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Maternal Experience Inhibits the Production of Immature Neurons in the Hippocampus During the Postpartum Period Through Elevations in Adrenal Steroids

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ABSTRACT: Motherhood is accompanied by alterations in numerous nonreproductive behaviors, including learning and memory, as well as anxiety and stress regulation. These functions have been linked to adult neurogenesis in the hippocampus, but the effect of maternal experience on this brain region has not been completely explored. To determine whether the production of new hippocampal granule cells is altered during the postpartum period, we examined the number of proliferating cells and their progeny in the dentate gyrus of primiparous female rats at various time points during the postpartum period while they were caring for their offspring, as well as after weaning. Additionally, we investigated whether cell proliferation in the postpartum female is affected by the presence of offspring and nursing-induced increases in glucocorticoids. Analysis of the number of BrdU-labeled cells revealed that cell proliferation in the dentate gyrus was suppressed in lactating postpartum females until the time of weaning. This effect was temporary; a difference was detectable at 1 week after BrdU-labeling, when the majority of cells expressed a marker of immature and mature granule neurons (TuJ1) but not at 2 weeks, when most cells expressed a marker of mature neurons (NeuN). The decrease in cell proliferation was dependent on elevated basal glucocorticoid levels associated with lactation; removal of nursing pups reduced basal corticosterone levels and prevented the decrease in the number of BrdU-labeled cells. Moreover, preventing increased basal corticosterone levels by means of adrenalectomy and low-dose corticosterone replacement eliminated the reduction in cell proliferation. These findings indicate that offspring interactions inhibit adult neurogenesis through changes in adrenal steroids, and further suggest a potential mechanism for alterations in hippocampal function during the postpartum period. © **2007 Wiley-Liss, Inc.**

KEY WORDS: dentate gyrus; cell proliferation; neurogenesis; glucocorticoids; lactation

INTRODUCTION

Numerous behavioral and physiological changes accompany motherhood, the most obvious being maternal care and lactation. In addition, several nonreproductive functions are altered during the postpartum period. For example, human and rodent studies have shown that the postpartum period is associated with changes in some types of learning and memory, anxiety regulation, and responsiveness to stress (Brett and Baxendale, 2001; Neumann, 2001; Lonstein, 2005; DeGroot et al., 2006; Leuner and Shors, 2006). All of these processes are known to involve the hippocampus (Jacobson and Sapolsky, 1991; Bannerman et al., 2004; Kesner and Hopkins, 2006), a brain region that shows a considerable degree of structural plasticity throughout adulthood. In the adult rodent, a large number of new granule neurons are produced each day in the dentate gyrus (DG) of the hippocampus (Cameron et al., 1993; Cameron and McKay, 2001). Although these new neurons have been implicated in many of the functions affected by motherhood (Santarelli et al., 2003; Leuner et al., 2006), previous studies have not explored the possibility that adult neurogenesis is altered during the postpartum period.

Motherhood is associated with profound changes to a variety of hormonal systems (Numan and Insel, 2003), including those of the hypothalamic–pituitary–adrenal (HPA) axis. Elevations in circulating levels of basal glucocorticoids occur postpartum, an effect that is directly related to the presence of nursing pups (Voogt et al., 1969; Stern et al., 1973; Walker et al., 1992; Fischer et al., 1995). Because adrenal steroids regulate adult neurogenesis (Cameron and Gould, 1994; Tanapat et al., 2001; Mirescu and Gould, 2006), it is possible that this form of structural plasticity may be altered by offspring contact, through the influence that this experience has on the HPA axis.

To investigate this possibility, we evaluated the number of proliferating cells and their progeny in the DG of primiparous female rats at various time points during the postpartum period while they were caring for their offspring, as well as after weaning. To more specifically examine the influence of offspring on postpartum-induced alterations in granule cell production, we assessed the number of proliferating cells in the DG of postpartum females that had offspring removed shortly after birth. In addition, to establish whether a causal relationship exists between changes in cell proliferation during the postpartum period and nursing-induced increases in adrenal steroids, the production of newly-born cells was examined after preventing the increase in basal corticosterone by bilateral adrenalectomy and replacement with low dose corticosterone.

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MATERIALS AND METHODS

Animal Care and Treatments

All experiments were approved by the Princeton University Institutional Animal Care and Use Committee and conformed to the US NIH Guide for the Care and Use of Laboratory Animals. Adult female Sprague–Dawley rats (~80–120 days of age) used in these experiments were from the breeding colony at Princeton University. Rats were maintained on a 12 h/12 h light/dark cycle (7 A.M. lights on) and provided with ad libitum access to food and water. Primiparous postpartum females were generated by pairing normally cycling virgin females with an adult male for five nights after which females were housed individually in translucent plastic cages lined with wood chip bedding and provided with nesting material. On the day of birth (postpartum day 0; PD0), litters were culled to 10 pups (5–6 males, 4–5 females).

Vaginal Cytology

In each experiment, stages of the estrous cycle were monitored in virgin females from cytology obtained through daily vaginal swabs (Everett, 1989). Only females that demonstrated at least two 4–5 day cycles were included in the experiment. Since the postpartum period is characterized by persistent diestrus and the cessation of ovarian cycling (Smith and Neill, 1977), vaginal cytology was not monitored in postpartum females. However, after weaning on PD28, vaginal cytology was examined in nonlactating postpartum females for at least 2 weeks to confirm the resumption of estrous cyclicity.

