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Sexual Experience Restores Age-Related Decline in Adult Neurogenesis and Hippocampal Function

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ABSTRACT: Aging is associated with compromised hippocampal function and reduced adult neurogenesis in the dentate gyrus. As new neurons have been linked to hippocampal functions, such as cognition, age-related decline in new neuron formation may contribute to impaired hippocampal function. We investigated whether a rewarding experience known to stimulate neurogenesis in young adult rats, namely sexual experience, would restore new neuron production and hippocampal function in middle-aged rats. Sexual experience enhanced the number of newly generated neurons in the dentate gyrus with both single and repeated exposures in middle-aged rats. Following continuous long-term exposure to sexual experience, cognitive function was improved. However, when a prolonged withdrawal period was introduced between the final mating experience and behavioral testing, the improvements in cognitive function were lost despite the presence of more new neurons. Taken together, these results suggest that repeated sexual experience can stimulate adult neurogenesis and restore cognitive function in the middle-aged rat as long as the experience persists throughout the testing period. The extent to which changes in adult neurogenesis underlie those in cognition remain unknown. © 2013 Wiley Periodicals, Inc.

KEY WORDS: dentate gyrus; object recognition; middle age; sexual experience

INTRODUCTION

In both humans and rodents, aging is associated with a decrement in cognitive function (Oler and Markus, 1998; Frick et al., 2003; Driscoll et al., 2006; Lewis et al., 2008; Soei and Daum, 2008; Bizon et al., 2009). Age-associated changes in cognition are evident as early as mid-life. The hippocampus has been associated with many of the cognitive functions that decline with aging (Geinisman et al., 1986; Smith et al., 2000). Although the neural substrates of age-associated changes in cognition remain unknown, changes in hippocampal plasticity may play a role.

The dentate gyrus of the hippocampus is a major site of adult neurogenesis in the mammalian brain (McEwen, 2001; Leuner and Gould, 2010). Evidence suggests that changes in adult neurogenesis can affect hippocampal functions (Shors, 2004; Leuner et al., 2006) with reports supporting claims that new neurons are involved in certain types of learning and memory (Gould et al., 1999b; Snyder et al., 2009; Gu et al., 2012), anxiety regulation (Leuner and Gould, 2010), and feedback of the stress response (Snyder et al., 2011). Aging is associated with a substantially reduced rate of neurogenesis in the dentate gyrus; this appears to be a general phenomenon among mammalian species, including rats, mice, tree shrews, dogs, marmosets, and macaques (Seki and Arai, 1995; Kuhn et al., 1996; Kempermann et al., 1998; Cameron and McKay, 1999; Gould et al., 1999a; Lemaire et al., 2000; Jin et al., 2003; Nacher et al., 2003; Heine et al., 2004; McDonald and Wojtowicz, 2005; Simon et al., 2005; Kronenberg et al., 2006; Rao et al., 2006; Leuner et al., 2007; Siwak-Tapp et al., 2007). Reduced adult neurogenesis becomes evident in mid-life, before the time when an age-associated decline in cognitive function has been reported (Knuttinen et al., 2001; Frick et al., 2003), suggesting that reduced adult neurogenesis may eventually contribute to these problems (Drapeau et al., 2003, 2007).

Experience has been shown to alter the rate of adult neurogenesis (Leuner and Gould, 2010). Some studies suggest that rewarding experiences, such as running (Stranahan et al., 2006) and intracranial self-stimulation (Takahashi et al., 2009) increase the number of new neurons in the dentate gyrus. Sexual behavior is known to be rewarding to rodents. Rats will bar press to gain access to a receptive female (Everitt et al., 1987; Beck and Bialy, 1993) and develop conditioned place preferences to locations previously associated with a sexually receptive female (Camacho et al., 2004). Additionally, sexual behavior activates reward centers within the brain (Agmo and Berenfeld, 1990; Damsma et al., 1992), much like those activated by exercise, intracranial self-stimulation, and environmental enrichment (Garris et al., 1999; Brene et al., 2007; Segovia et al., 2010).

In young adult male rats, sexual experience increases cell proliferation and adult neurogenesis in the hippocampus (Leuner et al., 2010) and also exerts anxiolytic effects in many behavioral paradigms (Fernandez-Guasti et al., 1989; Rodriguez-Manzo et al., 1999; Edinger and Frye, 2007; Waldherr and Neumann, 2007; Leuner et al., 2010), although its potential influence on cognition has not been examined. The possibility that sexual experience during mid-life can restore hippocampal structure and function to young adult levels has not been investigated. Here, we examined this possibility and found that even in older rats, sexual experience increases the numbers of new neurons and enhances cognitive function.

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MATERIALS AND METHODS

Ethics Statement

Procedures were conducted in accordance with Princeton University IACUC (protocol # 1756, approved July 2009) and The National Institutes of Health Guide for the Care and Use of Laboratory Animals.

