects (males: n = 11; females: n = 7) were also injected with BrdU and returned to their home cage.

To manipulate controllability, rats were placed in one of two electrically linked shuttleboxes (Med Associates, St. Albans, Vermont). Each shuttlebox (46 cm × 18 cm × 19 cm) was located within a sound attenuated illuminated (35 W) chamber (69 cm × 69 cm × 63 cm). A scrambled shock generator delivered 1-mA electric pulse through the grid floor and walls of the apparatus. Each shuttlebox consisted of grid flooring, steel walls, a Plexiglas top, and a doorway in the center. During training with an FR-1 task, one rat can escape a 1-mA footshock (controllable stress) by traversing the apparatus once, whereas the yoked rat cannot escape (uncontrollable stress) but is exposed to the same amount and duration of shock as the rat that can escape. The animal with control escapes by passing through a doorway and tripping a balance switch, which terminates the shock for both animals simultaneously. Rats were trained for 30 trials with a maximum latency to escape of 20 sec and an intertrial interval of 60 sec. Latency to escape was used as a measure of performance in rats exposed to controllable stress. All rats were returned to their home cage.

Two hours after the BrdU injection, rats were deeply anaesthetized with sodium pentobarbital (100 mg/kg) and intracardially perfused with 4% paraformaldehyde in .2 mol/L phosphate buffer. Brains were extracted and post-fixed in 4% paraformaldehyde for up to 48 hours and were later transferred to .1 mol/L phosphate buffer saline (PBS). Subsequently, brains were sliced and stained with immunohistochemistry for BrdU.

**Immunohistochemistry Methods.** Coronal sections (40 μm) were cut through the entire dentate gyrus with an oscillating tissue slicer and then stained with peroxidase methods. Under peroxidase protocol, brain tissue was heated in .1 mol/L citric acid, pH 6.0, incubated in trypsin, then incubated in 2 N hydrochloric acid, and incubated overnight in primary mouse anti-BrdU (1:200) and .5% Tween 20. The next day, tissue was incubated for 1 hour in biotinylated anti-mouse antibody (1:200), then in avidin-biotin-horseradish peroxidase (1:100), and lastly in diaminobenzidine. After rinsing in PBS, slides were counter-stained with cresyl violet and cover-slipped under Permount (Fisher Scientific, Fair Lawn, New Jersey). Slides were coded, and estimates of total numbers of BrdU-labeled cells were determined with modified unbiased stereology protocol (West et al. 1991). The BrdU-labeled cells on every 12th unilateral section throughout the entire rostrocaudal extent of the dentate gyrus (granule cell layer, subgranular zone and hilus) were counted at 1,000× (100× objective with a 10X ocular) on Nikon Eclipse E400 light microscope (Nikon, Melville, New York), avoiding cells in the outermost focal plane. The numbers of BrdU-labeled cells in the dentate gyrus were multiplied by 24 (number of intervening slices and hemispheres) and used as an estimate of total number of newly generated cells.