Experimental Design

Experiment 1

To determine whether the postpartum period is accompanied by alterations in cell proliferation in the DG, primiparous females at different time points during the postpartum period (PD2, $n = 7$; PD8, $n = 5$; PD28, $n = 8$) were injected with the S-phase marker bromodeoxyuridine (BrdU; 200 mg/kg bw i.p.) and were perfused after 2 h. This dose of BrdU was chosen based on prior work showing that lower doses label only a fraction of dividing cells (Cameron and McKay, 2001). The 2-h survival time was selected because it is sufficient for the incorporation of BrdU by cells in S-phase but not for the completion of mitosis or migration to occur (Cameron and McKay, 2001). These groups were compared to age-matched cycling virgin females in diestrus 2 ($n = 9$) and nonlactating, cycling postpartum females in diestrus 2, at least 2 weeks after litters were weaned on PD28 ($n = 6$). After perfusion, all brains were removed, postfixed, and processed for BrdU immunohistochemistry.

To determine whether neurogenesis in the DG is altered during the postpartum period, additional groups of age-matched PD2 and diestrus 2 virgin females were injected with BrdU and perfused 1 week or 2 weeks later ($n = 4$–6/group). The 1-week survival was chosen because by this time most BrdU-labeled cells in the DG are located in the granule cell layer and express class III β-tubulin (TuJ1), a marker of immature and mature neurons (Cameron and McKay, 2001; Tanapat et al., 2001). The 2-week survival was selected because by this time the majority of adult-generated cells express neuronal nuclei (NeuN), a marker of mature neurons (Stranahan et al., 2006). Postpartum females remained with their pups during the postinjection survival intervals. After perfusion, all brains were removed, postfixed, and processed for BrdU immunohistochemistry. Additional sections from the 1- and 2-week groups were processed for combined BrdU-labeling and immunohistochemistry for TuJ1 or NeuN.

Experiment 2

To determine if the presence of nursing pups mediates the reduction in cell proliferation during the postpartum period, groups of postpartum females remained with their pups ($n = 5$) or were separated from pups shortly after birth (PD0) and placed into clean cages ($n = 6$). Three days later (PD3), these rats were injected with BrdU along with age-matched diestrus 2 virgins ($n = 7$) and perfused 2 h later. After perfusion, all brains were removed, postfixed, and processed for BrdU immunohistochemistry. The three day time point was chosen because corticosterone returns to prepartum levels within this time if nursing and lactation do not occur (Walker et al., 1992). To confirm the influence of pup removal on corticosterone levels in postpartum females, trunk blood was collected by decapitation (3 P.M.) from separate groups of PD3 females with pups ($n = 5$), PD3 females that had pups removed on PD0 ($n = 7$) and diestrus 2 virgins ($n = 9$).

Experiment 3

To evaluate whether differences in cell proliferation during the postpartum period were related to elevated basal levels of adrenal steroids, age-matched virgin ($n = 7$) and postpartum (PD1; $n = 8$) rats were anesthetized with sodium pentobarbital anesthesia and adrenal glands removed bilaterally through small dorsal incisions. Virgin ($n = 5$) and postpartum ($n = 7$) rats assigned to sham surgery were subjected to the same surgical procedure except that the adrenal glands were left intact. During the surgery, litters were kept in plastic containers lined with bedding from the home cage and placed in an incubator maintained at nest temperature. ADX rats were given a low dose of corticosterone (Cort) in the drinking water (25 μg/ml in 0.9% saline) to reduce levels of adrenal steroids while maintaining the diurnal rhythm of glucocorticoids and normal plasma electrolytes as well as to prevent death of mature hippocampal granule neurons (Gould et al., 1990; Cameron and Gould, 1994).

To confirm that adrenal steroids are necessary for milk production (Tucker, 1994), overall litter weight gain was determined by weighing entire litters from the ADX + Cort and

Sham females before surgery on PD1 and on the day of perfusion (PD8). In addition, to evaluate whether maternal behavior was altered by adrenal steroid removal, ADX + Cort and Sham females were videotaped for 10 min on PD6 immediately following a brief (<5 min) separation from pups. Digital videos were analyzed for the following behaviors: latency to retrieve the first pup, time spent in nest, and duration of pup licking.

One week following surgery, virgin and postpartum (PD8) females from ADX + Cort and Sham groups were injected with BrdU and perfused 2 h later. In this experiment, virgin females were in different stages of estrous at the time of perfusion in order to keep the time between surgery and BrdU injection similar in all rats. However, the proportion of females in different stages of the estrous cycle was similar for ADX + Cort and Sham groups. After perfusion, all brains were removed, postfixed, and processed for BrdU immunohistochemistry. At the time of perfusion, blood samples were obtained to verify the efficacy of ADX.

Perfusion

All animals were deeply anesthetized with an overdose of sodium pentobarbital and transcardially perfused with 4.0% paraformaldehyde in 0.1 M phosphate buffer (pH 7.4). Brains were removed and postfixed for at least 2 days.