Experimental Animals

Young adult male and female Sprague Dawley rats (2–3 mo) and middle-aged retired breeder male Sprague-Dawley rats (9–11 mo) (Taconic, Germantown, NY) were provided ad libitum access to food and water, except as stated below (see "Object Recognition" Section) and maintained on a reverse 12:12 light-dark cycle (lights on 1900 h). The middle-aged retired breeder rats had a long history of sexual experience and were used for this study because they are much more sexually responsive than middle-aged virgin males, which are often completely resistant to mating (unpublished observation). Males (including retired breeders) were housed two per cage and were acclimated to the colony for 5 d before sexual experience began. No obvious fighting was observed between young adult or retired breeder rats. Rats housed together were included in the same experimental group. Females were individually housed following bilateral ovariectomy (OVX) under Nembutal anesthesia and allowed to recover for 1 week. Sexual receptivity was induced in OVX rats by subcutaneous injection of estrogen (200 mg/0.2 ml sesame oil) 48 h and progesterone (500 mg/0.2 ml sesame oil) 3 h before pairing the female with a male.

Sexual Behavior

Male rats were placed in a novel cage with a sexually receptive female or remained undisturbed in their home cage. Beginning from the first intromission, males were permitted to engage in sexual behavior for 30 min after which they were returned to their home cage. All exposures were monitored and videotaped in the dark under red-light illumination (1300–1600 h). If the rat did not initiate sexual behavior within 30 min, the session was terminated. All digital videos were analyzed for mounts, intromissions, and ejaculations (Leuner et al., 2010).

BrdU Administration and Perfusion

To assess the effects of sexual experience on cell proliferation in middle-aged rats, retired breeder male rats were injected intraperitoneally with 200 mg/kg of the DNA synthesis marker bromodeoxyuridine (BrdU) either directly after removal from the home cage (control) or 30 min after the first intromission (sexual experience). This dose of BrdU was used because it labels a maximal number of cells in the dentate gyrus (Cameron and McKay, 2001). Rats were then perfused 2 h after BrdU injection. This post-BrdU survival time is sufficient to label cells in S-phase but not to allow the labeled cells to divide, thus providing a measure of cell proliferation.

To assess whether continuous sexual experience alters the number of newly generated cells in the dentate gyrus of middle-aged rats, retired breeder rats were exposed to a sexually receptive female once daily, for 28 consecutive days. After each bout of sexual experience, during the first 14 d, males were injected intraperitoneally with 50 mg/kg of BrdU, along with controls and perfused following the final sexual experience on day 28. This dose of BrdU, and injection paradigm, was used to label a large number of proliferating cells to obtain a better estimate of the magnitude of the effect over time.

To assess whether discontinuous sexual experience produces an increase in adult neurogenesis, we exposed young adult and retired breeder male rats to a receptive female daily for 14 d. Following the final mating test on day 14, sexually experienced males and control rats were injected with 200 mg/kg of BrdU and perfused after a 2 week survival time [a time point when the majority of new cells in the dentate gyrus express the mature neuronal marker NeuN, (Cameron and McKay, 2001)].

To assess the effects of both continuous and discontinuous sexual experience on behaviors associated with the hippocampus, rats exposed to either 28 d of continuous sexual experience (tested immediately after sexual experience) or 14 d of sexual experience followed by 14 d of no sexual experience (tested 14 d after sexual experience) underwent a cognitive task associated with the hippocampus, novel object recognition testing. These rats were perfused 30 min after behavioral testing.

Rats were anesthetized with an overdose of Nembutal and transcardially perfused with 4% paraformaldehyde in 0.1 M phosphate buffer, pH 7.5.

Object Recognition

The novel object recognition test was performed as previously described (Bevins and Besheer, 2006; Dere et al., 2007). This version of the novel object recognition test has been shown to involve the hippocampus in rats (Dere et al., 2007). The testing apparatus consisted of an open-field box made of 1/2" plywood (50 cm^3) painted white. During the familiarization phase, rats explored two identical objects (Duplo structures) for 3 min and were returned to their home cages for 3 h (Jessberger et al., 2009). During the recognition phase, rats were returned to the testing apparatus, presented with a third copy of the familiar object and a novel object, and allowed to explore them for 3 min. The left/right position of the novel object was counterbalanced between each rat. Object exploration was defined as directing the nose toward the object at 2 cm and/or touching the object with the nose or paws. The following measures were calculated: time exploring familiar and novel object during object recognition test, total time exploring sample objects (total time spent exploring both identical objects during the familiarization phase), and difference score (time spent with the novel object minus time spent with the familiar object during the recognition phase). For comparison purposes, young adult male rats were also tested on novel object recognition.