A subset of male rats was injected with BrdU 30 min before being trained with one session of either controllable or uncontrollable stress (FR1). Rats were overdosed and perfused 3 weeks later along with naive control subjects. This time point was chosen because this approximates when newly generated cells express neuronal nuclei (NeuN), a marker of mature neurons (Christie and Cameron 2006). Because we were evaluating a decrease in proliferation, we did not conduct an extensive analysis of differentiation. Slices were incubated in 50% formamide/2x SSC (sodium chloride/sodium citrate buffer) for 2 hours at 65°C, rinsed with 2xSSC, and incubated in 2 N hydrochloric acid for 30 min at 37°C. They were then rinsed with borate buffer (pH 8.5) and incubated in 3% Normal Donkey Serum, .1% Triton X-100, in tris-buffered saline (TBS+, pH 7.5) for 1 hour at room temperature. Primary antibodies, rat anti-BrdU (1:50, Accurate Chemicals, Westbury, New York) and mouse anti-NeuN (1:200, Chemicon, Temecula, California) were incubated simultaneously in TBS+ overnight at 4°C. They were then rinsed with TBS and incubated in secondary antibodies (Jackson Immunoresearch, West Grove, Pennsylvania) sequentially: donkey anti-rat biotin-SP (1:200), fluorescein-DTAF-streptavidin (1:200), and donkey anti-mouse rhodamine red X (1:1000). Antibodies were diluted in TBS+ and tissue was rinsed with TBS between incubation periods. Sections were cover-slipped with Polyacquamount (Polysciences, Warrington, Pennsylvania). The BrdU–NeuN co-localization was determined with a Zeiss (Oberkochen, Germany) LSM 510 confocal laser scanning microscope. Sections were scanned using a Plan-Neofluar 25× water-immersion objective and dual-channel excitation with argon (488 nm) and helium-neon (545 nm). Co-localization analysis included visual inspection of size and shape of cell throughout a z-stack, orthogonal planes, and a profile of excitation intensity of the cell.

**Experiment 2**

Repeated Stress and Neurogenesis in Male Versus Female Hippocampus. The effect of seven sessions (30 trials/day) of either controllable or uncontrollable shock on cell proliferation in the dentate gyrus was examined. As before, males were yoked together in pairs (n = 15) and females were yoked according to the stage of estrus (n = 12). The training procedures were similar to those in the first experiment, except that rats were trained for 7 days instead of 1. Thirty minutes before the last session, rats were injected once with BrdU, trained, returned to their home cage, and overdosed and perfused 2 hours later, as before.

**Experiment 3**

Controllability and Helplessness Behavior in Males Versus Females. The effect of one session or seven sessions of controllable versus uncontrollable stress on helplessness behavior was examined. Pairs of male (n = 9 pairs for acute stress;
n = 8 pairs for repeated stress) and female rats (n = 8 pairs for each) were treated as before except that they were tested for helplessness rather than cell proliferation. Again, females were yoked according to stage of estrus. One day after the last FR1 training session, rats were tested on a fixed-ratio 2 (FR2) task, in which escape was possible for all subjects but required moving through the doorway twice in order to turn off the shock. Additional groups of male (n = 14) and female (n = 18) rats that had not been previously trained or exposed to any footshock were also tested in the FR2 task. The context for FR2 training was altered in the following ways: black and white stripes lined the walls of the chamber, an odor of menthol was placed in each chamber, and white bulbs were replaced with red bulbs. Latency to escape the FR2 was measured over 20 trials of training with a maximum latency of shock of 15 sec and an intertrial interval of 60 sec.

**Statistical Analysis**

Cell counts were analyzed with a paired dependent t test on data collected from yoked pairs of animals. One-way analyses of variance (ANOVAs) were performed to detect group differences. Performance during training on FR1 and FR2 operant conditioning tasks was analyzed with repeated measures ANOVA across sessions or blocks of five trials. Post hoc analyses with Newman-Keuls tests were conducted on significant interactions.

**Results**

**Experiment 1**

**Acute Stress Reduced Neurogenesis in the Male But Not Female Hippocampus.** Males and females performed differently during training on the FR1 task (Figures 1A and 1C). Males took longer than females to escape during the first five trials of FR1 training \(F(1,18) = 5.01; p < .05\), although the sex difference dissipated by the last trials of training \(p > .05\) (Figures 1A and 1C). Effects of acute stress on proliferation were also different in males versus females. Exposure to the acute stress altered the number of BrdU-labeled cells in males \(F(2,26) = 3.89; p < .05\), with naïve males producing more new cells than either of the stressed groups \(p < .05\), irrespective of whether they could control the shock \(t(8) = -3.65; p > .05\) (Figure 1B). In females, neither type of stressor altered the number of BrdU-labeled cells \(F(2,26) = 2.25; p > .05\) (Figure 1D). There was also no effect of controllability on the number of cells produced \(t(9) = -3.65; p > .05\) (Figure 1D). Stage of estrus did not alter numbers of the cells produced \(p > .05\), although the numbers of animals in each stage were too few to draw conclusions. In neither sex did the
weeks after the BrdU injection, 71% of the cells (indicate cells that were immunoreactive for both BrdU and NeuN. the dentate gyrus of hippocampus. Images illustrate BrdU-labeled cells Figure 2.