Immunohistochemistry

Coronal sections (40-μm) throughout the entire rostrocaudal extent of the DG were cut with a Vibratome from half brains into a bath of 0.1 M phosphate buffered saline (PBS) (pH 7.5). For BrdU peroxidase staining, a 1:12 series of sections were mounted onto glass slides, dried, and pretreated by heating in 0.1 M citric acid (pH 6.0). Slides were then rinsed in PBS, incubated in trypsin for 10 min, rinsed, denatured in 2 M HCl:PBS for 30 min, rinsed, and incubated overnight at 4°C in mouse monoclonal antibody against BrdU (diluted 1:250 with 0.5% Tween-20; Vector Laboratories, Burlingame, CA). The next day, slides were rinsed, incubated with biotinylated anti-mouse (1:200; Vector) for 60 min, rinsed, incubated with avidin–biotin complex (1:100; Vector) for 60 min, rinsed, and reacted in 0.01% diaminobenzidine with 0.003% H_2O_2 (Sigma-Aldrich, St. Louis, MO). Slides were counterstained with cresyl violet, dehydrated, cleared, and coverslipped under Permount (Fisher Scientific, Fair Lawn, NJ). Controls included processing brain sections as described earlier with omission of the primary antisera as well as brain sections from animals that did not receive BrdU injections; in either case, BrdU immunoreactive staining was not detected.

To determine the phenotype of BrdU-labeled cells, tissue was processed for double-labeling immunofluorescence for BrdU and the neuronal markers TuJ1 or NeuN. The following primary antibodies were used: (1) anti-BrdU (Accurate, Westbury, NY), a rat monoclonal antibody against BrdU that reacts with BrdU in single-stranded DNA and does not cross-react with thymidine. Staining was limited to cell nuclei and no staining was seen when the BrdU antibody was used on tissue from an animal that did not receive a BrdU injection. (2) Anti-TuJ1 (Covance, Princeton, NJ), a mouse monoclonal antibody that recognizes a neuron-specific class III β-tubulin, which is considered to be an early marker for cells that have begun to differentiate into neurons (Alexander et al., 1991). This antibody, raised against microtubules derived from rat brain, has been shown to recognize a single band of 50 kD molecular weight on Western blot and does not identify β-tubulin found in glial cells (Covance product datasheet). (3) Anti-NeuN (Chemicon International, Temecula, CA), a mouse monoclonal antibody that recognizes the neuron-specific nuclear protein NeuN. This antibody was raised against purified cell nuclei from mouse brain and has been shown to recognize 2–3 bands at 46–48 kDa by Western blot (Chemicon International product datasheet; Lind et al., 2005). Both anti-TuJ1 and anti-NeuN antibodies have been previously used to identify the phenotypes of adult-generated cells in the rat brain (Cameron and McKay, 2001; Stranahan et al., 2006) and showed staining patterns in neurons of the DG (TuJ1: cytoplasm; NeuN: nuclear and perinuclear) that was consistent with prior reports.

For immunofluorescence, free-floating sections were denatured in 2 M HCl:TBS for 30 min, rinsed in TBS, and incubated with rat anti-BrdU (1:200 with 0.5% Tween-20) plus mouse anti-TuJ1 (1:500) or mouse anti-NeuN (1:500) for 2 days. Sections were then rinsed, incubated with biotinylated anti-rat (1:250; Chemicon) for 90 min, rinsed, and incubated for 30 min in the dark with streptavidin-conjugated Alexa 568 (1:1,000; Molecular Probes, Eugene, OR) to visualize BrdU and with goat anti-mouse Alexa 488 (1:500; Molecular Probes) to visualize TuJ1 or NeuN. Some sections were counterstained with the DNA dye Hoechst 33342 (1:1,000; Molecular Probes). The sections were rinsed, mounted onto slides, and coverslipped using glycerol in TBS (3:1). Control sections were processed as described earlier with omission of the primary antisera.

Microscopic Data Analysis

All slides were coded prior to quantitative analysis.

BrdU Peroxidase

Estimates of total numbers of BrdU-labeled cells were determined using a modified stereology protocol (West et al., 1991). BrdU-labeled cells on every twelfth unilateral section throughout the entire rostrocaudal extent of the DG (granule cell layer, subgranular zone and hilus) were counted at 1,000× (100× objective with a 10× ocular) on an Olympus BX-50 light microscope, avoiding cells in the outermost focal plane. Counts were multiplied by 24 to obtain estimates of BrdU-labeled cells per brain. The total volume of the granule cell layer was calculated from cross-sectional area measurements obtained with Image-Pro Plus software (Media Cybernetics, Sliver Spring, MD) by using Cavalieri's principle (Gundersen et al., 1988).

For purposes of comparison, the density of BrdU-labeled cells was also determined in the SVZ. BrdU-labeled cells in the SVZ present on every twelfth coronal section throughout the DG (−1.88 to −4.52 mm anteroposterior; Paxinos and Watson, 1998) were counted, the volume of the analyzed region measured, and the data expressed as densities (number of BrdU-labeled cells/mm^3). This analysis included a substantial part of the SVZ but excluded the anterior portion.

