nature neuroscience

© 2004 Nature Publishing Group http://www.nature.com/natureneuroscience

Early life experience alters response of adult neurogenesis to stress

Christian Mirescu, Jennifer D Peters & Elizabeth Gould

Maternal deprivation produces persistent abnormalities in behavioral and neuroendocrine functions associated with the hippocampus, a brain region that shows considerable structural change in response to experience throughout life. Here we show that adverse experience early in life affects the regulation of adult neurogenesis in the hippocampus. More specifically, a decrease in cell proliferation and immature neuron production are observed in the dentate gyrus of adult rats that are maternally separated as pups. Although maternally separated rats show normal basal levels of corticosterone, the suppression of cell proliferation in these rats can be reversed by lowering corticosterone below the control value. In addition, normal stress-induced suppression of cell proliferation and neurogenesis, despite normal activation of the hypothalamic pituitary adrenal (HPA) axis, is not observed in maternally separated rats. Our results suggest that early adverse experience inhibits structural plasticity via hypersensitivity to glucocorticoids and diminishes the ability of the hippocampus to respond to stress in adulthood.

Early life stress is associated with changes in behavior, physiological responses to stress and susceptibility to psychopathology in adults[1]. Maternal separation is a well-characterized model of early life stress in rodents[2–4]. Prolonged maternal separation is sufficiently adverse to result in activation of the HPA axis during the stress hyporesponsive period[5], a neonatal time characterized by blunted glucocorticoid responses to most adult stressors[6].

Prolonged maternal separation during the early postnatal period does not affect basal measures of HPA function in adulthood, but distinct differences in regulation of the HPA axis emerge after exposure to stressful experiences. As adults, rodents previously subjected to extended maternal separation show protracted increases in corticotropin-releasing factor (CRF), adrenocorticotropin (ACTH) and corticosterone in response to stress[7,8]. By contrast, brief maternal separation (referred to as neonatal handling) results in reduced CRF mRNA, ACTH and corticosterone in response to stress[7–10]. In addition to these hormonal changes, maternal separation and neonatal handling are associated with behavioral differences in later life. Maternal separation increases measures of anxiety[11], impairs maternal care[12] and diminishes spatial navigation learning[5]. By contrast, neonatal handling generally alters these behaviors in directions opposite to those reported in maternally separated rodents.

The hippocampus has been implicated in many of the functions that are altered by early adverse experience. First, the hippocampus mediates negative feedback of the HPA axis. Lesions of the hippocampus or the fornix, its efferent hypothalamic projection, result in a slower restoration of basal measures of the HPA axis after stress[13–15]. Second, the hippocampus is an important structure in regulating behavioral measures of anxiety. Rats with hippocampal lesions appear to be less anxious when behaviors such as exploration of a new environment, social interaction and neophagia are measured[16,17]. Third, the hippocampus has a well-described role in spatial navigation learning[18]. Because all of these processes in the adult are influenced by maternal separation during early life, abnormal hippocampal development may underlie some, if not all, of these differences.

The hippocampus shows a remarkable degree of structural and functional plasticity in adulthood. One of the most fundamental forms of structural plasticity is adult neurogenesis, because the generation of new neurons leads to the growth of dendrites, axons and synapses[19]. In the dentate gyrus of adult rats, several thousand new granule cells are produced per day[20], suggesting that these new neurons participate in hippocampal function. Because adult neurogenesis is regulated in part by adrenal steroids[21,22], it is possible that this phenomenon may be related to maternal contact, through the persistent influence that this experience has on HPA axis function.

To investigate the possibility that early life events have a lasting effect on adult neurogenesis, we have examined the number of new cells in the hippocampus of adult rats exposed to different durations of maternal separation and stress in adulthood. We report that rats exposed to prolonged, but not brief, bouts of maternal separation show a long-lasting suppression of adult neurogenesis and diminished plasticity in this parameter after exposure to stress. Maternally separated rats do not show basal or stress levels of corticosterone that differ from those of control rats; rather, the reduction in cell proliferation associated with maternal separation results from a hypersensitivity to normal levels of corticosterone.

RESULTS

Effects of early experience on adult neurogenesis

Adult rats subjected to 180 min of maternal separation daily (HMS180) from postnatal day 1 (P1) to P14 showed significantly fewer BrdU-labeled cells in the dentate gyrus 2 h after bromod-

Department of Psychology, Princeton University, Princeton, New Jersey 08544, USA. Correspondence should be addressed to E.G. (goulde@princeton.edu).