number of cells generated in the dentate gyrus correlate with the amount of shock that the animals received \( (p > .05) \). Three weeks after the BrdU injection, 71% of the cells \((n = 4; > 10 \text{ cells/rat})\) were labeled with both BrdU and NeuN (Figure 2). Thus, the majority of cells did differentiate into neurons, and the percentage is consistent with those reported in previous studies (Gould et al. 1999; Leuner et al. 2004a). Because the number of cells produced was either unchanged or decreased by stress, the NeuN data simply indicate the percentage of new cells that would have expressed neuronal markers, if they had survived.

**Experiment 2**

**Controllability Over a Repeated Stressor Modulates Neurogenesis in the Male But Not Female Hippocampus.** As in Experiment 1, males took longer to escape during the initial trials of training in the first session but did not differ from females during training on the following days \( (p > .05) \) (Figure 3A and 3C). A paired dependent \( t \) test revealed that male rats that were trained to escape the shock for 7 days produced more cells than their yoked counterparts, who were exposed to the same amount of shock but did not have the opportunity to escape \( (t(14) = 2.42; p < .05) \) (Figures 3B and 4A). A dependent \( t \) test on similar data in yoked pairs of females was not significant \( (t(11) = -1.15; p = .28) \) (Figures 3D and 4B). The number of animals in each stage of estrus at the end of training was not sufficient to analyze results according to phase of the cycle. The number of BrdU-labeled cells did not correlate with the amount of shock that males or females were exposed to during the last session of stress \( (p > .05) \).

**Experiment 3**

**Sex Differences in Helplessness Behavior.** As in the first two experiments, males took longer than females to escape during the initial trials of training on the first session of the FR1 task \( (F(1,15) = 5.2; p < .05) \), but the sex difference dissipated within the 30 trials of training (Figures 5A and 5C). All groups learned to escape within the seven sessions, as evidenced by a decrease in escape latency across sessions culminating in very low latencies (Figures 5B and 5D) \( (F(6,42) = 8.76; p < .001) \). There was no sex difference in performance during training on the FR1 task across 7 days of training \( (p > .05) \) (Figures 5B and 5D). However, sex differences were evident during training on the FR2 task. Overall, females expressed shorter escape latencies than did males, irrespective of the pretraining condition \( (p < .001) \) (Figure 6). Females that were previously exposed to one session of FR1 training (irrespective of controllability) outperformed males, with performance analyzed in blocks of five trials each \( (F(1,32) = 18.95; p < .001) \). Males previously exposed to uncontrollable stress for one session did not alter their latency to escape across trials during testing on the FR2 task, even after they were exposed to seven sessions of uncontrollable stress \( (F(1,14) = 8.8; p < .01; \text{second block} \ F(1,14) = 5.96; p < .05; \text{third block} \ F(1,14) = 6.11; p < .05) \) (Figures 6C and 6F).

Controllability did influence learning to escape during the FR2 task in males and females, but the effect depended on the length of previous training. With one session of FR1 training in males, there was an interaction between the performance across trials and the pretraining condition \( (F(6,87) = 3.52; p < .005) \). Male rats that were trained with seven sessions of FR1 training (controllable stress) differed from those that could not escape (uncontrollable stress) or those that had no previous stress exposure \( (p < .001) \). Males previously exposed to uncontrollable or uncontrollable stress for one session did not alter their latency to escape across trials during testing on the FR2 task and thus did not learn \( (F(3,24) = 1.56; p > .05) \) (Figures 6B and 6E). Females also outperformed males during training on the FR2 task, even after they were exposed to seven sessions of uncontrollable stress \( (F(1,14) = 10.34; p < .005) \) (Figures 6B and 6E).

