Paternal Experience Suppresses Adult Neurogenesis without Altering Hippocampal Function in *Peromyscus californicus*

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ABSTRACT

Paternal care is rare among mammals, occurring in ≈6% of species. California mice (*Peromyscus californicus*) are unusual; fathers participate extensively in raising their young and display the same components of parental care as mothers, with the exception of nursing. Parenting is a complex experience, having stressful and enriching aspects. The hippocampus is sensitive to experience and responds to both stress and environmental enrichment with changes in structure and function. In rats, where females care exclusively for offspring, parenting is associated with suppressed hippocampal adult neurogenesis. Since this effect has been causally linked to lactation, it is unlikely that fathers would show a similar change. To investigate this issue, we examined adult neurogenesis in the hippocampus of California mouse fathers compared to males without pups and observed reduced adult neurogenesis. Similar effects were found in California mouse mothers. Next, we investigated whether behaviors linked to the hippocampus, namely, object recognition and novelty-suppressed feeding, were altered in fathers, and observed no substantial changes. During caregiving, suppressed adult neurogenesis does not appear to be related to changes in behaviors associated with the hippocampus, although it is possible that there are other effects on hippocampal function. J. Comp. Neurol. 519:2271–2281, 2011.

INDEXING TERMS: father; adult neurogenesis; California mouse; anxiety; learning; memory

In many human societies, fathers play a significant role in raising offspring (Kleiman and Malcolm, 1971; Hrdy, 2005). Among mammals, a role for fathers in caregiving is very rare (Numan and Insel, 2003). In monogamous species, like California mice (*Peromyscus californicus*), a strong bond exists between paired males and females, and males show high levels of parental investment. California mouse fathers participate extensively in raising the young, engaging in all maternal behaviors, including licking/grooming, nest building, pup retrieval, and huddling, with the exception of nursing (Dudley, 1974; Gubernick and Alberts, 1987; Gubernick and Nelson, 1989; Ribble, 1991; Gubernick and Nordby, 1993).

Experience modulates the structure and function of the hippocampus (Leuner et al., 2010). In mother rats, the postpartum period has been associated with decreased adult neurogenesis in the dentate gyrus (Leuner et al., 2007; Pawluski and Galea, 2007) and changes in behaviors linked to the hippocampus, like spatial navigation (Darnaudery et al., 2007) and anxiety regulation (Lonstein, 2005). Some studies have investigated the influence of paternal care on adult neurogenesis in rodents and observed increased new neuron production, but these studies have induced paternal care either in virgins (Ruscio et al., 2008) or in males that do not normally display caregiving behavior (Mak and Weiss, 2010). To date, the effects of paternal experience in a biparental species on adult neurogenesis and hippocampal function remain unexplored. We show that paternal experience reduces the production of new neurons in the dentate gyrus in the absence of substantial changes in some behaviors linked to the hippocampus.

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MATERIALS AND METHODS

Animals

Adult male and female California mice (*Peromyscus californicus*), descendants of mice purchased from the Peromyscus Genetic Stock Center (University of South Carolina, Columbia, SC) were born in our colony, weaned at 4 weeks, and housed in same-sex dyads until >60 days of age. The gestation period for laboratory stock of *P. californicus* ranges from 21 to 25 days. There was no obvious variability in the timing of pregnancy/birth after breeding pairs were housed together.

All mice were maintained on a 12:12 light-dark cycle, with lights on at 0700, and allowed ad libitum access to food and water, except where noted below. This study was conducted in accordance with the National Institutes of Health *Guidelines for the Care and Use of Laboratory Animals* and was approved by the Princeton University Institutional Animal Care and Use Committee.

Experimental design

California mouse mothers and fathers, as well as their age-matched same-sex controls ($n = 4–5$/group), were injected with bromodeoxyuridine (BrdU, 200 mg/kg, Sigma-Aldrich, Milwaukee, WI, cat. no. B5002), a marker of DNA synthesis, 1 week following the birth of offspring. All BrdU-injected mice were perfused 3 weeks later, at the time of weaning. To control for reproductive experience, an additional group of male California mice were either sham-operated or vasectomized ($n = 4–5$) at 60–90 days of age, 3 weeks prior to pairing with a female. Three weeks later, males were paired with a female and left undisturbed, except for routine cage changes. These animals were injected with BrdU as described above and tested on a hippocampus-dependent version of a novel object recognition task. All mice were perfused 30 minutes after object recognition testing. A 3-week post-BrdU survival time was selected because it is sufficient for BrdU-labeled cells to express markers of mature neurons (Cameron and McKay, 2001) and for new cells to exhibit signs of functional integration, including experience-dependent expression of immediate early genes (Snyder et al., 2009a,b). A second cohort of vasectomized male controls and sham-operated fathers ($n = 11$) underwent the same timeline as mentioned above, without injection of BrdU, and novelty-suppressed feeding testing occurred around the time of weaning.

Paternal behavior

Following the birth of pups, parental behavior was assessed for 15 minutes every other day between 2200 and 2400 hours on postnatal days 3–25. The following behaviors were observed and percentages calculated: presence in nest, huddling, retrieving, grooming, and nest building.