BrdU Immunofluorescence

The percentage of BrdU-labeled cells in the DG (granule cell layer and subgranular zone) that expressed NeuN or TuJ1 was determined using a Zeiss Axiovert confocal laser scanning microscope (510 LSM; lasers, Argon 458/488 and HeNe 543; Zeiss, Oberkochen, Germany). For each brain, 25 randomly selected BrdU-labeled cells per marker (TuJ1 and NeuN) were analyzed. 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

All blood samples were centrifuged at 14,000 rpm, plasma obtained, and stored at −20°C. Circulating levels of corticosterone (free plus bound) were later measured by radioimmunoassay using a Coat-a-Count Rat Corticosterone kit (detection limit, 5.7 ng/ml; Diagnostics Products, Los Angeles, CA).

Statistical Analysis

Data were subjected to a two-tailed *t*-test, one-way (reproductive status) or two-way (reproductive status X glucocorticoid status) analysis of variance (ANOVA) followed by Newman–Keuls post hoc comparisons.

RESULTS

Reduced Cell Proliferation and Immature Neuron Production During the Postpartum Period

PD2 and PD8 postpartum females had significantly fewer BrdU-labeled proliferating cells in the DG than diestrus 2 virgins 2 h after BrdU administration ($F_{(4,30)} = 7.7$, $P = 0.0002$; Figs. 1 and 2). By the time of weaning (PD28) and at least two weeks later when postpartum females had resumed normal estrous cycles and were in diestrus 2, the suppression in cell proliferation was no longer evident; both of these groups were not significantly different from diestrus 2 virgins. In contrast to the DG, the density of BrdU-labeled cells in the caudal SVZ did not differ statistically across groups 2 h after BrdU administration (diestrus 2 virgin, 17,840 ± 1,728 cells/mm^3; PD2, 14,280 ± 2,076 cells/mm^3; PD8, 18,820 ± 3,481 cells/mm^3; PD28, 17,810 ± 4,334 cells/mm^3; diestrus postweaning,

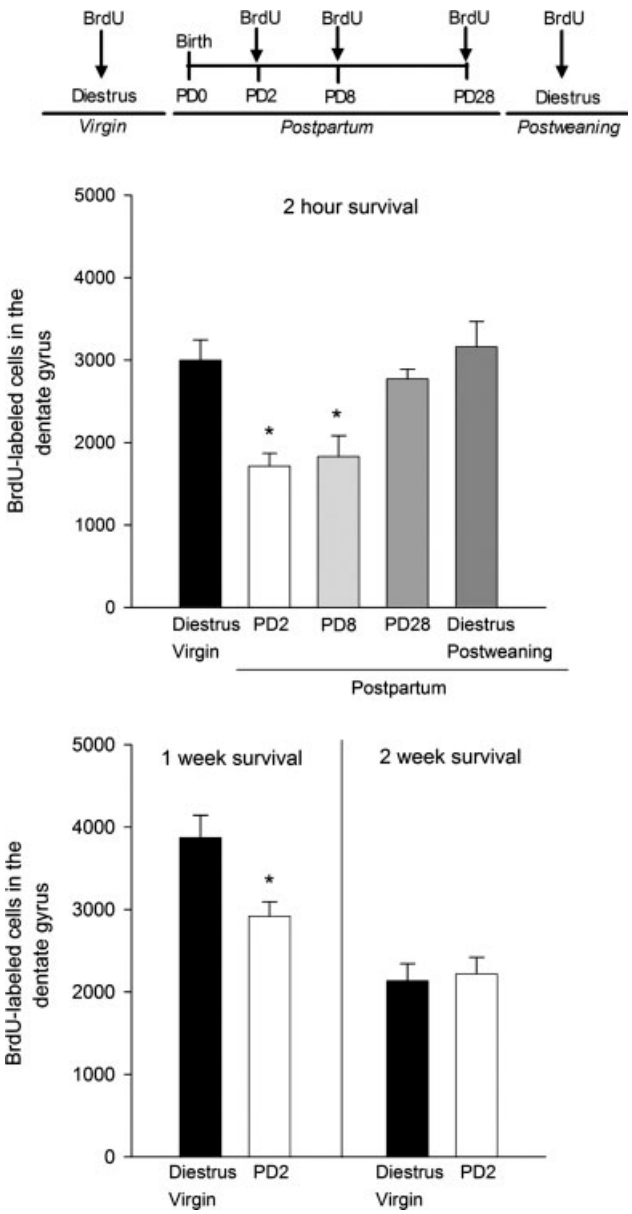

FIGURE 1. Suppression of cell proliferation and immature neuron production during the postpartum period. Top, Postpartum females on PD2, PD8, or PD28 were injected with BrdU along with age-matched diestrus 2 virgin females and nonlactating, cycling postpartum females at least 2 weeks after weaning in diestrus 2. All groups were perfused 2 h after BrdU injection. Additional groups of age-matched PD2 females and diestrus 2 virgins were injected with BrdU and perfused after 1 or 2 weeks. Middle, PD2 and PD8 females had fewer BrdU-labeled cells in the DG 2 h after BrdU injection than diestrus 2 virgins. The suppression in cell proliferation ended by PD28—both PD28 and postweaning diestrus 2 females had more BrdU-labeled cells than PD2 or PD8 females but did not differ from diestrus 2 virgins. Bottom, At 1 week, fewer numbers of BrdU-labeled cells were found in PD2 females relative to diestrus 2 virgins. By 2 weeks, no difference in the number of BrdU-labeled cells was evident. Bars represent mean ± standard error of mean (SEM). *$P < 0.05$.