Published online 25 July 2004; doi:10.1038/nn1290

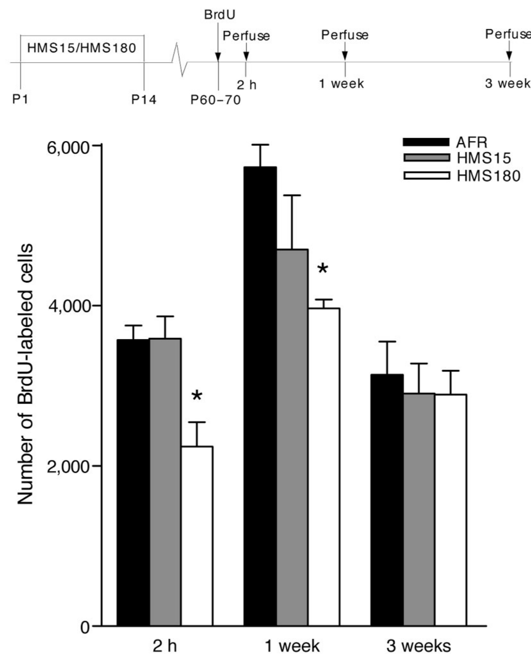

Figure 1 Suppression of baseline cell proliferation and immature neuron production by prolonged perinatal maternal deprivation. Top, on P60–P70 rats received a single injection of BrdU (200 mg/kg i.p.) and were perfused after 2 h, 1 week or 3 weeks. Bottom, as compared with AFR and HMS15 rats, HMS180 rats had fewer numbers of BrdU-positive cells in the dentate gyrus at 2 h (AFR, $n = 10$; HMS15, $n = 9$; HMS180, $n = 7$). At 1 week, significantly lower numbers of BrdU-positive cells were found in HMS180 ($n = 7$), relative to both AFR ($n = 5$) and HMS15 ($n = 5$) rats. However, no difference in the numbers of BrdU-labeled cells was evident by 3 weeks (AFR, $n = 4$; HMS15, $n = 5$; HMS180, $n = 4$). Error bars indicate the s.e.m. $*P < 0.05$.

eoxyuridine (BrdU) injection ($F_{2,23} = 8.47$, $P = 0.002$) than did age-matched controls subjected to 15 min of maternal separation daily (HMS15, $P = 0.004$) or animal facility rearing (AFR, $P = 0.003$) (**Fig. 1**). This effect seemed to be specific to the dentate gyrus, as BrdU-labeled cell counts in the subventricular zone did not differ statistically across groups ($F_{2,12} = 0.46$, $P = 0.64$).

A week after BrdU injection, HMS180 rats had fewer BrdU-labeled cells in the dentate gyrus ($F_{2,14} = 8.27$, $P = 0.004$) than both the HMS15 ($P = 0.04$) and AFR ($P = 0.003$) groups. In all groups, most of these cells (AFR, 76.8 ± 6.4%; HMS15, 64.8 ± 2.3%; HMS180, 74.4 ± 5.7%; mean ± s.e.m.) expressed Tuj1 (**Fig. 2**), a marker of immature and mature neurons. There were no differences in the percentage of BrdU-labeled cells that expressed Tuj1 across the groups ($F_{2,12} = 1.53$, $P = 0.256$), suggesting that the decrease in BrdU-labeled cells represents, at least in part, a decrease in the number of immature neurons (**Fig. 1**). At 1 week, a relatively low percentage of BrdU-labeled cells expressed the mature neuronal marker NeuN (AFR, 15.0 ± 1.9%; HMS15, 16.0 ± 2.3%; HMS180, 19.0 ± 5.0%). This proportion did not differ significantly across groups ($F_{2,9} = 0.38$, $P = 0.69$).

By 3 weeks after BrdU labeling, however, the difference in the number of BrdU-labeled cells between HMS180 and control rats was no longer apparent ($F_{2,10} = 0.12$, $P = 0.90$). At this time point, all groups had similar numbers of BrdU-labeled cells (**Fig. 1**). In all groups, most BrdU-labeled cells expressed NeuN at 3 weeks (AFR, 87.0 ± 2.5%; HMS15, 85.0 ± 3.0%; HMS180, 84.0 ± 1.6%; **Fig. 2**). By contrast, a low proportion of BrdU-labeled cells were colabeled with the astrocyte marker GFAP at this time point (AFR, 8.0 ± 1.6%; HMS15, 7.0 ± 1.9%; HMS180, 7.0 ± 1.9%; **Fig. 2**).

No relationship between basal plasma corticosterone and cell proliferation was observed in HMS180, HMS15 and AFR rats. As previously reported by others[7], we found no differences in circulating corticosterone between AFR and HMS180 rats under undisturbed conditions.

Effects of decreasing glucocorticoid levels in adulthood

In all groups, plasma corticosterone was significantly reduced by adrenalectomy followed by low-dose corticosterone in the drinking water, as compared with sham-operated controls (**Fig. 3**). No differences in BrdU-labeled cell counts were observed among AFR and HMS15 adult rats with lowered corticosterone levels and those with control corticosterone levels. In HMS180 rats, however, lower corticosterone resulted in a significant increase in the number of BrdU-labeled cells ($P < 0.05$, **Fig. 3**). In HMS180 rats, adrenalectomy followed by low-dose corticosterone replacement resulted in BrdU-