Object recognition

The object recognition test was performed as previously described (Bevins and Besheer, 2006). This version of the object recognition test with a 24-hour delay was selected because it was shown to require the hippocampus in laboratory mice (Hammond et al., 2004). Object recognition testing occurred between 0900 and 1200 hours, 3 weeks following the birth of pups, just before the time of weaning. The testing apparatus was an open-field box (50 cm^3). During the familiarization phase, California mice were habituated to the box on 2 consecutive days for 5 minutes. The following day, mice explored two identical objects (Duplo structures) for 5 minutes and were returned to their home cages for 24 hours. During the recognition phase, mice were returned to the testing apparatus and presented with a third copy of the familiarization phase objects and a novel object for 5 minutes. The left/right position of the novel object was counterbalanced between mice. Object exploration was defined as directing the nose toward an object at 2 cm and/or touching the object with the nose or paws. The following were calculated: object interaction during the familiarization phase (time spent with sample objects) and discrimination ratio (novel object interaction time/total interaction time with sample object and novel object).

Novelty-suppressed feeding

The novelty-suppressed feeding test was performed as previously described (Santarelli et al., 2003) to assess anxiety-like behavior. Twenty-four hours prior to testing, food was removed from the home cage. Testing occurred between 0900 and 1200 hours. A single food pellet was placed on white filter paper (3 cm diameter) in the center of the open-field covered with ≈2 cm of bedding. The mouse was placed in a corner of the open-field and the latency to approach and consume the food pellet was determined. If the mouse did not begin feeding within 300 seconds, the test was terminated and a latency of 300 seconds was assigned. Immediately after the test the mouse was transferred to its home cage.

Immunohistochemistry

Mice were anesthetized with sodium pentobarbital and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer (PB) following behavioral testing. Brains were dissected from the skulls and postfixed for 48 hours. Coronal sections (40 μm) were cut throughout the entire rostrocaudal extent of the dentate gyrus on a vibratome into a bath of 0.1 M phosphate-buffered saline (PBS), pH 7.5. Sections containing the dentate gyrus

were identified using a *Peromyscus* stereotaxic brain atlas (Eleftheriou and Zolovick, 1965).

Immunoperoxidase staining for BrdU

For BrdU peroxidase staining, a 1:8 series of sections was 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 minutes, denatured in 2 M HCl:PBS for 30 minutes, rinsed, and incubated overnight with the following monoclonal antibody: BD BioSciences anti-BrdU (1:200) (San Jose, CA, cat. no. 347580, clone B44). The next day, slides were rinsed, incubated with biotinylated horse antimouse (1:200; Vector, Burlingame, CA, cat. no. BA2000) for 60 minutes, rinsed, incubated with avidin-biotin complex (1:100; Vector, cat. no. PK6100) for 60 minutes, rinsed, and reacted in 0.01% diaminobenzidine with 0.003% H_2O_2 (Sigma-Aldrich, cat. no. D4293).Free-floating tissue was mounted onto glass slides and allowed to dry for 2 hours. All slides were counterstained with cresyl violet, dehydrated, cleared with Citrisolv (Fisher Scientific, Fair Lawn, NJ), and coverslipped with Permount (Fisher Scientific).

Immunofluorescent colabeling with BrdU

Double labeling with immunofluorescence for BrdU and neuronal nuclei (NeuN), neuron-specific class III beta tubulin (TuJ1), and glial fibrillary acidic protein (GFAP) were carried out to determine the fate of the new cells. Double labeling for BrdU and the immediate early gene (IEG) activity regulated cytoskeleton associated protein (arc) was used to determine whether new cells were activated by object recognition. arc is an IEG that has been linked to synaptic plasticity (Bramham et al., 2008) and used as a marker of neural activity in the hippocampus following learning and memory tasks (Soule et al., 2008).

For double labeling immunofluorescence of BrdU and NeuN, free-floating sections were rinsed in 0.1 M Tris-buffered saline (TBS), pH 7.5, denatured in 2 M HCl:TBS for 30 minutes, rinsed in TBS, and incubated in anti-BrdU (1:200) (Accurate, Westbury, NY, cat. no. OBT0030, clone BU1/75 ICR1) and anti-NeuN (Chemicon, Temecula, CA, cat. no. MAB377) diluted 1:500 for 2 nights. For double labeling immunofluorescence of BrdU and TuJ1, free-floating sections were rinsed in 0.1 M TBS, denatured in 2 M HCl:TBS for 30 minutes, rinsed in TBS, and incubated in anti-BrdU (1:200) and anti-TuJ1 1:500 (Covance, Princeton, NJ, cat. no. MMS-435P) for 2 nights.

For double labeling immunofluorescence of BrdU and GFAP, free-floating sections were rinsed in 0.1 M TBS, denatured in 2 M HCl:TBS for 30 minutes, rinsed in TBS, and incubated in anti-BrdU (1:200) and anti-GFAP (1:1,000) (Advanced Immunochemical, Long Beach, CA, cat. no. 31223) for 2 nights.

