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Cell proliferation and survival in the mating circuit of adult male hamsters: Effects of testosterone and sexual behavior

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ABSTRACT

The transient actions of gonadal steroids on the adult brain facilitate social behaviors, including reproduction. In male rodents, testosterone acts in the posterior medial amygdala (MeP) and medial preoptic area (MPOA) to promote mating. Adult neurogenesis occurs in both regions. The current study determined if testosterone and/or sexual behavior promote cell proliferation and survival in MeP and MPOA. Two experiments were conducted using the thymidine analog BrdU. First, gonad-intact and castrated male hamsters (n=6/group) were compared 24 h or 7 weeks after BrdU. In MeP, testosterone-stimulated cell proliferation 24 h after BrdU (intact: 22.8±3.9 cells/mm^2, castrate: 13.2±1.4 cells/mm^2). Testosterone did not promote cell proliferation in MPOA. Seven weeks after BrdU, cell survival was sparse in both regions (MeP: 2.5±0.6 and MPOA: 1.7±0.2 cells/mm^2), and was not enhanced by testosterone. In Experiment 2, gonad-intact sexually-experienced animals were mated weekly to determine if regular neural activation enhances cell survival 7 weeks after BrdU in MeP and MPOA. Weekly mating failed to increase cell survival in MeP (8.1±1.6 vs. 9.9±3.2 cells/mm^2) or MPOA (3.9±0.7 vs. 3.4±0.3 cells/mm^2). Furthermore, mating at the time of BrdU injection did not stimulate cell proliferation in MeP (8.9±1.7 vs. 8.1±1.6 cells/mm^2) or MPOA (3.6±0.5 vs. 3.9±0.7 cells/mm^2). Taken together, our results demonstrate a limited capacity for neurogenesis in the mating circuitry. Specifically, cell proliferation in MeP and MPOA are differentially influenced by testosterone, and the birth and survival of new cells in either region are not enhanced by reproductive activity.

Introduction

Gonadal steroid hormones exert activational effects on the adult brain that sculpt neural circuits for expression of adult behavior. Hormones act in steroid-responsive brain regions, where they exert neurotrophic effects to enhance neuronal morphology and synaptic connectivity (Cooke and Woolley, 2005). In addition, gonadal hormones stimulate neurogenesis in adult mammals (Fowler et al., 2008; Galea et al., 2006). These structural changes are thought to be a principal mechanism through which hormones promote social behaviors, including mating.

Adult mammalian neurogenesis includes both cell proliferation and cell survival. It is notably demonstrated in the hippocampus (Gould, 2007). In male rodents, testosterone promotes the survival of new neurons in the dentate gyrus (Spritzer and Galea, 2007). Cell proliferation has also been demonstrated elsewhere in the male brain, including the olfactory bulb (Peretto et al., 2001), posterior medial nucleus of the amygdala (MeP) (Fowler et al., 2003), medial preoptic area (MPOA) and bed nucleus of the stria terminalis (BST) (Huang and Bittman, 2002). MeP, BST, and MPOA are essential for male rodent sexual behavior. Although this mating circuit exhibits a lower capacity for neurogenesis, it serves as an excellent model to study hormone-driven cell proliferation and survival. The actions of testosterone throughout the mating circuit are essential for the expression of reproductive behavior in the presence of an appropriate sexual partner (reviewed in Wood and Swann, 2000).

Both MeP and medial MPOA transduce testosterone via androgen and estrogen receptors to stimulate mating (reviewed in Wood and Newman, 1995a). Neurogenesis occurs in both regions (Fowler et al., 2003; Huang and Bittman, 2002). In MeP, testosterone increases cell proliferation (Fowler et al., 2003). It is unknown whether testosterone influences new cell birth in MPOA. Therefore, it is important to determine whether testosterone-enhanced cell proliferation extends to MPOA. Additionally, we examined whether the survival of newly-born cells parallels testosterone's long-term effects on behavior. Gonadal hormones exert lasting effects throughout the male brain. For example, mating behavior in male rodents is not immediately abolished after castration (reviewed in Wood and Newman, 1995a). Conversely, the full recovery of sexual behavior in long-term castrates requires weeks of testosterone exposure. Thus, newly-born cells are more likely to be functionally important if they persist through the time course known to influence behavior. Using the cell proliferation marker BrdU, the first experiment determined if testosterone-driven

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cell proliferation is similar in MeP and MPOA. Additionally, we determined if testosterone promotes cell survival in MeP and MPOA.

In a second experiment, we determined if the regular activation of reproductive circuits enhances the survival of cells in MeP and MPOA by comparing male hamsters that were allowed to mate on a weekly basis with males that did not mate. Several investigators have put forth the hypothesis that the birth and/or survival of new cells in the adult brain are enhanced in regions that are regularly activated by experience or environment (Gould et al., 2000; Lledo et al., 2006; Prickaerts et al., 2004). Animals engaged in hippocampal-dependent learning tasks exhibit higher levels of cell proliferation and survival in the dentate gyrus compared to animals that have not engaged in these tasks (Dalla et al., 2007; Shors, 2004). Additionally, cell proliferation is enhanced in the olfactory bulb after rodents are repeatedly exposed to novel olfactory stimuli (Rochefort et al., 2002). If the activity-dependent hypothesis applies to the hamster mating behavior circuit, then regular sexual activity should enhance cell proliferation and survival in MeP and MPOA.