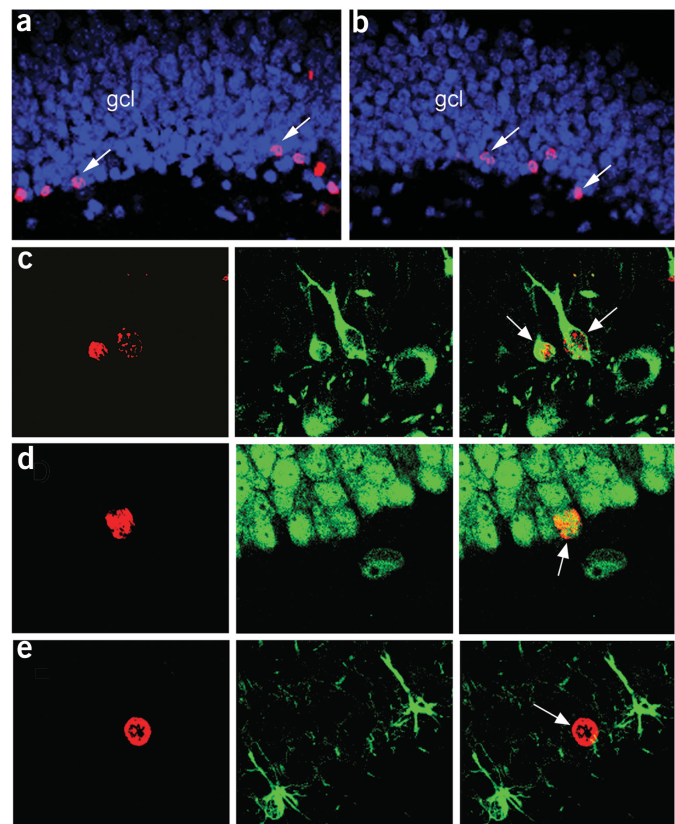

Figure 2 Prolonged maternal separation results in a reduction in immature neurons in the dentate gyrus. (**a**,**b**) Confocal laser scanning microscopic images of BrdU-labeled cells (arrows) showed a significant difference between control AFR (**a**) and HMS180 (**b**) adult rats, 1 week after BrdU administration. gcl, granule cell layer. (**c**–**e**) At this time, most cells examined, in AFR, HMS15 and HMS180 rats showed morphological characteristics of granule cells and were immunoreactive for Tuj1 (**c**), a marker of immature neurons. By 3 weeks, most BrdU-positive cells were colabeled with NeuN (**d**), a marker of mature neurons, but not GFAP (**e**), an astroglial marker. Left panels, BrdU-labeled cells (red; **c**–**e**). Middle panels, TuJ1- (**c**), NeuN- (**d**) and GFAP-labeled (**e**) cells. Right panels, these images merged (BrdU-labeled cells are indicated by arrows).

© 2004 Nature Publishing Group http://www.nature.com/natureneuroscience

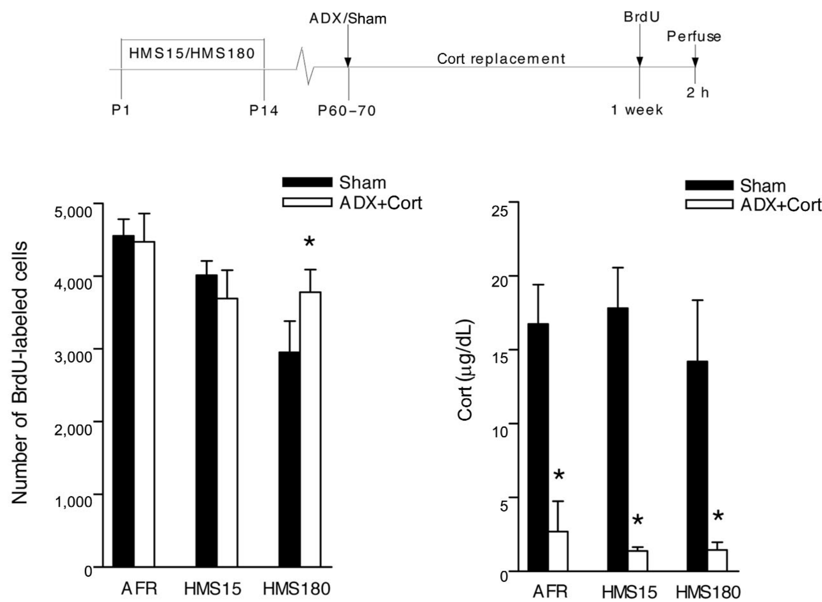

Figure 3 Reversal of suppressed baseline cell proliferation in maternally deprived rats by lowering corticosterone (Cort) in adulthood. Top, on P60–P70 rats underwent bilateral surgical removal of the adrenal glands (ADX) or were sham-operated. All ADX-treated rats were given drinking water containing Cort (25 µg/ml in 0.9% saline). A week after surgery, all rats received a single injection of BrdU (200 mg/kg, i.p.) and were killed 2 h later. Bottom, ADX plus Cort had no effect on cell proliferation in AFR (sham, $n = 3$; ADX + Cort, $n = 3$) or HMS15 (sham, $n = 4$; ADX + Cort, $n = 5$) rats, but enhanced cell proliferation in HMS180 rats (sham, $n = 5$; ADX + Cort, $n = 4$) to a level comparable to that seen in the AFR and HMS15 groups. To verify that ADX plus Cort replacement results in a decrease in plasma Cort on the day of death, trunk blood was collected from ADX plus Cort–treated (AFR, $n = 5$; HMS15, $n = 6$; HMS180, $n = 5$) and sham-operated (AFR, $n = 5$; HMS15, $n = 6$; HMS180, $n = 6$) rats. In all rearing groups, ADX plus Cort resulted in significantly lower but detectable concentrations of circulating Cort. Error bars indicate the s.e.m. $^*P < 0.05$.

labeled cell counts that were not significantly different from those in AFR and HMS15 rats, suggesting that lowering total corticosterone has a restorative effect on cell proliferation.

Effects of stress in adulthood

In all groups, exposure to fox odor resulted in significant increases in circulating corticosterone after 30 min. Fox odor exposure decreased the number of BrdU-labeled cells in the dentate gyrus in HMS15 and AFR rats at both 2 h ($F_{1,32} = 28.77$, $P < 0.001$) and 1 week ($F_{1,21} = 14.03$, $P < 0.01$) after BrdU injection (**Fig. 4**). These differences were comparable to those observed in a previous study using control (AFR) rats[23]. Double-labeling with BrdU and Tuj1 revealed no differences in the percentage of new cells that expressed this neuronal marker among groups, suggesting that the decrease in BrdU-labeled cell number reflects diminished adult neurogenesis.