For double labeling immunofluorescence of BrdU and arc, free-floating sections were rinsed in 0.1 M TBS, denatured in 2 M HCl:TBS for 30 minutes, rinsed in TBS, and incubated in anti-BrdU (1:200) and anti-arc (1:1,000) (Synaptic Systems, Gottingen, Germany, cat. no. 156 003) for 3 nights.

All sections were rinsed, incubated with biotinylated goat anti-rat (1:250; Chemicon, cat. no. AP183B) for 90 minutes, rinsed, and incubated for 30 minutes in the dark with streptavidin-conjugated Alexa 568 (1:1,000; Invitrogen Molecular Probes, Carlsbad, CA, cat. no. S11226) to visualize BrdU and goat antimouse Alexa 488 (1:500; Invitrogen Molecular Probes, cat. no. A11029) to visualize NeuN, TuJ1, or GFAP, or goat antirabbit Alexa 488 (1:500; Invitrogen Molecular Probes, cat. no. A11008) to visualize arc. Finally, sections were rinsed, mounted onto glass slides, dried, and coverslipped using glycerol in TBS (3:1).

Antibody characterization (Table 1)

The BD anti-BrdU antibody specifically recognizes BrdU in single-stranded and not double-stranded DNA. Histogram analysis showed that intensity of BrdU labeling is reduced to half every 20 hours, suggesting that the majority of the BrdU-labeled cells were actively involved in cell cycle progression following BrdU incorporation (Lee et al., 2006).

The Accurate anti-BrdU antibody recognizes BrdU incorporated into single-stranded DNA, attached to a protein carrier and free BrdU. It does not crossreact with thymidine but does react weakly with chlorodeoxyuridine (manufacturer's technical information). The antibody does not recognize either halogenated base in double-stranded DNA nor does it react with uracil or bromocytidine (Vanderlaan and Thomas, 1985).

The Chemicon anti-NeuN antibody recognizes 2-3 bands at 46–48 kDa and possibly another band at ≈66 kDa by western blot (manufacturer's technical information; Lind et al., 2005).

The Covance anti-TuJ1 antibody is well characterized and highly reactive to neuron-specific Class III β-tubulin (βIII). TuJ1 does not identify β-tubulin found in glial cells. In western blots, using rat brain lysates it is recognized as a 50 kDa band (manufacturer's technical information; Madhavan et al., 2009).

The Advanced Immunochemical anti-GFAP antibody detected a band of 43–45 kDa by western blot of human brain and spinal cord lysates, corresponding to the predicted molecular weight of GFAP (manufacturer's technical information) and stained an expected pattern of cellular morphology and distribution in mouse brain sections (Stillman et al., 2009).

The Synaptic Systems anti-Arc antibody recognizes a 50-kDa band in western blots using enriched

TABLE 1.

Summary of the Source, Properties, and Dilution of the Antibodies Used

Table of Antibodies Used

Antigen	Immunogen	Manufacturer	Dilution used
anti-BrdU	derived from hybridization of mouse Sp2/0-Ag14 myeloma cells with spleen cells from mice immunized with iodouridine-conjugated ovalbumin	BD Biosciences, San Jose, CA, mouse monoclonal, clone B44, cat. no. 347580	1:200
anti-BrdU	purified IgG prepared by affinity chromatography on Protein G	AbD Serotec, distributed by Accurate, Westbury, NY, rat monoclonal, clone BU1/75 (ICR1), cat. no. OBT0030	1:200
anti-NeuN	purified cell nuclei from mouse brain	Chemicon, Temecula, CA, mouse monoclonal, cat. no. MAB377	1:500
anti-TuJ1	microtubules derived from rat brain	Covance, Princeton, NJ, mouse monoclonal, cat. no. MMS-435P	1:500
anti-GFAP	purified glial fibrillary acidic protein from human brain	Advanced Immunochemical, Long Beach, CA, guinea pig polyclonal, cat. no. 31223	1:1000
anti-Arc	enriched synaptosome fraction of rat brain (P2)	Synaptic Systems, Gottingen, Germany, rabbit polyclonal; cat. no. 156 003	1:1000

synaptosome fraction of rat brain (P2) (manufacturer's technical information).

Data analysis

Quantitative analysis was conducted on coded slides. The numbers of BrdU-labeled cells on every eighth unilateral section throughout the entire rostrocaudal extent of the dentate gyrus (granule cell layer, subgranular zone, and hilus) were counted at 100× with an Olympus BX-50 light microscope by using a modified version of the optical fractionator method (West et al., 1991; Ngwenya et al., 2005). The estimated total number (N) of BrdU-labeled cells was calculated according to the following formula: $N = \Sigma Q \times (t/h) \times (1/asf) \times (1/ssf)$ where ΣQ is the sum of counted labeled cells (excluding cells in the outermost focal plane), t is the mean section thickness, h is the height of the optical dissector, asf is the area sampling fraction, and ssf is the section sampling fraction. Optical fractionator rules typically require the use of guard zones at the upper and lower surfaces of the section. However, tissue sections shrink substantially (to ≈15 μm) in the z-dimension when mounted on slides, dried, processed for immunohistochemical staining, counterstained, dehydrated, and cleared. Consequently, we used a modified optical fractionator technique in which we did not use guard zones, making the height of the counting frame equal to the section thickness. Because we used an exhaustive sampling scheme, asf was equal to one. Thus, the simplified formula for the estimated total number of labeled cells was: $N = \Sigma Q \times (1/ssf)$, which is the total number of labeled cells counted multiplied by the reciprocal of the section sampling fraction (1/16) (Leuner et al., 2009).