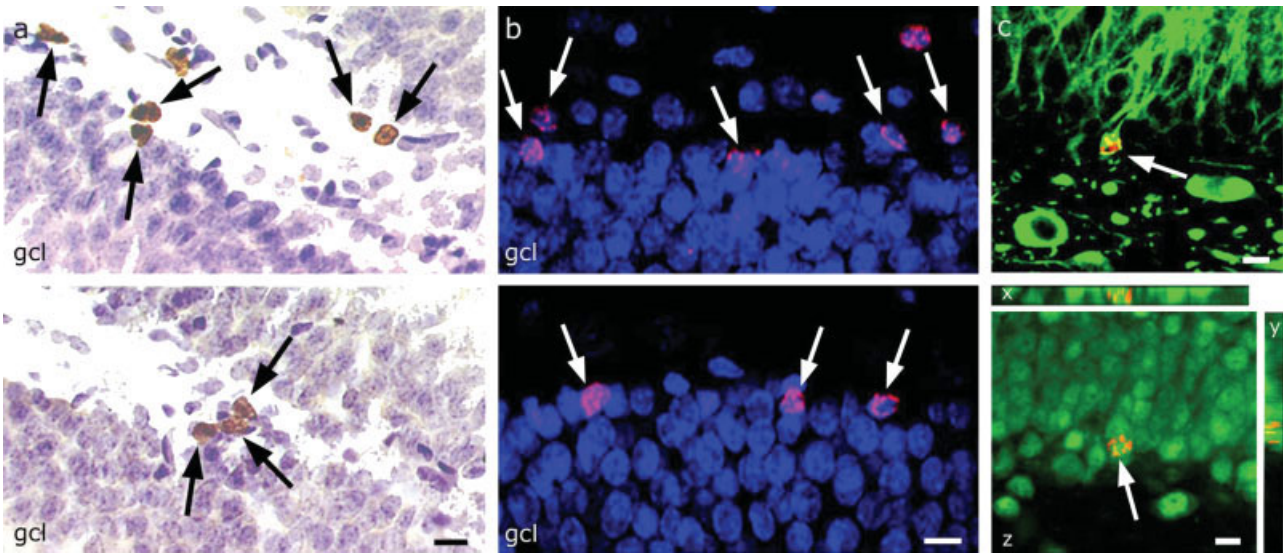

FIGURE 2. The postpartum period is associated with a reduction in immature neurons in the DG. (a) Compared to diestrus virgin females (top), BrdU cell counts in the DG were significantly lower in PD2 and PD8 postpartum females (bottom) 2 h after BrdU administration. (b) Confocal laser scanning microscopic images of BrdU-labeled cells 1 week after BrdU administration. At this time point, PD2 postpartum females (bottom) had fewer BrdU-labeled cells than diestrus virgins (top). (c) At 1 week, most BrdU-labeled cells expressed the marker of immature and mature neurons, TuJ1 (top). By 2 weeks, most BrdU-labeled cells were colabeled with NeuN, a marker of mature neuronal phenotype (bottom). The image is rotated in orthogonal planes (*x*, *y*, *z*) to verify double labeling throughout its extent. Arrows indicate labeled or double-labeled cells. gcl, granule cell layer. Scale bars, 10 μm. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

18,620 ± 826 cells/mm^3; $F_{(4,30)} = 0.40$, $P = 0.81$) suggesting that the reduction of cell proliferation during the postpartum period exhibits regional specificity.

A similar decrease in labeled cells was observed in PD2 mothers with a 1-week post-BrdU injection survival time ($t_{(8)} = 2.99$, $P = 0.02$; Figs. 1 and 2). Most of these cells (diestrus 2 virgin, 72.8% ± 3.2%; PD2, 68.8% ± 2.3%; $t_{(8)} = 1.01$, $P = 0.34$; Fig. 2) expressed TuJ1, a marker of immature and mature neurons. At 1 week, a relatively low percentage of BrdU-labeled cells expressed the mature neuronal marker NeuN (diestrus 2 virgin, 18.4% ± 2.0%; PD2, 16.0% ± 2.2%; $t_{(8)} = 0.80$, $P = 0.45$). These results demonstrate that the decrease in BrdU-labeled cells during the postpartum period is indicative of a decrease in the number of immature neurons.

However, following a 2-week post-BrdU injection survival time, the difference in numbers of BrdU-labeled cells between virgin and postpartum females was no longer evident ($t_{(8)} = 0.28$, $P = 0.79$; Fig. 1). The majority of BrdU-labeled cells expressed NeuN at 2 weeks (diestrus 2 virgin, 67.2% ± 5.9%; postpartum PD2, 68.0% ± 4.3%; $t_{(7)} = 0.10$, $P = 0.92$; Fig. 2). No differences in the total volume of the granule cell layer were detected among the groups at any time point.