Fox odor exposure did not result in a significant decrease in BrdU-labeled cell number in HMS180 rats at either time point (**Fig. 3**). Thus, an inverse relationship between corticosterone and cell proliferation was observed in HMS15 and AFR rats, but this relationship was not observed in HMS180 rats.

DISCUSSION

Reduced cell proliferation and neurogenesis occur in the hippocampus of adult rats after stressful experiences[23–26]. Evidence suggests that the stress-induced suppression of neurogenesis is dependent on adrenal steroids[23]. The reduction in basal numbers of proliferating cells and immature neurons that we observed in the dentate gyrus of HMS180 adults is similar to the decreases in cell proliferation observed in control rats after acute stressor exposure. No differences in basal corticosterone were found across rearing groups, however, despite different basal levels of cell proliferation. Thus, these data suggest that glucocorticoids are not directly responsible for the reduced adult neurogenesis associated with early adverse experience. Instead, the persistent suppression of cell proliferation in maternally deprived rats resembles the state observed in adult rats that show learned helplessness to inescapable stress, namely, reduced cell production in the absence of increased corticosterone[26]. No differences between AFR and HMS15 rats were observed under control or stressed conditions, suggesting that only extreme changes in mother-pup contact produce lasting alterations to adult neurogenesis.

This apparent dissociation of cell proliferation and basal corticosterone does not eliminate the possibility that maternal separation renders the adult rat hypersensitive to relatively normal levels of circulating glucocorticoids. In support of this view, we found that lowering corticosterone below normal levels reversed the effect of maternal separation on cell proliferation. This hormone manipulation did not increase cell proliferation in AFR or HMS15 adult rats, but it did increase the number of BrdU-labeled cells in HMS180 rats to values similar to those in non-deprived rats. Because maternal separation is known to decrease corticosteroid-binding globulin[27], cell proliferation may be suppressed by higher levels of circulating free corticosterone in HMS180 rats than in AFR and HMS15 rats.

Despite this hypersensitivity to normal levels of circulating glucocorticoids, HMS180 rats did not show a further decrease in neuron production when exposed to stress levels of glucocorticoids in adulthood. In fact, these rats completely lacked a stress-induced suppression of cell proliferation and neurogenesis, despite a normal corticosterone response to predator odor. Given the suppression of cell proliferation in HMS180 rats, the absence of a further decrease in response to stress suggests that there is a floor effect in the proportion of progenitor cells that can be inhibited at a given time. The inability of stress to suppress adult neurogenesis further may be an adaptive mechanism that prevents this form of structural plasticity from being completely abolished. Alternatively, the lack of a further decrease in new neuron production in HMS180 rats exposed to stress may represent a maladaptive response that contributes to abnormal functioning of the hippocampus.

Notably, maternal deprivation decreased the number of immature, but not mature, neurons added to the dentate gyrus in adulthood. In HMS180 rats, fewer BrdU-labeled cells were observed at the 1-week time point. This difference did not persist to a time when most new cells express markers of mature neurons (3 weeks), however, indicating that the functional consequences of early adverse experience may involve a diminished pool of nascent neurons. Some evidence suggests that immature neurons have a unique and relatively potent role in the functioning of the adult hippocampus.

© 2004 Nature Publishing Group http://www.nature.com/natureneuroscience

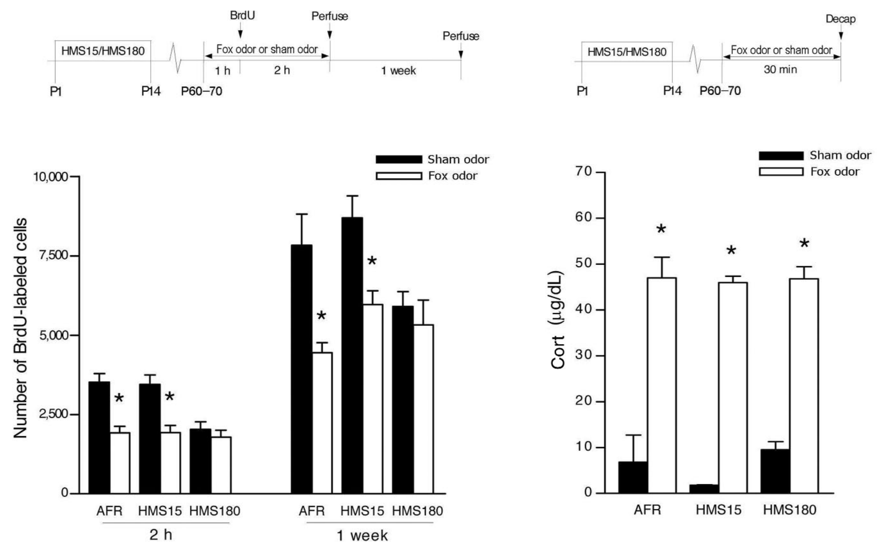

Figure 4 There is no significant stress-induced suppression of cell proliferation and neurogenesis in maternally deprived adult rats, despite normal activation of the HPA axis to fox odor. Top, on P60–P70 rats were exposed to fox odor or sham odor, injected with BrdU (200 mg/kg, i.p.) after 1 h, and killed 2 h or 1 week later. Exposure to fox odor suppressed cell proliferation in AFR (sham odor, $n = 6$; fox odor, $n = 5$) and HMS15 (sham odor, $n = 6$; fox odor, $n = 6$) but not HMS180 (sham odor, $n = 7$; fox odor, $n = 7$) rats. At 1 week, numbers of BrdU-labeled cells were also reduced by fox odor exposure in AFR (sham odor, $n = 4$; fox odor, $n = 4$) and HMS15 (sham odor, $n = 4$; fox odor, $n = 4$) but not HMS180 (sham odor, $n = 5$; fox odor, $n = 5$) rats. To verify that early rearing conditions do not affect the efficacy of fox odor in eliciting a corticosterone response, separate groups of adult rats were exposed to either fox odor (AFR, $n = 3$; HMS15, $n = 3$; HMS180, $n = 3$) or sham odor (AFR, $n = 4$; HMS15, $n = 3$; HMS180, $n = 4$) and then rapidly decapitated after 30 min, a time point when stress-induced corticosterone increases are maximal. In all rearing groups, exposure to fox odor resulted in a significant rise in circulating corticosterone. Error bars indicate the s.e.m. $*P < 0.05$.