Immunohistochemistry.

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.

For BrdU peroxidase staining, a 1:12 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 min, denatured in 2 M HCl:PBS for 30 min, rinsed, and incubated with a mouse monoclonal antibody to BrdU (1:200 with 0.5% Tween 20; Vector, Burlingame, CA, cat. no. VPB209). The next day, slides were rinsed, incubated with biotinylated anti-mouse (1:200; Vector, Burlingame, CA, cat. no. BA2000) for 60 min, rinsed, incubated with avidin–biotin complex (1:100; Vector, cat. no. PK6100) for 60 min, rinsed, and reacted in 0.01% diaminobenzidine with 0.003% H2O2 (Sigma-Aldrich, cat. no. D4293). Slides were counterstained with cresyl violet, dehydrated, cleared with Citrisolv (Fisher Scientific, Fair Lawn, NJ), and coverslipped under Permount (Fisher Scientific).

Double labeling with immunofluorescence for BrdU and the neuronal marker neuronal nuclei (NeuN) was carried out to determine whether the newly labeled cells were neurons. 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 min, rinsed in TBS, and incubated with rat anti-BrdU (1:200 with 0.5% Tween 20; Accurate, Westbury, NY, cat. no. OBT0030) and mouse anti-NeuN (1:500; Chemicon, Temecula, CA, cat. no. MAB377). Sections were then rinsed, incubated with biotinylated anti-rat (1:250; Chemicon, cat. no. AP183B) for 90 min, rinsed, and incubated for 30 min in the dark with streptavidin-conjugated Alexa 568 (1:1,000; Invitrogen) to visualize BrdU and with goat anti-mouse Alexa 488 (1:500; Invitrogen Molecular Probes, cat. no. A11029) to visualize NeuN. Finally, sections were rinsed, mounted onto glass slides, dried, and coverslipped using glycerol in TBS (3:1).

Microscopic Data Analysis

Quantitative analysis was conducted on coded slides to keep group assignments unknown. The numbers of BrdU-labeled cells on every 12th unilateral section throughout the entire rostrocaudal extent of the dentate gyrus (granule cell layer, subgranular zone, and hilus) were counted at 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 simplified formula for the estimated total number of labeled cells was: N Σ Q x (1/ssf), which is the total number of labeled cells (N Σ Q) counted multiplied by the reciprocal of the section sampling fraction (1/ssf or 1/12) (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).

For purposes of comparison, the density of BrdU-labeled cells was also determined in the subventricular zone (SVZ) of middle-aged rats assessed for cell proliferation (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 12th unilateral coronal section of the dentate gyrus were counted [1.8–4.8 mm from Bregma; (Paxinos and Watson, 1998)] and expressed as the number of cells per cubic millimeter. The volume of the analyzed area was determined by 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 NeuN analysis, the percentage of BrdU+ cells that were NeuN+ was determined from 25 randomly selected BrdU-labeled cells in the dentate gyrus. 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

All data were analyzed using unpaired Student's *t*-tests or one way analysis of variance (ANOVA) followed by Bonferroni post hoc analysis. Welch's correction for unequal variance was applied when necessary. Pearson correlations were performed where appropriate.

RESULTS

Middle-Age Retired Breeder Rats Readily Engage in Sexual Behavior

Like young adult virgins, middle-aged retired breeders reliably, but not always, engaged in sexual behavior when exposed to a sexually receptive female. For the single sexual experience study, only rats which copulated (∼60%) were included in the BrdU analysis. For the 28 and 14 d on/14 d off sexual experience studies, all rats copulated within the first 2 d of exposure to a sexually receptive female. Among rats in the continuous sexual experience group (28 d), the latency to mount, intromit, and ejaculate did not significantly differ. Additionally, no significant difference in the frequency of each behavior was observed ($P > 0.05$, for all comparisons; Table 1).

Among rats in the discontinuous sexual experience group (14 d on/14 d off), which received one BrdU injection after completing 14 d of exposure to a sexually receptive female, no differences in the latency to mount and number of mounts were observed across days ($P > 0.05$, for all comparisons). However, significant differences were observed in the latency to intromit ($F(2,42) = 10.4$, $P < 0.001$) and the number of intromissions ($F(2,42) = 13.0$, $P < 0.0001$), as well as the la-

TABLE 1.