Prevention of Suppressed Cell Proliferation During the Postpartum Period by Removal of Nursing Pups

Removal of pups shortly after birth prevented the decrease in cell proliferation in postpartum females. Two hours after BrdU injection, PD3 postpartum females without pups had more BrdU-labeled cells than PD3 postpartum females with pups but did not differ from diestrus 2 virgins ($F_{(2,15)} = 4.1$, $P = 0.04$; Fig. 3). Moreover, removal of pups from postpartum females was accompanied by a decrease in basal corticosterone levels. PD3 postpartum females with pups had higher levels of corticosterone than virgin females ($F_{(2,20)} = 5.15$, $P = 0.01$; Fig. 3). Removal of pups from PD3 postpartum females decreased corticosterone to levels observed in virgins.

Elimination of Suppressed Cell Proliferation During the Postpartum Period by Decreasing Basal Corticosterone Levels

Preventing the pup-induced elevation in corticosterone by adrenalectomy and low-dose Cort replacement eliminated the decrease in the number of BrdU-labeled cells observed during the postpartum period ($F_{(1,23)} = 5.1$, $P = 0.04$; Fig. 4). In postpartum females, ADX + Cort resulted in BrdU-labeled cell counts that were not significantly different from Sham or ADX + Cort virgin females, suggesting that prevention of the increase in basal corticosterone restored cell proliferation. ADX + Cort had no effect on cell proliferation in virgin females.

In both virgin and postpartum females, plasma corticosterone was significantly reduced by ADX + Cort, as compared with Sham controls ($F_{(1,22)} = 98.36$, $P = 0.000005$). No differences in CORT levels were noted among virgin (51.27 ± 10.15 ng/ml) or postpartum (54.15 ± 4.13 ng/ml) adrenalectomized animals, validating the efficacy of glucocorticoid normalization by this method and this dose of hormone replacement.

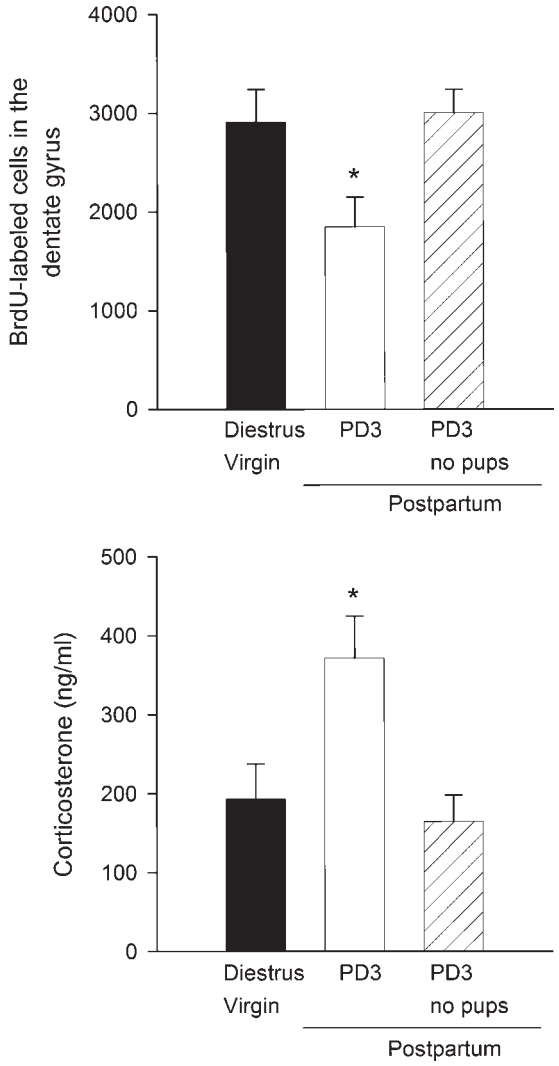

FIGURE 3. Prevention of suppressed cell proliferation and elevated basal corticosterone levels during the postpartum period by removal of nursing pups. Groups of postpartum females remained with their pups or were separated from pups shortly after birth on PD0. Three days later (PD3), these animals were injected with BrdU and perfused after 2 h along with age-matched diestrus 2 virgins. Top, PD3 postpartum females without pups had more BrdU-labeled cells than PD3 postpartum females with pups, but did not differ from diestrus 2 virgins. Bottom, PD3 postpartum females with pups had higher basal levels of corticosterone than virgin females. Removal of pups from PD3 postpartum females decreased corticosterone to levels similar to that of virgins. Bars represent mean ± SEM. *$P < 0.05$.

Consistent with a role for glucocorticoids in milk production (Tucker, 1994), overall litter weight gain was lower in the pups of ADX + Cort postpartum females (Sham, 139.4 ± 6.2 g; ADX + Cort, 97.4 ± 6.9 g; $t_{(13)} = 4.49$, $P = 0.0006$) but our behavioral analysis indicated no differences in maternal behavior in ADX + Cort mothers. No effects on pup retrieval latency ($t_{(11)} = 0.03$, $P = 0.98$), time spent by the mother in the nest ($t_{(11)} = 0.33$, $P = 0.74$), or the duration of pup licking ($t_{(11)} = 1.24$, $P = 0.24$) were observed.