First, young neurons appear to make new connections very rapidly; within a week of mitosis, immature neurons possess dendrites and extend axons into the distal CA3 region[20,28]. Second, immature neurons in the adult dentate gyrus lack inhibitory responses to GABA[29–31]. Last, new neurons show more robust associative LTP that can be induced at a lower threshold as compared with mature neurons[29–31]. These characteristics may render new neurons capable of influencing the hippocampus to a greater extent than the same number of mature neurons. Because immature neurons may make disproportionately large contributions to the overall function of the hippocampus, decreases in their number may underlie some behavioral and neuroendocrine changes associated with early adverse experience.

Along these lines, maternal deprivation has been shown to alter behaviors that may be associated with immature neurons in the hippocampus. Evidence suggests that immature granule neurons may be involved in anxiety regulation[32] as well as learning[33–36]. HMS180 rats show heightened anxiety[5,11] and diminished capacity to learn some tasks[5]—functions that involve the hippocampus[16–18]. Maternal deprivation also alters negative feedback of the HPA axis[7,8], a lesser known function of the hippocampus[13–15]. The decrease in adult-generated immature neurons associated with maternal deprivation may also contribute to impaired hippocampal function in HPA feedback.

Stress-related neuropsychiatric disorders such as depression have been associated with reductions in hippocampal volume[37–39]. Adult women who have been sexually abused as children also show a decrease in hippocampal volume[40,41] as compared with women with no history of abuse. This suggests that not only are existing depressive disorders associated with reduced hippocampal volume but traumatic events in early life trigger lasting structural changes in this brain region.

The validity of maternal separation as a model for studying the interaction between early adverse experience and the development of psychopathology is strengthened by findings that some of the abnormalities associated with maternal separation—namely, heightened anxiety and altered responsiveness of the HPA axis—can be reversed with antidepressant treatment[11]. Although it is unclear whether stress-induced adaptations of hippocampal structure underlie the etiology of depression, observations that adult neurogenesis in the hippocampus is decreased by stress and is requisite for some behavioral effects of chronic antidepressants suggest that this process may be involved in the modulation of mood disorders[32].

METHODS

Rat treatments. All experiments were done in accordance with Princeton University guidelines and with the US National Institutes of Health (NIH) Guide for the Care and Use of Laboratory Animals.

Early life experience. The maternal separation and handling procedures were based on standardized protocols[7], except that Sprague-Dawley rather than Long Evans rats were used because substantial baseline data on adult neurogenesis has been obtained from this strain in our laboratory[23,28,33] Litters bred in-house were cross-fostered and culled on P1, the day after birth. Litter composition was standardized to 10 pups (5–6 males, 4–5 females). We assigned whole litters to one of three rearing conditions from P1–P14: AFR, handled with brief maternal separation (HMS15) or handled with prolonged maternal separation (HMS180). During this period, regular cage cleaning was ceased to avoid disturbing home cage nests. Rats were maintained on a 12 h/12 h light/dark cycle (lights on 07:00) and provided with *ad libitum* access to food and water.

For HMS15 and HMS180 litters, dams were first removed and placed in an adjacent cage. Litters were then transferred to a plastic container lined with bedding from the home cage and placed in an incubator maintained at roughly 34 °C, a temperature consistent with nest measurements. After the separation period, HMS15 and HMS180 pups were returned to their home cage, where they were reunited with the dam. We treated rats in both groups identically except for the duration of maternal separation. HMS15 litters were separated from the dam for 15 min, which corresponds to the average amount of time that mother rats reportedly leave the nest in the field[42]. HMS180 litters were separated for a period of 180 min. AFR litters were left undisturbed until P14, at which time normal cage cleaning resumed. On P22, all rats were weaned and housed as whole litters until P45. At this stage, rats were housed as same-sex and same-litter groups of two or three per cage. All experiments were carried out on sexually mature male rats beginning at P60–P70.

Lowering glucocorticoid levels. To determine whether the suppression of cell proliferation could be reversed by reducing levels of circulating corticosterone, rats from each group were either bilaterally adrenalectomized or sham-operated under deep sodium pentobarbital anesthesia on P60–P70. Adrenalectomized rats were given a low dose of corticosterone in the drinking

© 2004 Nature Publishing Group http://www.nature.com/natureneuroscience

water (25 μg/ml in 0.9% saline; Sigma) to reduce levels of adrenal steroids while maintaining normal plasma electrolytes and preventing granule cell death[43].

Fox odor exposure in adulthood. To examine the influence of a natural stressor on adult HMS15, HMS180 and AFR rats, groups of rats were exposed to trimethyl thiazoline (Pherotech), a main component of fox feces[44]. Before odor exposure, rats were placed into clean cages in one of two separately ventilated experimental rooms and acclimated to this environment for at least 2 h. A vial containing gauze and 150 μl of saline (sham odor) or trimethyl thiazoline was then placed in the middle of the cage. Some of these rats were injected with BrdU after 1 h and perfused 2 h or 1 week later. Other groups were exposed to either sham or fox odor and rapidly decapitated after 30 min for blood measures of corticosterone. Blood was not collected from rats injected with BrdU because of the potential stress associated with this procedure.