Sexual Behavior of Middle-Aged Male Rats Exposed to Repeated BrdU Injections

	Day of sexual experience		
	1	14	28
Latency to mount (sec)	49.1 ± 9.8	146.9 ± 62.0	72.9 ± 53.5
Number of mounts	24.33 ± 4.80	30.3 ± 5.4	21.9 ± 4.7
Latency to intromit (sec)	424.4 ± 192.0	648.3 ± 172.5	297.7 ± 146.4
Number of intromissions	26.1 ± 4.7	19.3 ± 6.4	41.2 ± 7.9
Latency to ejaculate (sec)	841.7 ± 187.4	1164.0± 230.1	867.8 ± 176.0
Number of ejaculations	2.2 ± 0.5	0.9 ± 0.4	1.3 ± 0.3
% copulation	83.3 ± 11.2	83.3 ± 11.2	91.7 ± 8.3

tency to ejaculate ($F(2,42) = 10.0$, $P < 0.001$) and the number of ejaculations ($F(2,42) = 8.0$, $P = 0.001$; Table 2). A positive correlation was observed between the average latency to intromit over the 14 d paradigm and the number of days each retired breeder copulated with the sexually receptive female ($r_2 = 0.97$, $P < 0.0001$).

Sexual Experience Increases Cell Proliferation and Neurogenesis in the Middle-Aged Hippocampus

A single mating session enhanced cell proliferation in the dentate gyrus of middle-aged retired breeders, compared to age-matched controls. Retired breeder rats with 30 min of sexual experience, followed by a single injection of BrdU, and perfused after 2 h exhibited an increase in the number of BrdU-labeled cells in the dentate gyrus compared to control retired breeders ($t(20) = 1.98$, $P < 0.05$; Fig. 1). It should be noted that the controls in the present study remained confined to their home cages, except for routine cage changes. Previous studies in our lab suggest that naïve home cage controls serve as a suitable control group, as no differences in BrdU-labeled cells exist between naive rats and those exposed to nonreceptive females (Leuner et al., 2010).

The number of BrdU-labeled cells did not correlate with any measure of sexual behavior during the single mating session, including latencies to mount, intromit and ejaculate and numbers of mounts, intromissions, and ejaculations ($P > 0.05$, for each comparison).

No significant difference was observed in the density of BrdU-labeled cells in the SVZ between controls and middle-aged rats with sexual experience (Control: 136.6 ± 26.5, Sexual Experience: 176.7 ± 51.0, $P > 0.05$).

A similar increase in BrdU-labeled cells in the dentate gyrus was observed in middle-aged retired breeders with 14 d on/14 d off and 28 d of sexual experience (Fig. 1). Male retired breeders exposed to a sexually receptive female for 14 consecutive days, injected with BrdU, followed by 14 d without sexual experience displayed more BrdU-labeled cells compared to middle-aged controls ($F(2,22) = 5.82$, $P < 0.01$, one way ANOVA; Fig. 1), and did not differ from young adult control rats. At this time point, most BrdU-labeled cells expressed the neuronal marker NeuN in both groups (Control: 88.0 ± 7.3%, Sexual Experience: 76.2 ± 4.9%; Fig. 1). There was no difference in the proportion of BrdU-labeled cells expressing NeuN among groups ($P > 0.05$, for each comparison) suggesting that the mating- induced increase in BrdU-labeled cells represents an increase in adult neurogenesis. The number of BrdU-labeled cells that survived 2 weeks post injection correlated positively with the total number of intromissions on the last day of sexual experience (day 14; $r_2 = 0.34$, $P < 0.05$). Other measures of sexual behavior were not correlated with the number of BrdU-labeled cells 14 d after mating. Male retired breeders also exposed to a receptive female for 28 consecutive days displayed significantly more BrdU labeled cells compared to controls ($t(9) = 3.42$, $P < 0.05$, unpaired t-test with Welch's correction; Fig. 1). The number of BrdU-labeled cells that survived up to 4 weeks post injection did not significantly correlate with any measure of sexual behavior ($P > 0.05$, for all comparisons).

Sexual Experience Alters Novel Object Recognition

Young adult control males showed evidence of object recognition during the testing phase but middle-aged control males from either experiment (14 d on/14 d off or 28 d) did not. Exposure to sexual experience for 14 d followed by a 14 d survival time did not alter novel object recognition in middle-aged rats (Fig. 2) but sexual experience for 28 d did such that middle-aged rats performed similarly to the young adults (Fig. 3). In the 14 d group, time spent exploring the familiar and novel objects was similar between controls and sexually experienced middle-aged retired breeders ($P > 0.05$, Fig. 2), with no difference in sample object exploration, which serves as a general indication of exploratory behavior (Fig. 2). In addition, difference scores were identical between groups (Control: 0.5 ± 0.03,

TABLE 2.