Brightfield photomicrographs were taken with an Olympus U-PMTUC camera attached to the microscope using ImagePro software (Media Cybernetics, Bethesda, MD). Images were cropped and optimized by adjusting brightness and color balance in Adobe Photoshop 7.0 (San Jose, CA).

The density of BrdU-labeled cells was determined in the subventricular zone (SVZ; Kuhn et al., 1996; Mirescu et al., 2004). This analysis included a substantial part of the SVZ, but excluded the anterior portion. BrdU-labeled cells in the SVZ present on every eighth unilateral coronal section of the DG were counted at 100× on a Olympus BX-50 light microscope (−2.12 to −4.60 mm from bregma; Eleftheriou and Zolovick, 1965) and expressed as the number of cells per mm^3. The volume of the analyzed area was determined using Cavalieri's principle on video-projected images with cross-sectional area measurements performed using ImagePro software (Gundersen et al., 1999).

Immunofluorescence analyses were carried out with a Zeiss Axiovert confocal microscope with Argon 458/488 and HeNe 543 lasers and 510LSM software. For the phenotypic fate analyses, the percentage of BrdU+ cells that were NeuN+, TuJ1+, or GFAP+ were determined from 25 randomly selected BrdU-labeled cells in the DG. For arc analysis, all BrdU+ and/or arc+ cells were counted. Optical stacks of 1-μm thick sections were obtained through all putatively double-labeled cells. To verify double labeling throughout their extent, cells were examined in orthogonal planes.

Statistics

Unpaired *t*-tests were used to compare all histological and behavioral data. When variances were unequal, unpaired *t*-tests with Welch's correction were performed.