For males tested on the FR2 task after 7 days of FR1 training, there was an effect of blocks \( (F(6,81) = 4.30, p < .01) \), the pretraining condition \( (F(2,27) = 10.34; p < .001) \), and an interaction between the two factors \( (F(6,81) = 4.00; p < .001) \). Post hoc analysis revealed that performance of males that had learned to escape the footshock over the seven sessions of FR1 training (controllable stress) differed from those that could not escape (uncontrollable stress) or those that had no previous stress exposure \( (p < .001) \). Male rats that were trained with seven sessions of FR1 (controllable stress) reduced their escape latencies across blocks of trials of FR2 task and thus expressed evidence of learning \( (F(3,21) = 3.64; p < .05) \) (Figure 6C). Those that could not escape over the seven sessions of FR1 training (uncontrollable stress) did not reduce their latency to escape.
during FR2 task, did not learn, and thus expressed evidence of helplessness $F(3,21) = 1.58; p > .05$ (Figure 6C). However, as noted, male rats that were naïve before training on the FR2 task also did not show evidence of learning and therefore also expressed helplessness behavior $F(3,39) = 7.33; p < .001$ (Figure 6A). In fact, only males exposed to seven sessions of escape training in FR1 (controllable stress) learned to escape during testing in the FR2 task (Figure 6C). Again, these data suggest that if the males are not previously trained to escape, they express evidence of learned helplessness behavior.

In females, the response to repeated training was quite different. After one session of FR1 training, there was an effect of the pretraining condition on performance during training on the FR2 task $F(2,31) = 6.97; p < .01$ with no interactions (Figure 6E). Those that were trained to escape an FR1 for one session (controllable stress) expressed a decrease in escape latency and thus learned to escape during training on the FR2 task and did so faster than the females that were exposed to the uncontrollable stress for one session $F(1,14) = 6.11; p < .05; F(1,14) = 16.9; p < .001$, for the two first blocks of trials, respectively, although the groups were not different when assessed across the 20 trials of training. Both groups (controllable and uncontrollable stress) responded sooner than the naïve females, especially on the first trials of training with the FR2 task $F(2,21) = 4.39; p < .05$; $F(2,21) = 14.9; p < .001$ for the two first blocks of trials, respectively. Escape latencies among the three groups did not differ during the last 10 trials of training on the FR2 task (Figures 6D and 6E). In contrast to males, females with no previous FR1 training (no stress group) expressed a decrease in latency across trials and thus learned to escape during training on the FR2 task $F(3,51) = 4.24; p < .005$ (Figure 6D). Although escape latencies increased between the first 5 and 10 trials ($p < .001$), they decreased over the remaining trials ($p < .005$).

The escape latencies expressed by females in the FR2 task after seven sessions of FR1 training indicated an effect of trials $F(3,93) = 4.97; p < .005$, pretraining condition $F(2,31) = 5.93; p < .01$, and an interaction between the two factors $F(6,93) = 2.68; p < .05$. Post hoc analysis revealed that the escape latencies of females trained for seven sessions of escapable footshock in FR1 (controllable stress) differed from those exposed to the same amount of inescapable footshock (uncontrollable stress) ($p < .05$). However, females that were exposed to seven sessions of the uncontrollable stress decreased their escape latencies over trials in the FR2 task $F(3,21) = 4.35; p < .05$ and thus learned to escape (Figure 6F), as did the females that were not exposed to any stress before training on the FR2 $F(3,51) = 4.24; p < .005$ (Figure 6D). Females that had learned to escape during FR1 training (controllable stress) did not reduce their latency to
escape during testing on the FR2 task \( F(3,21) = 1.88; p > .05 \) (Figure 6F), probably because they performed so well during the early trials of FR2 testing. Although females previously exposed to controllable stress performed differently than those exposed to uncontrollable stress, both groups showed evidence of learning to escape the footshock in the FR2 task.