Sexual Behavior of Middle-Aged Male Rats Exposed to a Receptive Female for 14 days

	Day of sexual experience		
	1	7	14
Latency to mount	94.6 ± 25.8	179.6 ± 82.9	256.2 ± 123.9
Number of mounts	29.5 ± 3.8	21.4 ± 4.5	31.3 ± 4.3
Latency to intromit	511.5 ± 124.6*	1196.0 ± 166.5***	495.4 ± 141.5
Number of intromissions	24.3 ± 3.0**	20.9 ± 5.7***	49.5 ± 6.1
Latency to ejaculate	815.3 ± 125.6*	1335.0 ± 125.5***	665.9 ± 114.5
Number of ejaculations	2.0 ± 0.3*	0.8 ± 0.2***	1.7 ± 0.2
% copulation	81.8 ± 8.4	59.1 ± 10.7	81.8 ± 8.4

*$P < 0.05$, **$P < 0.01$, ***$P < 0.001$.

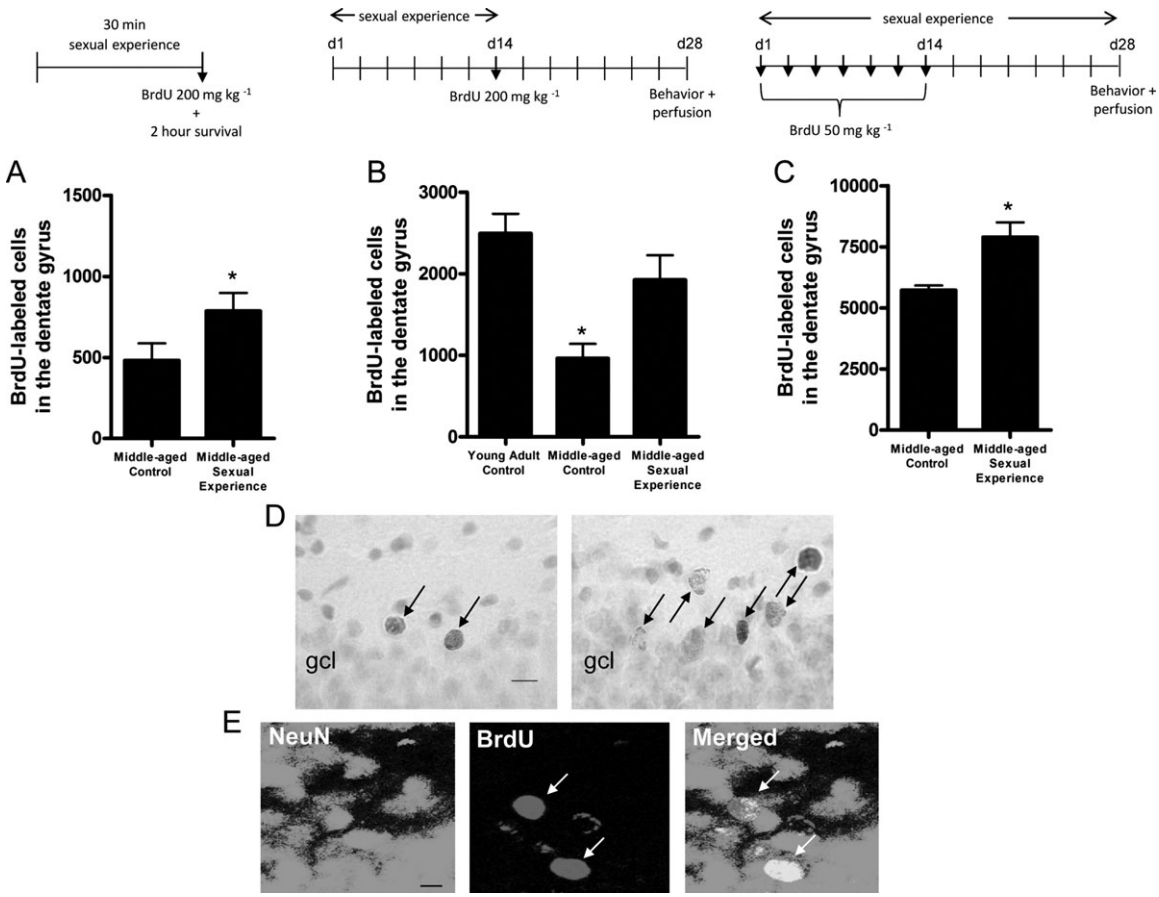

FIGURE 1. Sexual experience increases the production of new granule cells in the dentate gyrus of the hippocampus. (A) A brief exposure to a sexually receptive female increases the number of newly labeled cells in the dentate gyrus of middle-aged retired breeder rats (n = 11/group). (B) Daily sexual experience, for 14 d, followed by a 14 d survival time, restored adult neurogenesis to young adult levels in the middle-aged retired breeder rat (n = 7–12/group). (C) Continuous sexual experience for 28 d produced a large number of newly labeled cells in the dentate gyrus of the middle-aged rat (n = 8–9/group). (D) Continuous exposure to a sexually receptive female increased the number of newly labeled cells in the dentate gyrus of middle-aged retired breeder rats (right panel) compared to control (left panel). Arrows point to BrdU-labeled cells. gcl = granule cell layer. Scale bar = 10 μm. (E) Most BrdU labeled cells expressed the neuronal markers NeuN, with no difference in the proportion of BrdU-labeled cells expressing NeuN between groups. Arrows point to BrdU-labeled cells. Yellow indicates colocalization of BrdU and NeuN. Scale bar = 10 μm. Bars represent mean ± SEM. * indicates P < 0.05. [Color figure can be viewed in the online issue, which is available at wileyonlinelibrary.com.]