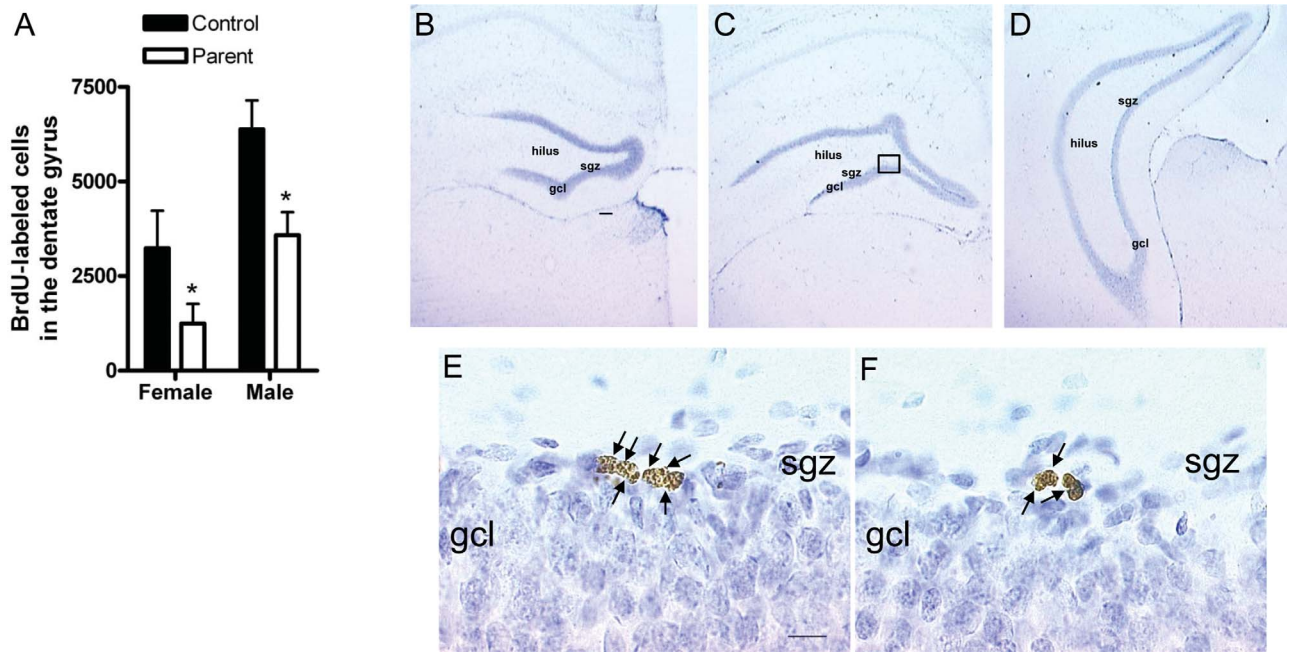

Figure 1. Parenting decreases the number of BrdU-labeled cells in the dentate gyrus of California mice. **A**: Maternal and paternal experience significantly reduced the number of BrdU-labeled cells in the dentate gyrus of the hippocampus, compared to controls. Bars represent mean ± SEM, $*P < 0.05$. **B-D**: Photomicrographs of the Nissl stained dentate gyrus of the California mouse at different levels throughout the extent of the hippocampus. Box indicates location from which higher magnification photomicrographs shown in E,F were taken. **E,F**: Photomicrographs of BrdU-labeled cells (arrows) in the dentate gyrus of control (E) and father (F) California mice. Arrows indicate BrdU-labeled cells: gcl, granule cell layer; sgz, subgranular zone. Scale bar in B = 100 um and pertains to C-D; Scale bar in E = 10 um and pertains to E,F. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Correlational analyses were performed on BrdU-labeled cell number and paternal behavioral measures. Power analyses were performed in order to see whether the observed behavioral results had sufficient mice/group to draw statistical conclusions.

RESULTS

Parents have fewer new neurons than nonparents

BrdU-labeled cells were present at all rostrocaudal levels of the dentate gyrus of California mice. As observed for laboratory mice and rats, the majority of BrdU-labeled cells were located in the granule cell layer and subgranular zone (Fig. 1). California mouse mothers displayed a decrease in the number of BrdU-labeled cells in the dentate gyrus compared to controls ($t_{(7)} = 2.88$, $P < 0.05$; Fig. 1A). Fewer BrdU-labeled cells were also observed in the dentate gyrus of fathers compared to controls ($t_{(8)} = 2.87$, $P < 0.05$; Fig. 1A). The inhibitory effect of paternal experience on the number of BrdU-labeled cells was also present in the second experiment where the controls were vasectomized (vasectomized control 5,645 ± 892.0; sham-operated father 3,462 ± 272.9, ($t_{(7)} = 2.59$, $P < 0.05$)).

The majority of BrdU-labeled cells in the dentate gyrus of fathers expressed the neuronal markers NeuN (Control: 67.5% ± 7.4%, Father: 68.9 ± 4.9; Fig. 2, Supplementary Fig. 1) and TuJ1 (Control: 81.1% ± 4.4, Father: 85.8 ± 6.3; Fig. 2, Supplementary Fig. 1). No difference existed between controls and fathers in the percentage of cells expressing neuronal markers ($P > 0.05$, for each comparison), suggesting that the decreased number of BrdU-labeled cells associated with paternal experience represents decreased adult neurogenesis. Few BrdU-labeled cells expressed the astrocytic marker GFAP (Control: 4.8% ± 2.9, Father: 4.0% ± 2.3, Fig. 2, Supplementary Fig. 1), with no differences in this measure between controls and fathers ($P > 0.05$). No significant difference was observed in the density of BrdU-labeled cells in the subventricular zone between controls and fathers (Control: 4,114 ± 1,087, Father: 4,354 ± 1,574; $P > 0.05$).

Paternal care does not correlate with number of BrdU-labeled cells

California mice fathers spent a majority of their time in the nest with pups (>65%), displaying parental behaviors such as huddling and grooming (Fig. 3A,B). Fathers also participated in other parental behaviors, such as nest