**Discussion**

The present results indicate that controllability modulates neurogenesis in the adult hippocampus in males but not in females, at least with the conditions described here. The males that were able to learn to control a stressor presented over seven days produced more cells in their hippocampus than their yoked counterparts, an effect that was observed in 11 of 15 pairs. In contrast and on average, females that were able to learn to control the same stressor over 7 days produced as many cells as their yoked counterparts; only 2 of the 12 females with control produced more cells than their yoked counterparts. We also found that exposure to just one session of stress, irrespective of controllability, decreased proliferation in males, whereas it did not affect proliferation in females. Because females escaped sooner than males in the first trials of FR1 task, they were exposed to less duration of shock, which could explain the sex differences in proliferation after acute stress. Thus, the behavior of the females apparently affected their response to acute stress at the cellular level—something to consider when evaluating animal models of depression. In a finding consistent with this, exposure to controllable stress alleviated the expression of helplessness behavior in both females and males, but females learned to escape more rapidly than males, irrespective of their opportunity to control the stressor. Thus, females more readily learned the FR2 task and were less likely to become helpless. In contrast, even males that were not previously stressed did not learn to escape in the FR2 task and thus exhibited evidence of helplessness, despite the fact that they had the opportunity to escape. It could be argued that the males learned better than females because they were less likely to reenter the chamber where they had been shocked. In the end, it can only be said that controllability had a more pronounced effect in males, because those that learned to control the stress were “protected” from helplessness behavior and the reduced proliferation effect. Males that were exposed to uncontrollable stress or males that had not been trained before (no stress group) were more likely to “give up” during training on the FR2 task than were females exposed to the same conditions.

The present study confirms previous findings that uncontrollable stress induces learned helplessness behavior and decreases hippocampal cell proliferation in male rats (Malberg and Duman 2003) and that cell proliferation in the hippocampus is affected by uncontrollable but not controllable stress (Bland et al. 2006). The means by which uncontrollable stress reduces proliferation are unknown, but serotonin is likely involved. Chronic treatment...
with serotonergic drugs can reverse or prevent the effects of uncontrollable stress on neurogenesis (Malberg and Duman 2003; Santarelli et al. 2003). Also, exposure to similar types of stressors as used here activates neurons in the dorsal raphe nucleus (Maier and Watkins 2005), which send serotonergic projections to the hippocampus (Conrad et al. 1974; Maier and Watkins 2005) and affect neurogenesis (Brezun and Daszuta 1999; Huang and Herbert 2005). The stress hormone corticosterone could also be involved, because it decreases cell proliferation in the adult hippocampus (Cameron and Gould 1994). If it is necessary, it is probably not sufficient, because controllable and uncontrollable stress increases corticosterone to similar levels that remain elevated even in the animals that learn to control the stressor (Shors et al. 1989).

The present results suggest that learning to control a stressor over days can alter the production of new cells in the male hippocampus, but they do not necessarily indicate that new cells are involved in learning how to control the stressor. This scenario is unlikely for several reasons. First, the cells would be very immature at the time of the training experience. The cells become incorporated into the granule cell layer more than 1 week after birth and well after the training experience has ended (Cameron et al. 1993; Zhao et al. 2006). When BrdU was injected during training, we observed no increase in cell numbers (Gould et al. 1999). Also, processes of learning that increase neurogenesis have generally been limited to those that depend on the hippocampus or are difficult to learn (Leuner et al. 2006). Because learning the operant response tested here does not depend on the hippocampus and the FR1 task is relatively easy to learn, it is unlikely that such learning would increase neurogenesis. That said, males that learned to control the stressor over 7 days of training produced more cells than the other groups, including their respective females. Thus, it is possible that the training regimen was a form of environmental enrichment that enhanced proliferation, as has been shown before (Kempermann et al. 1997).