Sexual Experience: 0.5 ± 0.03, $P > 0.05$). By contrast, in the 28 d group, while time spent exploring the familiar object did not significantly differ across days ($P > 0.05$), time spent exploring the novel object differed between middle-aged controls and sexually experienced males ($F(2,22) = 8.84$, $P < 0.05$; Fig. 3). No difference in sample object exploration was observed, suggesting no difference in general exploratory behavior ($P > 0.05$; Fig. 3). The performance of middle-aged rats exposed to 28 d of continuous sexual experience was similar to that of young adult controls ($P > 0.05$; Fig. 3) [It should be noted that the amount of time spent exploring objects by the 14 d on/ 14 d off rats was considerably higher than that observed in the 28 d rats, regardless of whether rats had sexual experience or not. This difference may be due to the fact that the 14 d on/14 d off rats had a single injection of BrdU whereas the 28 d rats received 14 injections of BrdU on each of the first 14 d of the experiment. Repeated injections may have produced differences in exploratory behavior between studies as a result of injection stress].

DISCUSSION

Here we have shown that single and repeated sexual experience increase cell proliferation and adult neurogenesis in the dentate gyrus of middle-aged rats. Since cell proliferation and adult neurogenesis are known to drop precipitously by midlife (Kuhn et al., 1996; van Praag et al., 2005), these findings suggest that sexual experience may partially restore adult neurogenesis to young adult levels. Continuous daily exposure (28 d) to sexual experience had beneficial effects on cognitive behavior, restoring object recognition in middle-aged rats to young adult levels. However, repeated but discontinuous sexual experience

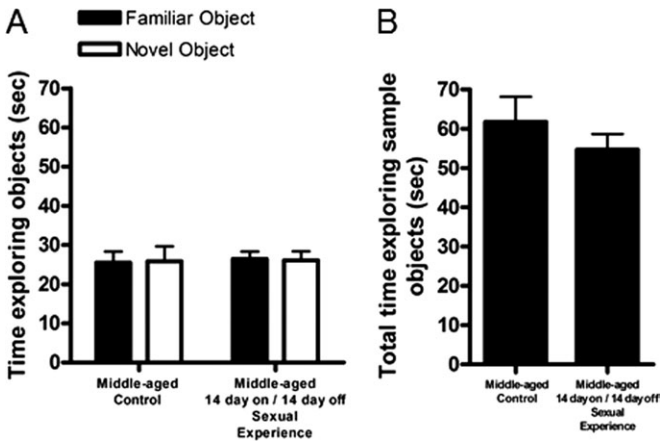

FIGURE 2. Repeated, but discontinuous sexual behavior, does not alter object recognition memory among middle-aged retired breeder rats. (A) Time exploring the familiar and novel objects was similar between middle-aged controls and retired breeder males with discontinuous sexual experience. (B)Time exploring the sample objects was similar between middle-aged controls and retired breeder males with discontinuous sexual experience (n = 9–11/ group).

(14 d of sexual experience followed by 14 d rest) produced no benefits on object recognition. Instead, sexual experience followed by a comparable time with no such experience prevented any improvements in novel object recognition.

Rats are highly motivated to engage in sexual behavior (Everitt and Stacey, 1987; Everitt et al., 1987; Beck and Bialy, 1993; Camacho et al., 2004; Kruger et al., 2005; Camacho et al., 2009) raising the possibility that the rewarding component of mating is responsible for increased neurogenesis and enhanced cognition. Indeed, other experiences associated with reward, such as running and intracranial self-stimulation, are associated with enhanced neurogenesis and improved cognition (Stranahan et al., 2006; Takahashi et al., 2009; Leuner and Gould, 2010). Evidence suggests that a ceiling may exist in the degree to which reward may enhance neurogenesis. That is, providing a food reward does not further enhance neurogenesis associated with running (Klaus et al., 2009) but whether this is due to the rewarding aspects of running remains unknown. It is important to note that sexual experience is a form of enrichment and also increases physical activity. Both environmental enrichment and running have been shown to stimulate adult neurogenesis and enhance cognition (Leuner and Gould, 2010), raising the possibility that mating has similar effects because it shares characteristics with these other experiences. However, some data suggest that the enriching and physically activating aspects of mating cannot account for the effects on adult neurogenesis. First, environmental enrichment increases neurogenesis by enhancing cell survival, not cell proliferation (reviewed in van Praag et al., 2000). This stands in contrast to our findings that sexual experience increases cell proliferation. Second, physical activity seems to require a longer period of time for the induction of increased cell proliferation than we observed for sexual experience (several days—Van der Borght et al., 2009 compared to 30 min—present study). Future studies will be needed to determine whether there is overlap in the mechanisms that drive changes in adult neurogenesis and cognition among mating, environmental enrichment and physical activity.