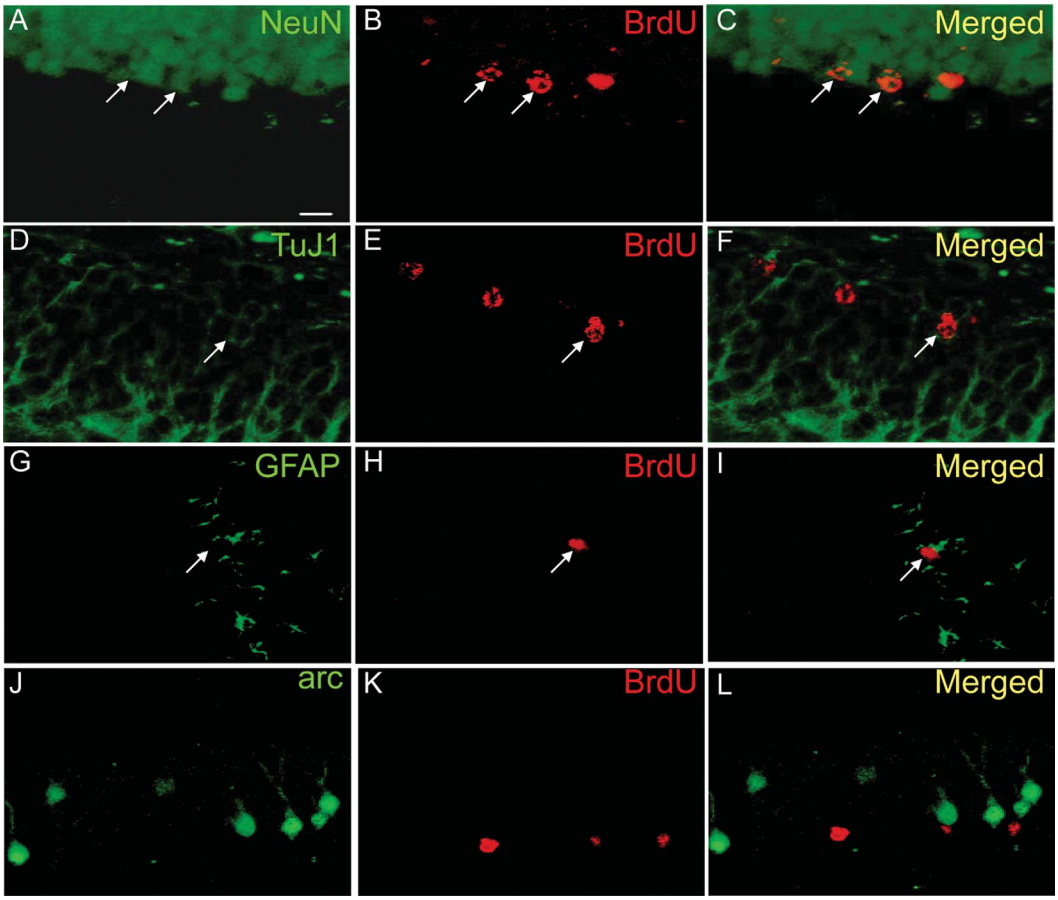

Figure 2. Paternal experience decreases adult neurogenesis without altering the number of cells expressing the immediate early gene arc. Many BrdU-labeled cells expressed neuronal nuclei (NeuN, **A–C**) and neuron-specific class III beta tubulin (TuJ1, **D–F**), while few coexpressed glial fibrillary acidic protein (GFAP, **G–I**). None of the BrdU-labeled cells expressed the immediate early gene, activity regulated cytoskeleton associated protein (arc, **J–L**), in response to novel object exposure. Arrows indicate double-labeled cells. Magenta-green copies of this figure are available as Supplementary Figure 1. Scale bar = 10 μm. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

building and retrieval. No correlation existed between BrdU-labeled cells and the above parental behaviors ($P > 0.05$, for all comparisons).

No change in the number of neurons expressing the immediate early gene arc after object recognition testing

Paternal experience did not significantly alter the number of arc+ cells in the dentate gyrus, CA1 or CA3 regions of the hippocampus ($P > 0.05$, for each comparison; Table 2, Fig. 2, Supplementary Fig. 1). Additionally, no BrdU-labeled cells were observed to co-express arc following object recognition testing in either group.

Paternal experience does not alter object exploration, recognition, nor novelty-suppressed feeding

Time spent exploring sample objects did not differ significantly between controls and fathers ($P > 0.05$; Fig. 3C). Twenty-four hours later, time exploring the familiar and novel objects was also similar for both controls and fathers ($P > 0.05$; Fig. 3D). Neither the controls nor the fathers showed a preference for the novel object. Paternal experience did not alter the latency to approach the food pellet in the novelty-suppressed feeding task ($P > 0.05$, Welch's corrected; Fig. 3E). Additionally, fathers did not demonstrate a significant change in latency to begin eating in the novel environment ($P > 0.05$; Fig. 3F). Because the variability was high on data collected for both behavioral tasks examined, we carried out power analyses to determine whether a reasonable increase in *n* size might alter our conclusions. Power analyses revealed that in order to obtain a statistically significant result, an *n* size of 198 mice per group would be necessary for the object recognition task and an *n* size of 31 mice per group would be necessary for the novelty-suppressed feeding task. Since these are atypically high numbers of experimental animals for a study of this nature, it is