BrdU administration. To determine whether early life experience affects baseline cell proliferation and neurogenesis in the hippocampus, HMS15, HMS180 and AFR rats were injected with BrdU (200 mg per kg body weight, intraperitoneally (i.p.), in saline plus 0.007 M NaOH; Sigma), and perfused after 2 h, 1 week or 3 weeks. We used the 2-h time point because it is sufficient for uptake of BrdU but not for mitosis, migration or differentiation[20]. The 1-week time point was used because by this time most BrdU-labeled cells in the dentate gyrus are located in the granule cell layer and express Tuj1, a marker of immature and mature neurons[20,23]. The 3-week time point was used because by this time most BrdU-labeled cells express NeuN, a marker of mature neurons[23].

To determine whether group differences in baseline cell proliferation were related to adrenal steroid levels, rats from each rearing group were subjected to either adrenalectomy or sham-surgery in adulthood. After recovery, all rats were injected with BrdU and killed after 2 h.

To determine whether early life experience alters cell proliferation and neurogenesis in response to adult stress, rats from all three rearing groups were exposed to fox odor or sham odor, injected with BrdU after 1 h and perfused 2 h or 1 week later. In a previous study, this regimen of fox odor exposure yielded a significant decrease in the number of BrdU-labeled cells in the dentate gyrus of control rats at both time points[23]. All rats injected with BrdU were deeply anesthetized with an overdose of sodium pentobarbital- (Nembutal; 100 mg/kg, i.p.) and transcardially perfused with 4.0% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were post-fixed for 3 d.

BrdU immunohistochemistry. For each brain, we cut 40-μm unilateral sections through the whole dentate gyrus into a bath of 0.1 M PBS (pH 7.5) with an OTS-4000 oscillating tissue slicer (Electron Microscopy Sciences).

For BrdU peroxidase staining, sections were mounted onto glass slides, dried and heated in 0.1 M citric acid (pH 6.0). After being rinsed, the slides were incubated in trypsin (1 mg/ml in Tris buffer plus 10 μl/ml of 10% calcium chloride). Sections were denatured in 2 M HCl in PBS for 30 min, rinsed and incubated with mouse antibodies to BrdU (diluted 1:200 with 0.5% Tween-20; Vector). The next day, the slides were rinsed, incubated with biotinylated anti-mouse (1:200; Vector) for 60 min, rinsed, incubated with avidin-biotin complex, rinsed and reacted with diaminobenzidine and H_2O_2 (Sigma-Aldrich). Slides were counterstained with cresyl violet, dehydrated, cleared and covered with a coverslip under Permount (Fisher Scientific).

For BrdU immunofluorescence, sections were denatured in 2 M HCl in TBS for 30 min, rinsed and incubated with rat anti-BrdU (1:200 with 0.5% Tween-20; Accurate Chemical) plus mouse anti-NeuN (1:500; Chemicon), mouse anti-Tuj1 (1:500; Covance) or guinea-pig anti-GFAP (1:500; Advanced Immunochemical) for 2 d. Then sections were rinsed, incubated with biotinylated anti-rat (1:250; Vector) for 90 min, rinsed, and incubated for 30 min in the dark with streptavidin-conjugated Alexa 568 (1:1,000; Molecular Probes) to visualize BrdU and with goat anti–mouse Alexa 488 or goat anti–guinea-pig Alexa 488 (1:500; Molecular Probes) to visualize Tuj1, NeuN or GFAP. We counterstained some sections with the DNA dye Hoechst 33342 (1:1,000; Molecular Probes). The sections were rinsed, mounted onto slides, and covered under TBS and glycerol.

Data analysis. All slides were coded before analysis, and the code was not broken until each analysis was complete.

BrdU peroxidase. BrdU-positive cells on every twelfth unilateral section through the whole dentate gyrus were counted at ×1,000 magnification, avoiding cells in the outermost plane of focus. The number of BrdU-labeled cells per dentate gyrus was then multiplied by 24 to estimate the total number of BrdU-positive cells through the dentate gyrus. The numbers of BrdU-positive cells in the caudal subventricular zone of HMS15, HMS180 and AFR rats were counted and expressed as the number of cells per mm^3. We calculated volumes from cross-sectional area measurements obtained with Neurolucida (MicroBrightField, Williston, VT) by using Cavalieri's principle.

BrdU immunofluorescence. For each cell type marker, 25 BrdU-labeled cells in the granule cell layer of each rat were randomly selected and examined by confocal microscopy (Axiovert 510 LSM; lasers, Argon 458/488 and HeNe 543; Zeiss). Optical stacks of 1-μm-thick sections were obtained through putatively double-labeled cells. To verify double-labeling throughout their extent, cells were examined in orthogonal planes.

Corticosterone radioimmunoassay. To determine whether there was a relationship between corticosterone and BrdU cell counts, we obtained measures of corticosterone from trunk blood collected from decapitated unanesthetized rats in all three rearing conditions under basal conditions and after exposure to either sham or fox odor. Trunk blood was also analyzed from adrenalectomized, corticosterone-treated rats and sham-operated rats to verify that adrenalectomy plus corticosterone treatment resulted in reduced circulating corticosterone as compared with that in sham-operated rats. Circulating corticosterone (free plus bound) was determined by radioimmunoassay using a Coat-a-Count Rat Corticosterone kit (detection limit, 5.7 ng/ml; cross-reactivity, <3.0% for 11-deoxycorticosterone and <1.0% for all other steroid hormones; Diagnostics Product Corporation).