Sexual experience produces substantial elevations in circulating glucocorticoids in both naïve and sexually experienced young adult rats (Szechtman et al., 1974; Bonilla-Jaime et al., 2006; Leuner et al., 2010) along with enhanced adult neurogenesis in young adults (Spritzer et al., 2009; Leuner et al., 2010). These findings are unexpected given that elevated glucocorticoids are associated with growth suppression in the hippocampus (reviewed in Schoenfeld and Gould, 2012). The effects of sexual experience on circulating glucocorticoid levels were not measured in this study, due to the possible confound of age-related elevations in serum corticosterone (Cameron and McKay, 1999). However, the role of glucocorticoids in the observed results should be further explored. Sexual experience shares many other characteristics with running, including elevated glucocorticoid levels (Brown et al., 2007; Droste et al., 2007) and increased neuronal growth (van Praag et al., 1999; Eadie et al., 2005; Stranahan et al., 2006, 2007). Sexual experience and running effects resemble those of intracranial self-stimulation—increased glucocorticoid levels (Terry and Martin, 1978; Burgess et al., 1993) and enhanced adult neurogenesis (Takahashi et al., 2009). Although prolonged elevated corticosteroid levels are typically associated with reduced cognitive abilities (Plaschke et al., 2006; Wuppen et al., 2010), no previous studies, however, have examined the influence of sexual experience on learning and memory in male rodents of any age. Our study demonstrates a significant improvement in object recognition in older male rats following continuous sexual experience. Taken together, these findings suggest that sexual experience can override the potentially suppressive effects of

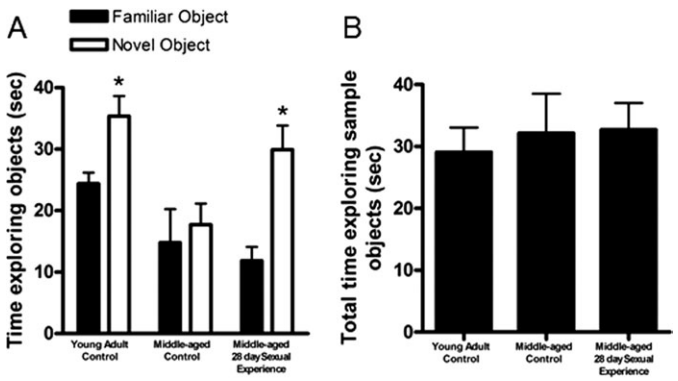

FIGURE 3. Continuous, and prolonged, sexual experience restores object recognition in the middle-aged rat. (A) Continuous sexual experience positively influenced recognition memory on the novel object preference test in the middle-aged rat. Retired breeder rats with 4 weeks of sexual experience spent more time exploring the novel object than the familiar object, compared to middle-aged controls. No difference was observed between young adult controls and middle-aged retired breeders with sexual experience (n = 4–6/ group). (B) Total time exploring the sample objects did not differ between groups. Bars represent mean ± SEM. * indicates $P < 0.05$.

elevated glucocorticoids on adult neurogenesis in the middle-aged rodent, and possibly on behaviors related to the hippocampus, like cognitive function (Schoenfeld and Gould, 2012).

Regarding the mechanism by which rewarding experiences may prevent the negative effects of elevated glucocorticoids, there are a number of obvious candidates including, but not limited to, dopamine, endogenous opiates, and oxytocin. With regard to the latter possibility, oxytocin is particularly intriguing with respect to sexual experience. Oxytocin is released in the hippocampus during mating (Waldherr and Neumann, 2007), enhances synaptic plasticity (Theodosis et al., 1986; Monks et al., 2003), and has recently been shown to enhance cell proliferation and neurogenesis in the hippocampus even in the presence of elevated glucocorticoids (Leuner et al., 2012).