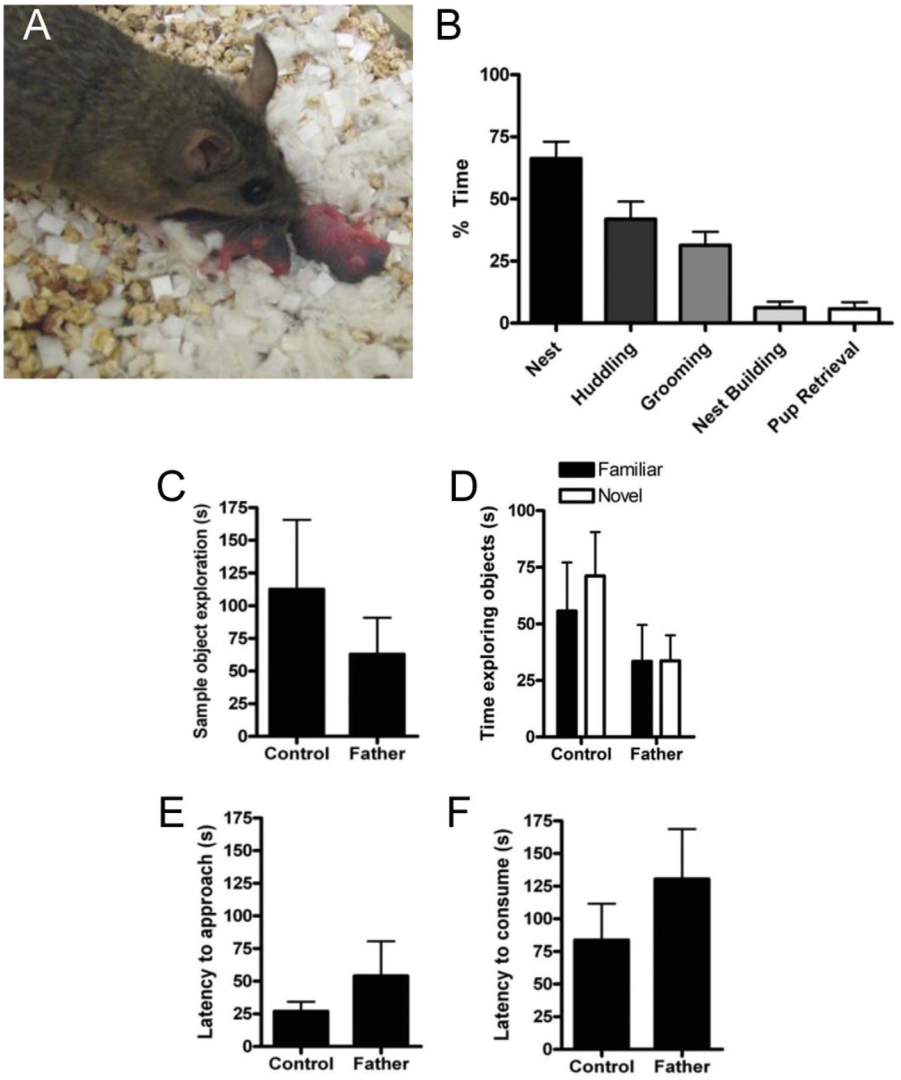

Figure 3. Parenting, object recognition and anxiety-like behavior in the male California mouse. **A**: Photograph of a California mouse father caring for his day-old pups. **B**: California mouse fathers engage in many parental behaviors typical of mothers, including spending time in the nest, huddling, grooming, nest building and pup retrieval. **C**: Sample object exploration was not significantly different between control and father California mice. **D**: Time exploring the familiar and novel objects on the testing day was similar for control and father California mice, suggesting that object preference did not exist in either group. **E,F**: Novelty-suppressed feeding testing revealed no significant difference in the latency to approach or consume a food pellet. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

TABLE 2.

Paternal Experience Does Not Significantly Alter the Number of Cells Expressing the Immediate Early Gene arc

	Dentate gyrus			CA1	CA3
	arc+ cells	BrdU+ cells	arc+/BrdU+ cells	arc+ cells	arc+ cells
Control	480.0 ± 321.2	1728.0 ± 327.3	0	136.0 ± 73.2	16.0 ± 6.5
Father	928.0 ± 455.5	1011.0 ± 152.0*	0	217.6 ± 135.2	57.6 ± 31.4

Paternal experience significantly decreased the number of BrdU-labeled cells in the dentate gyrus; however, the expression of the immediate early gene activity regulated cytoskeleton associated protein (arc) was not substantially altered. Additionally, no BrdU-labeled cells were observed to coexpress arc after object recognition testing in either group. Mean ± SEM.

*$P < 0.05$.

unlikely that we would have detected a significant difference with a reasonable increase in *n* size.

DISCUSSION

Our data show that California mouse mothers and fathers exhibit decreased adult neurogenesis in the dentate gyrus. Despite these structural changes, some behaviors associated with the hippocampus, such as object recognition and novelty-suppressed feeding, appear to be unaltered by paternal experience.

Parenting affects adult neurogenesis but not overall hippocampal function

We know that the postpartum period in female rats is characterized by diminished adult neurogenesis (Leuner et al., 2007; Pawluski and Galea, 2007), which is dependent on pup contact and directly linked to lactation-induced elevations in glucocorticoids. Pup removal or normalization of elevated corticosterone levels prevents the suppression of adult neurogenesis in mothers (Leuner et al., 2007). The present study extends these findings to California mouse mothers. Since fathers do not lactate, the additional finding that California mouse fathers display decreased new neuron formation comparable to that of mothers seems unexpected. However, given that California mouse fathers engage in all parental behaviors other than nursing, there are marked similarities in parenting experience between the sexes of this species. Because of the metabolic demands of parenting, it is possible that similar mechanisms, involving elevated glucocorticoids but without lactation, contribute to reduced adult neurogenesis in fathers.

The California mouse is responsive to day length changes (Trainor et al., 2010). In the present study, we chose a light:dark cycle of 12:12, which is neither a long nor short photoperiod. Corticosterone levels vary depending on photoperiod in male California mice (Trainor et al., 2010); given this, the response of corticosterone levels to an intermediate 12:12 light:dark cycle remains unknown. Future studies will be necessary to address whether photoperiod is an issue here, and may provide an important clue to the mechanism behind the fatherhood-induced reduction in adult neurogenesis in California mice.

In the female rat, the postpartum period is associated with reduced adult neurogenesis (Leuner et al., 2007; Pawluski and Galea, 2007) as well as diminished cognitive abilities (Darnaudery et al., 2007). These effects are temporary—following weaning, spatial working and reference memory are improved over virgin controls (Pawluski et al., 2006a,b). These benefits persist—primiparous and multiparous female rats outperform virgins on many spatial learning tasks (Kinsley et al., 1999; Love et al., 2005; Lemaire et al., 2006; Pawluski et al., 2006b; Paris and Frye, 2008). Lactating female rodents have reduced anxiety-like behavior and decreased fear behavior while exposed to pups (Lonstein, 2005; Pereira et al., 2005; Agrati et al., 2008; Kinsley and Lambert, 2008). These findings raise the possibility that reduced adult neurogenesis contributes to changes in hippocampal functions in mothers, such as cognition and anxiety regulation.