The current data suggest that the new cells in the female hippocampus are less responsive to stress, as suggested by others. For example, neurogenesis in adult females is reportedly not affected by predator odors, whereas it is decreased in males (Falconer and Galea 2003). Similarly, hippocampal cell proliferation in females was not affected by 8 days of uncontrollable footshock stress and reportedly increased after longer exposure (21 days) (Westenbroek et al. 2004). Because we did not compare the animals trained over 7 days with naïve control subjects, we cannot state with certainty that neurogenesis was not reduced in both groups—those exposed to uncontrollable and controllable stress over the 7 days. This explanation seems unlikely, because one session of stress did not alter proliferation relative to naïve control subjects. Also, we did not assess the effects of stress on neurogenesis for each stage of the estrus cycle but did yoke the females according to the stage of estrus at the beginning of training, and in most cases, the pairs were in the same stage at the time of sacrifice. With this strategy, there was no effect of controllability on neurogenesis in the female hippocampus.

In these studies, we attempted to relate sex differences in neurogenesis with the expression of helplessness behavior. First, there were pronounced sex differences in helplessness behavior, and to our surprise, sex differences were evident in naïve animals.
that were simply trained on the two types of operant conditioning tasks. In general, females outperformed males during the early trials of training, irrespective of whether they were previously exposed to a stressor. Sex differences in performance during the FR1 task dissipated quickly but persisted for animals trained on the FR2 task. In fact, males provided no evidence that they would learn to escape. It is as if they became helpless during the course of training. Sex differences in avoidance behavior have been noted previously and might reflect sex differences in activity and/or different strategies in response to the footshock (Beatty and Beatty 1970; Kirk and Blampied 1985; Steenbergen et al. 1990). Females often respond to the shock with increased activity, whereas males tend to freeze (Shors 1998). Some of these effects are dependent on the stage of estrus in which the training occurs (Jenkins et al. 2001), although those reported here are probably not. We have recent data showing that these sex differences in helplessness behavior remain even in ovariec-
tomized females and thus occur in the absence of estrogen and progesterone (Dalla et al, unpublished data, 2006).

The data presented suggest that females are resistant to the negative effects of stress on learning an operant response, in which volitional motor activity is required to learn. Such effects are not observed for example with classical conditioning and are in fact quite the opposite. With a classically conditioned eyeblink task, we have found that exposure to uncontrollable stress significantly reduces learning ability in females and actually enhances learning in males (Leuner et al. 2004b). Thus, it cannot be said that stress does not affect females as much as males; rather, the effects of stress on learning in females are often different from those expressed by males. These differences add to the growing body of evidence suggesting that females respond differently to stressful experience than do males (Bale 2006; Cahill 2006; McCarthy and Konkle 2005), differences that might relate to sex differences in mental illness, especially those associated with depression.

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Figure 6. Males express learned helplessness behavior, whereas females do not. (A) Escape latencies in males that were only trained on the operant task in which they had to cross the shuttle-box apparatus twice to escape (fixed-ratio 2 [FR2]) (means of five trials ± SEM). (B) Escape latencies during training on the FR2 task (means of five trials ± SEM) after exposure to one session of controllable or uncontrollable stress (30 trials). (C) Escape latencies in males that were trained on the FR2 task (means of five trials ± SEM) after exposure to seven sessions of controllable or uncontrollable stress. (D) Escape latencies in females that were only trained on the operant task in which they had to cross the shuttle-box twice to escape (FR2) (means of five trials ± SEM). (E) Escape latencies during training on the FR2 task (means of five trials ± SEM) in females after exposure to one session of controllable or uncontrollable stress. (E) Escape latencies in the FR2 test (means of five trials ± SEM) after exposure to seven sessions of controllable or uncontrollable stress.