Statistical analysis. The mean ± s.e.m. was determined for each group, and the data were subjected to one- or two-way (early life experience × stress in adulthood, early life experience × surgical procedure) analysis of variance and Tukey-HSD *post hoc* comparisons.

ACKNOWLEDGMENTS
We thank to D.L. Samburg, N.B. Hastings, P. Tanapat and Y. Kozorovitskiy for help with this manuscript. This work was supported by funding from the NIH (grant MH59740) and the Conte Center for the Neuroscience of Mental Disorders (MH58922).

COMPETING INTERESTS STATEMENT
The authors declare that they have no competing financial interests.

Received 8 March; accepted 15 June 2004
Published online at http://www.nature.com/natureneuroscience/

1. Heim, C. & Nemeroff, C.B. The role of childhood trauma in the neurobiology of mood and anxiety disorders: preclinical and clinical studies. *Biol. Psychiatry* **49**, 1023–1039 (2001).
2. Sanchez, M.M., Ladd, C.O. & Plotsky, P.M. Early adverse experience as a developmental risk factor for later psychopathology: evidence from rodent and primate models. *Dev. Psychopathol.* **13**, 419–449 (2001).
3. Newport, D.J., Stowe, Z.N. & Nemeroff, C.B. Parental depression: animal models of an adverse life event. *Am. J. Psychiatry* **159**, 1265–1283 (2002).
4. Levine, S. Influence of psychological variables on the activity of the hypothalamic-pituitary-adrenal axis. *Eur. J. Pharmacol.* **405**, 149–160 (2000).
5. Huot, R.L., Plotsky, P.M., Lenox, R.H. & McNamara, R.K. Neonatal maternal separation reduces hippocampal mossy fiber density in adult Long Evans rats. *Brain Res.* **950**, 52–63 (2002).
6. Sapolsky, R.M. & Meaney, M.J. Maturation of the adrenocortical stress response: neuroendocrine control mechanisms and the stress hyporesponsive period. *Brain Res.* **396**, 64–76 (1986).
7. Plotsky, P.M & Meaney, M.J. Early, postnatal experience alters hypothalamic corticotropin-releasing factor (CRF) mRNA, median eminence CRF content and stress-induced release in adult rats. *Brain Res. Mol. Brain Res.* **18**, 195–200 (1993).
8. Liu, D., Caldji, C., Sharma, S., Plotsky, P.M. & Meaney, M.J. Influence of neonatal rearing conditions on stress-induced adrenocorticotropin responses and norepinephrine release in the hypothalamic paraventricular nucleus. *J. Neuroendocrinol.* **12**, 5–12 (2000).
9. Meaney, M.J., Aitken, D.H., Viau, V., Sharma, S. & Sarrieau, A. Neonatal handling alters adrenocortical negative feedback sensitivity and hippocampal type II glucocorticoid receptor binding in the rat. *Neuroendocrinology* **50**, 597–604 (1989).