DISCUSSION

Our findings demonstrate that the postpartum period is associated with a decrease in cell proliferation and immature neuron production. Two lines of evidence indicate that this suppressive effect is dependent on nursing pups: (1) it subsides around the time of weaning, when mothers no longer nurse; and (2) it can be completely prevented by removing the offspring shortly after birth. Moreover, our findings identify the adrenal steroid corticosterone as the factor responsible for this inhibition of immature neuron production. Basal levels of circulating corticosterone are high after birth and this elevation requires the presence of nursing pups (our results and Voogt et al., 1969; Stern et al., 1973; Walker et al., 1992; Fischer et al., 1995). Prevention of the rise in corticosterone (by adrenalectomy and low dose hormone replacement) eliminated the postpartum-induced decrease in cell proliferation despite the continued presence of nursing pups and no modification in maternal behavior. Collectively, these findings suggest that nursing pups and lactation elevate stress hormones and have a negative effect on structural plasticity in the postpartum hippocampus.

Regulation of Cell Proliferation During the Postpartum Period

Our data point to corticosterone as the primary mediator of suppressed cell proliferation during the postpartum period.

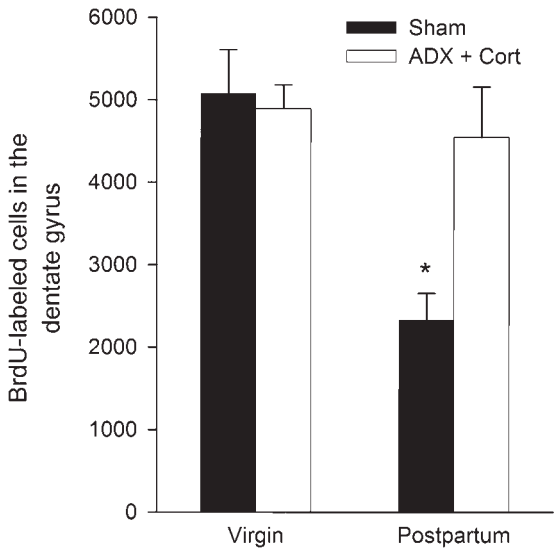

FIGURE 4. Elimination of suppressed cell proliferation during the postpartum period by lowering corticosterone. Age-matched virgin and postpartum females (PD1) were either sham-operated (Sham) or bilaterally adrenalectomized and given low dose corticosterone in the drinking water (ADX + Cort). One week after surgery, virgin and postpartum (PD8) females from the Sham and ADX + Cort groups were injected with BrdU and perfused after a 2-h survival. Lowering glucocorticoid levels had no effect on the number of BrdU-labeled cells in virgin females, but enhanced the number of BrdU-labeled cells in postpartum females to a level comparable to that in the virgin groups. Bars represent mean ± SEM. *$P < 0.05$.

These findings are consistent with previous studies demonstrating inhibition of adult neurogenesis under conditions of elevated glucocorticoid secretion such as stress (Tanapat et al., 2001; Mirescu and Gould, 2006; Hill et al., 2006; Mitra et al., 2006) and aging (Cameron and McKay, 1999). It has been suggested that the mechanisms underlying glucocorticoid-induced reductions in cell proliferation may involve direct effects through glucocorticoids receptors or may engage an intermediary factor, such as the *N*-methyl-D-aspartic acid (NMDA) receptor-dependent excitatory pathway (Mirescu and Gould, 2006). In addition, it remains possible that during the postpartum period, glucocorticoids interact with some other, as yet unidentified, biochemical change associated with lactation or maternal care to suppress cell proliferation.

Previous studies that examined cell proliferation in the hippocampus during pregnancy have failed to find any effect (Banasr et al., 2001; Shingo et al., 2003; Furuta and Bridges, 2005), suggesting that the postpartum reduction in cell proliferation and immature neuron production that we observed occurred after parturition. This may seem paradoxical as corticosterone levels are also elevated during late pregnancy (Voogt et al., 1969). However, corticosteroid-binding globulin (CBG), which normally serves to buffer the amount of biologically active glucocorticoids, is decreased postpartum but not during pregnancy (Gala and Westphal, 1965; Walker et al., 1992). Thus, the overall effect of increased basal corticosterone may be exacerbated by altered levels of CBG during the postpartum period. Other factors that differ between pregnancy and the postpartum period may also influence hippocampal cell production. For example, estrogen, which has been shown to stimulate cell proliferation in the DG (Tanapat et al., 1999, 2005; Banasr et al., 2001; Ormerod et al., 2003; Mazzucco et al., 2006), declines from high levels during late pregnancy to low levels postpartum (Smith and Neill, 1977). Thus, the dramatic shifts in two hormonal axes known to be important in regulating cell proliferation may render the hippocampus particularly sensitive during the postpartum period. It should also be noted that whereas our study points to a connection between elevated basal glucocorticoid levels and diminished cell production during the postpartum period, a previous study did not find a change in the number of BrdU-labeled cells in the postpartum mouse DG (Shingo et al., 2003). This difference is very likely related to the fact that mice do not exhibit elevated glucocorticoid levels during lactation (Douglas et al., 2003). Our study examined rats, which, like humans, have elevated levels of glucocorticoids while nursing (Bonnin, 1992).