However, the extent to which oxytocin may mediate the effects of sexual experience (and possibly those of other rewarding experiences) on the aging brain remains unknown. Another possibility for the enhancement of adult neurogenesis following sexual experience in middle-aged rats is that mating-induced increases in testosterone, stimulate cell proliferation and adult neurogenesis. Testosterone levels increase with sexual experience in experienced male rats (Bonilla-Jaime et al., 2006)—this hormone has been associated with increased adult neurogenesis (Spritzer and Galea, 2007). Despite producing opposite effects on object recognition, both continuous and discontinuous repeated sexual experience produced an increase in the number of new neurons in the hippocampus. This pattern of findings raises the possibility that increasing the pool of new neurons is not causally linked to changes in cognitive function following sexual experience. Although this conclusion may be correct, it remains possible that new neurons participate in recognition memory but that their activation is altered in rats which experience a period of withdrawal from an established pattern of sexual experience. For example, new neurons generated during a period of sexual experience may be active only under conditions of repeated reward. An additional possibility is that changes in behavior could be linked to changes in a different population of new neurons than the ones presently measured in this study, namely younger neurons. These new neurons would be up to 2 weeks of age at the time of behavioral testing—a time when neuronal activation can occur (Snyder et al., 2009). Future studies will be necessary to determine whether new neurons in the hippocampus are important for recognition memory following repeated sexual experience and whether the activation of these new neurons plays a significant role.

Previous studies have shown that discriminating between familiar and novel objects using a 3 h intertrial delay is dependent on the hippocampus in young adult rats (Hammond et al., 2004; Ainge et al., 2006; Jessberger et al., 2009), but that older rats can only perform the hippocampus independent version of this task (with a shorter delay) (Burke et al., 2010; Leite et al., 2011; Terry et al., 2011). Along these lines, we found that young adult controls, but not middle-aged controls, were able to distinguish between the novel and familiar object following a 3 h delay. Among middle-aged rats subjected to continuous mating experience, object recognition memory improved to a level similar to young adult controls in the hippocampus-dependent version of the one trial object recognition task. That is, middle-aged rats with continuous sexual experience (28 d) spent significantly more time exploring the novel object compared to the familiar one. By contrast, discontinuous sexual experience (14 d on, 14 d off) did not exhibit any beneficial effects of sexual experience on cognition.

Living in an enriched environment, where rodents have the ability to run on a wheel and interact with novel objects in a general way, as well as running itself, leads to increased cognitive ability (Burghardt et al., 2004; Cao et al., 2004; Lambert et al., 2005; O'Callaghan et al., 2007; Leasure and Jones, 2008; Trejo et al., 2008; Griffin et al., 2009) with most of these studies testing cognitive function immediately after exposure to the enriched environment or running wheel. Positive effects of enriched environment living and running on cognitive abilities have also been observed in aged rodents (Kempermann et al., 2002; van Praag et al., 2005; Kronenberg et al., 2006; Leasure and Jones, 2008). Although the control retired breeders in our study had an adult life-time exposure to sexual experience, the stimulatory effects of continuous sexual experience were not present once the mating stopped after 14 d, resulting in these retired breeder rats showing no change in object recognition compared to age-matched controls. These findings raise the question of whether the beneficial effects of sexual experience require the near term presence of the mating stimuli or whether the detrimental influence of a "withdrawal" period may be blocking the positive influence of prior mating. For example, new neurons generated during the period of mating, as well as other mechanisms that support learning, may be rendered inactive due to the engagement of mechanisms associated with reward withdrawal. In this potential scenario, the substrate for enhanced cognition would exist but remain dormant until the blocking effects of withdrawal were overcome.

Although it is common to see an age-induced reduction in adult neurogenesis in laboratory animals, not all aged animals demonstrate a loss in cognitive function (Bizon et al., 2004), an observation that is also made in humans. In humans, intellectually and physically active lifestyles are thought to protect from cognitive impairment (Middleton et al., 2010; Treiber et al., 2011). However, it remains unclear whether such beneficial effects last significantly beyond the time of their occurrence. Some evidence suggests that a higher level of education protects against age-related cognitive decline (Valenzuela et al., 2011), but it is not clear whether formal education builds a fortress of protection or rather, a foundation which engenders a lifelong propensity to seek out cognitively enriching experiences. Although the results of the present study showing that laboratory rats with a very narrow range of opportunities over a lifetime require continuous exposure to rewarding experience to reap persistent beneficial effects on cognition, it is not clear how these findings translate to humans with a lifetime of complex experiences. At present, it is not fully understood why a subpopulation of aging animals are able to retain cognitive function despite a reduction is adult hippocampal neurogenesis. Clearly, a complex interaction between environment, age, and

cognitive function exists and should be further investigated. It should also be noted that no evidence suggests that sexual experience has beneficial effects on hippocampal structure and function in humans. As humans demonstrate a much greater range in what a given individual finds rewarding than do laboratory rodents, it seems prudent to translate the results of the present study only in the broadest possible context. That is, our findings may suggest that any experience, providing it is interpreted as rewarding by the individual, may stimulate neuronal growth and prevent cognitive decline in the aging population. The mechanisms which promote these changes and whether these effects are causally linked to one another remain the subject of future experimentation.

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