© 2004 Nature Publishing Group http://www.nature.com/natureneuroscience

10. Viau, V., Sharma, S., Plotsky, P.M. & Meaney, M.J. Increased plasma ACTH responses to stress in nonhandled compared with handled rats require basal levels of corticosterone and are associated with increased levels of ACTH secretagogues in the median eminence. *J. Neurosci.* **13**, 1097–1105 (1993).
11. Huot, R.L., Thrivikraman, K.V., Meaney, M.J. & Plotsky, P.M. Development of adult ethanol preference and anxiety as a consequence of neonatal maternal separation in Long Evans rats and reversal with antidepressant treatment. *Psychopharmacology* **158**, 366–373 (2001).
12. Lovic, V., Gonzalez, A. & Fleming, A.S. Maternally separated rats show deficits in maternal care in adulthood. *Dev. Psychobiol.* **39**, 19–33 (2001).
13. Magarinos, A.M., Somoza, G. & De Nicola, A.F. Glucocorticoid negative feedback and glucocorticoid receptors after hippocampectomy in rats. *Horm. Metab. Res.* **19**, 105–109 (1987).
14. Sapolsky, R.M., Armanini, M.P., Sutton, S.W. & Plotsky, P.M. Elevation of hypophysial portal concentrations of adrenocorticotropin secretagogues after fornix transection. *Endocrinology* **125**, 2881–2887 (1989).
15. Herman, J.P., Cullinan, W.E., Morano, M.I., Akil, H. & Watson, S.J. Contribution of the ventral subiculum to inhibitory regulation of the hypothalamo-pituitary-adrenocortical axis. *J. Neuroendocrinol.* **7**, 475–482 (1995).
16. Deacon, R.M., Bannerman, D.M. & Rawlins, J.N. Anxiolytic effects of cytotoxic hippocampal lesions in rats. *Behav. Neurosci.* **116**, 494–497 (2002).
17. Bannerman, D.M. *et al.* Double dissociation of function within the hippocampus: spatial memory and hyponeophagia. *Behav. Neurosci.* **116**, 884–901 (2003).
18. Riedel, G. *et al.* Reversible neural inactivation reveals hippocampal participation in several memory processes. *Nat. Neurosci.* **2**, 898–905 (1999).
19. Gould, E. & Gross, C.G. Neurogenesis in adult mammals: some progress and problems. *J. Neurosci.* **22**, 619–623 (2002).
20. Cameron, H.A. & McKay, R.D. Adult neurogenesis produces a large pool of new granule cells in the dentate gyrus. *J. Comp. Neurol.* **435**, 406–417 (2001).
21. Cameron, H.A. & Gould, E. Adult neurogenesis is regulated by adrenal steroids in the dentate gyrus. *Neuroscience* **61**, 203–209 (1994).
22. Gould, E., Cameron, H.A., Daniels, D.C., Woolley, C.S. & McEwen, B.S. Adrenal hormones suppress cell division in the adult rat dentate gyrus. *J. Neurosci.* **12**, 3642–2650 (1992).
23. Tanapat, P., Hastings, N.B., Rydel, T.A., Galea, L.A. & Gould, E. Exposure to fox odor inhibits cell proliferation in the hippocampus of adult rats via an adrenal hormone-dependent mechanism. *J. Comp. Neurol.* **437**, 496–504 (2001).
24. Gould, E., McEwen, B.S., Tanapat, P., Galea, L.A. & Fuchs, E. Neurogenesis in the dentate gyrus of the adult tree shrew is regulated by psychosocial stress and NMDA receptor activation. *J. Neurosci.* **17**, 2492–2498 (1997).
25. Gould, E., Tanapat, P., McEwen, B.S., Flugge, G. & Fuchs, E. Proliferation of granule cell precursors in the dentate gyrus of adult monkeys is diminished by stress.*Proc. Natl. Acad. Sci. USA* **95**, 3168–3171 (1998).
26. Malberg, J.E. & Duman, R.S. Cell proliferation in adult hippocampus is decreased by inescapable stress: reversal by fluoxetine treatment. *Neuropsychopharmacology* **28**, 1562–1571 (2003).
27. Viau, V., Sharma, S. & Meaney, M.J. Changes in plasma adrenocorticotropin, corticosterone, corticosteroid-binding globulin, and hippocampal glucocorticoid receptor occupancy/translocation in rat pups in response to stress. *J. Neuroendocrinol.* **8**, 1–8 (1996).
28. Hastings, N.B. & Gould, E. Rapid extension of axons into the CA3 region by adult-generated granule cells. *J. Comp. Neurol.* **413**, 146–154 (1999).
29. Snyder, J.S., Kee, N. & Wojtowicz, J.M. Effects of adult neurogenesis on synaptic plasticity in the rat dentate gyrus. *J. Neurophysiol.* **85**, 2423–2431 (2001).
30. Wang, S., Scott, B.W. & Wojtowicz, J.M. Heterogenous properties of dentate granule neurons in the adult rat. *J. Neurobiol.* **42**, 248–257 (2000).
31. Schmidt-Hieber, C., Jonas, P. & Bischofberger, J. Enhanced synaptic plasticity in newly generated granule cells of the adult hippocampus. *Nature* **429**, 184–187 (2004).
32. Santarelli, L. *et al.* Requirement of hippocampal neurogenesis for the behavioral effects of antidepressants. *Science* **301**, 805–809 (2003).
33. Gould, E., Beylin, A., Tanapat, P., Reeves, A. & Shors, T.J. Learning enhances adult neurogenesis in the hippocampal formation. *Nat. Neurosci.* **2**, 260–265 (1999).
34. Dobrossy, M.D. *et al.* Differential effects of learning on neurogenesis: learning increases or decreases the number of newly born cells depending on their birth date. *Mol. Psychiatry* **8**, 974–982 (2003).
35. Shors, T.J., Townsend, D.A., Zhao, M., Kozorovitskiy, Y. & Gould, E. Neurogenesis may relate to some but not all types of hippocampal-dependent learning. *Hippocampus* **12**, 578–584 (2002).
36. Madsen, T.M., Kristjansen, P.E., Bolwig, T.G. & Wortwein, G. Arrested neuronal proliferation and impaired hippocampal function following fractionated brain irradiation in the adult rat. *Neuroscience* **119**, 635–642 (2003).
37. Sheline, Y.I. Hippocampal atrophy in major depression: a result of depression-induced neurotoxicity? *Mol. Psychiatry* **1**, 298–299 (1996).
38. Sheline, Y.I., Sanghavi, M., Mintun, M.A. & Gado, M.H. Depression duration but not age predicts hippocampal volume loss in medically healthy women with recurrent major depression. *J. Neurosci.* **19**, 5034–5043 (1999).
39. Bremner, J.D. *et al.* Hippocampal volume reduction in major depression. *Am. J. Psychiatry* **158**, 652–653 (2000).
40. Stein, M.B., Koverola, C., Hanna, C., Torchia, M.G. & McClarty, B. Hippocampal volume in women victimized by childhood sexual abuse. *Psychol. Med.* **27**, 951–959 (1997).
41. Vythilingam, M. *et al.* Childhood trauma associated with smaller hippocampal volume in women with major depression. *Am. J. Psychiatry* **159**, 2072–2080 (2002).
42. Jans, J.E. & Woodside, B.C. Nest temperature: effects on maternal behavior, pup development, and interactions with handling. *Dev. Psychobiol.* **23**, 519–534 (1990).
43. Gould, E., Woolley, C.S. & McEwen, B.S. Short-term glucocorticoid manipulations affect neuronal morphology and survival in the adult dentate gyrus. *Neuroscience* **37**, 367–375 (1990).
44. Vernet-Maury, E., Polak, E.H. & Demael, A. Structure-activity relationship of stress-inducing odorants in the rat. *J. Chem. Ecol.* **10**, 1007–1019 (1984).

© 2004 Nature Publishing Group http://www.nature.com/natureneuroscience