We also found that the number of BrdU-labeled cells was unchanged in the SVZ throughout the postpartum period, suggesting that the suppression of cell proliferation may be specific to the DG. This is perhaps not surprising given that other experiences, such as stress and physical exercise, which elevate corticosterone do not alter the number of BrdU-labeled cells in the SVZ (Tanapat et al., 2001; Brown et al., 2003). Although another study reported increased cell production in the SVZ of postpartum mice on PD7 (Shingo et al., 2003), it is important to note that we examined the caudal SVZ whereas this study examined the anterior SVZ, the portion that gives rise to cells that travel along the rostral migratory stream to the olfactory bulb (Luskin, 1993). In contrast to the involvement of glucocorticoids in the postpartum reduction of neurogenesis in the DG, the increase in olfactory bulb neurogenesis appears to be mediated by prolactin (Shingo et al., 2003). Taken together, these findings demonstrate that maternal experience can have opposite effects on the same form of structural plasticity in two different brain regions, through distinct hormonal mechanisms.

Reduced Immature Neuron Production During the Postpartum Period

The decrease in the number of BrdU-labeled cells during the postpartum period was observed 2 h after labeling, indicating a reduction in cell proliferation. Similar to the 2-h time point, fewer BrdU-labeled cells were detected 1-week after labeling. The majority of these cells expressed TuJ1, suggesting that inhibition of cell proliferation diminishes the pool of new cells that express a neuronal phenotype. However, the lack of a detectable difference in the number of BrdU-labeled cells at the 2-week time point indicates that this decrease in the number of new neurons is only temporary. One explanation for these effects may be related to differences in the survival of new cells between postpartum and virgin females. As shown here and in other studies (Cameron et al., 1993; Tanapat et al., 2001), the number of BrdU-labeled cells increases between 2 h and 1 week after DNA synthesis, and then declines dramatically by 2 weeks after labeling in control animals under standard laboratory conditions. Therefore, it appears that a greater percentage of new cells are rescued from death during this period in postpartum animals relative to virgin controls. Perhaps the presence of offspring and the experiences of motherhood provide the environmental features that are sufficient to increase cell survival in postpartum females, an effect that may be akin to the enhancement in new neuron survival associated with living in an enriched environment (Kempermann et al., 1997).

Functional Implications of Suppressed Hippocampal Cell Production During the Postpartum Period

While the increase in olfactory bulb neurogenesis reported in other studies may be related to the fact that mother rats undergo a substantial change in their response to the odors of pups and rely heavily on olfactory cues to guide normal maternal behavior (Shingo et al., 2003; Furuta and Bridges, 2005), the significance of reduced numbers of proliferating cells in the hippocampus during the postpartum period remains unknown. Since elevations in glucocorticoid secretion are necessary for milk production (Tucker, 1994), it is possible that adrenal–steroid inhibition of cell proliferation may allow for the allocation of metabolic resources away from growth processes toward successful lactation.

As noted, the decrease in the number of BrdU-labeled cells during the postpartum period did not persist to a time when most new cells express markers of mature neurons (2 weeks). Yet, evidence suggests that while decreased cell proliferation may not immediately impact hippocampal function, new hippocampal neurons may be functionally significant before they achieve full maturity. Adult-generated neurons may extend mossy fibers into area CA3 shortly after their production (Hastings and Gould, 1999; Zhao et al., 2006) and have also been shown to exhibit robust long-term potentiation (LTP) that, unlike in mature cells, is insensitive to GABAergic inhibition (Wang et al., 2000; Snyder et al., 2001; Schmidt-Heber et al., 2004). These properties may enable new neurons to affect hippocampal function even at an immature state (Aimone et al., 2006). As a result, decreases in their numbers during the postpartum period may contribute to some of the endocrine and behavioral changes that occur during this time.

One possibility is that new neurons in the hippocampus participate in the regulation of the HPA axis. In this regard, reductions in the number of newly generated neurons could play a role in the altered stress responsiveness observed during the postpartum period (Neumann, 2001). Additionally, there is some evidence that new granule neurons may be involved in learning (see Leuner et al., 2006 for review), suggesting that the decrease in adult-generated immature neurons during the postpartum period may contribute to cognitive deficits often reported in new mothers (Brett and Baxendale, 2001; DeGroot et al., 2006). There are also data showing that females are less anxious during the postpartum period (Sjogren et al., 2000; Neumann, 2001; Lonstein, 2005). Nevertheless, for many women, it also represents a time of enhanced susceptibility to mental illness with an estimated 10–15% of new mothers experiencing postpartum depression (Llewellyn et al., 1997). Given reports suggesting a link between adult neurogenesis and mood disorders (Santarelli et al., 2003), our findings present a potential mechanism that may contribute to the increased incidence of such disorders during the postpartum period. When considering the potential functional implications of changes in cell proliferation during the postpartum period, however, it is important to bear in mind that dendritic architecture (Pawluski and Galea, 2006; Kinsley et al., 2006) and synaptic plasticity (Tomizawa et al., 2003) in the hippocampus are also altered as a result of motherhood. Thus, it is likely that the decrease in immature neuron number may interact with these other changes to alter one or more of aspects of hippocampal function during the postpartum period.

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