By contrast, despite substantial reductions in adult neurogenesis in fathers, we did not observe any significant difference between fathers and controls in two behavioral tasks linked to hippocampal function—novel object recognition and novelty-suppressed feeding. It should be emphasized here that considerable variability exists in the behavioral data we acquired testing California mice on both of these tasks. This variability may have precluded detection of differences between controls and fathers, but also as noted above, power analyses showed that increasing the *n* size to address this possibility would have required atypically large numbers of animals. A possible reason that differences in object recognition behavior were not observed between the control and fathers is that the retention interval was too long. In the one-trial object recognition task, retention intervals can range from minutes up to many hours. The ability to distinguish novel from familiar objects deteriorates as the delay between the sample and the test trial increases (Bertaina-Anglade et al., 2006). The 24-hour interval was selected because previous studies have shown that laboratory mice (*Mus musculus*) can distinguish between familiar and novel objects at this timepoint and, importantly, object recognition with a 24-hour interval requires the hippocampus (Bredy et al., 2007). Our results showed that not only was there no significant difference between controls and fathers in object recognition, but that neither group spent significantly more time exploring the novel versus the familiar object. It is possible that species differences exist between laboratory mice and California mice such that a 24-hour delay was too long for retention of the familiar object memory trace in the latter species. A shorter delay may have yielded significant differences between the control and father mice; however, even a delay of 15 minutes produces high variability among this species (Bredy et al., 2007) and object recognition with such an interval does not require the hippocampus.

We also did not observe an increase in the number of new cells that expressed the immediate early gene arc in response to novel object exposure within the time period examined. It remains possible, however, that different timepoints after exposure to the novel object would have revealed significant differences in the number of arc-expressing cells. Although these findings collectively suggest that a reduction in the number of new neurons in

California mouse fathers does not substantially alter hippocampal function, at least up until the time of weaning, it is important to consider that other behaviors linked to the hippocampus may be affected. It remains additionally feasible that the generation of fewer new neurons in fathers may only become functionally relevant after weaning. These caveats aside, an additional distinct possibility is that a large pool of new neurons is not necessary to maintain hippocampal function in this wild species of mouse. Other mechanisms supporting optimal hippocampal function, such as synaptic plasticity and additional types of structural plasticity, such as synaptogenesis and changes in dendritic spines, may compensate for the presence of fewer new neurons during the time of parenting.

Species differences in adult neurogenesis may be related to social behavior

Reduced adult neurogenesis in response to parenting experience in males does not appear to be universal. Some studies have shown that exposure of male rodents to pups can stimulate adult neurogenesis. Exposure of naïve adult male prairie voles to foster pups enhances cell proliferation in the dentate gyrus (Ruscio et al., 2008). Opposing responses of adult neurogenesis to pup exposure between prairie voles and California mice may be related to behavioral differences in response to pups. Virgin male prairie voles, when exposed to pups, readily demonstrate parental behavior, rarely attack pups, and are usually spontaneously paternal (Carter et al., 1995). Naïve male California mice, in contrast, react in an aggressive way toward pups and become parental only after copulating with a female or after the birth of their own litter (Gubernick et al., 1994). Relatively few naïve male California mice are spontaneously paternal (Gubernick and Nelson, 1989). Thus, the opposite response of adult neurogenesis to pup exposure in prairie voles and California mice may be due to the emotional valence of the experience—in virgin prairie voles but not California mice, pup exposure may have an enriching effect. In laboratory rats and mice, environmental enrichment has been shown to enhance adult neurogenesis (Olson et al., 2006).

Repeated exposure of male laboratory mice also induces paternal behavior that is not a natural characteristic of this species (Svare and Mann, 1981). Like virgin male prairie voles, laboratory mice displaying paternal behaviors also show increased adult neurogenesis in response to pups (Mak and Weiss, 2010). This increase in new neuron formation with repeated pup exposure appears to serve a useful function in laboratory mice—the new neurons play an important role in kin recognition once offspring reach reproductive age. Thus, new neurons may serve to limit the deleterious consequences of consanguineous breeding, a problem that may arise in a promiscuous species like *Mus musculus* (Costello et al., 2009). California mice, on the other hand, are not only socially but genetically monogamous (Ribble, 1991). Thus, for California mice the production of more new neurons that are organized to participate in offspring recognition postweaning may not be worth the energy expenditure. Instead, California mice may shunt energy away from neuronal growth and toward parenting behaviors that provide the best possible chance of offspring survival. Species differences in the response of adult neurogenesis to parenting raise the question of what might be the response in primates, especially humans, where parenting is governed less by olfactory cues than it is in rodents. Thus, the role of new neurons and parenting-induced plasticity in the hippocampus may vary depending on the species.

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