Materials and methods

Subjects

Forty-four adult male Syrian hamsters (*Mesocricetus auratus*, 130–150 g) were obtained from Charles River Laboratories (Wilmington, MA). Hamsters were singly-housed on a long day photoperiod (14:10 LD) with access to food and water *ad libitum*. Experimental procedures were approved by USC's Institutional Animal Care and Use Committee and conducted in accordance with the NIH Guide for the Care and Use of Laboratory Animals (DHEW Publication 80-23, revised 1985, Office of Science and Health reports, DRR/NIH, Bethesda, MD 20205).

Experiment 1

Gonad-intact and castrated male hamsters were compared at 24 h and 7 weeks post-BrdU to determine if testosterone promotes cell proliferation and survival in MeP and MPOA. Twenty-eight animals were used. Three weeks prior to BrdU injections, half of the animals were castrated. Castration causes a profound loss in circulating androgens, and is accompanied by the elimination of sexual behavior (reviewed in Siegel, 1985; Wood and Newman, 1995a). The other half remained gonad-intact. Proliferating cell populations were labeled 3 weeks following castration, when mating behavior is severely diminished (reviewed in Wood and Newman, 1995a).

All animals received a single injection of BrdU [300 mg/kg BW ip (Sigma, St. Louis, MO) in 0.9% saline with 0.007 N NaOH] to maximally incorporate BrdU into dividing cells (Cameron and McKay, 2001; Fowler et al., 2003). Half of the hamsters in each group (n=7 each) were sacrificed 24 h later to determine if testosterone promotes cell proliferation in MPOA and MeP. The remaining males were sacrificed 7 week post-BrdU to determine if testosterone enhances the survival of newly-born cells. The remaining animals were sacrificed 7 week post-BrdU to determine if testosterone enhances the survival of newly-born cells. Seven weeks is sufficient for testosterone to restore mating behavior in long-term castrates and for newly-born olfactory neurons to express Fos in response to estrous females (Huang and Bittman, 2002; Morin and Zucker, 1978). By examining cell survival after 7 weeks, the current study seeks to bridge short-term (Fowler et al., 2003) and long-term (Huang and Bittman, 2002) studies. The hypothesis is that testosterone enhances long-term cell survival at 7 weeks similar to its effects on short-term cell proliferation, as measured at 24 h.

Experiment 2

Gonad-intact, sexually-experienced male hamsters (n=18) were mated weekly or left unstimulated to determine if regular sexual activity enhances cell proliferation and survival in MeP and MPOA. Sexual interactions acutely stimulate testosterone in male hamsters (Pfeiffer and Johnston, 1992). Accordingly, we hypothesize that the consistent activation of mating circuits will increase cell survival in MeP and MPOA. Males were included in the study only if they mated to ejaculation in two of three preliminary tests for sexual experience.

Mating behavior tests were conducted during the first hours of the dark phase under dim light. Eight female hamsters were used as stimulus animals for mating behavior. All females were ovariectomized via bilateral dorsal flank incision, and received a 4-mm Silastic estradiol implant sc (id: 1.98 mm, od: 3.18 mm; Dow Corning, MI) to maintain chronic physiologic levels of estrogen. To stimulate lordosis, females received 250 μg progesterone in cottonseed oil sc 4 h prior to testing (see Carter, 1985). For testing, an estrous female was introduced into the male's home cage for 10 min. Behavior of the test male was recorded including mounts, intromissions, and ejaculations. For weekly mating experience after BrdU injection in Experiment 2, males were required to ejaculate at least once during each test. If necessary, males were given extra time and/or placed with a different stimulus female to facilitate ejaculation. The week-long period between mating exposures ensured that males would successfully copulate at each mating test without becoming sexually satiated (Arteaga et al., 2000; Beach and Rabedeau, 1959).

As in Experiment 1, all males received a single injection of BrdU (300 mg/kg BW ip). The hamsters were separated into three groups (n=6/group). Males in the Control group did not mate after BrdU administration. The remaining hamsters were allowed to mate weekly for 7 weeks. However, to determine whether reproduction stimulates cell proliferation, hamsters in the Immediate Mating (IM) group were allowed to mate 10 min after BrdU administration. Animals in the Delayed Mating (DM) group mated 24 h later. Thus, males in the IM and DM groups received the same amount of sexual activity. However, only males in the IM group mated during BrdU incorporation, which occurs over 2 h following BrdU administration (Takahashi et al., 1992). Therefore, BrdU labeling in IM hamsters should reflect mating-induced cell proliferation and survival, while BrdU labeling in DM hamsters should indicate activity-dependent cell survival only. After the final sexual behavior test, all hamsters were sacrificed.

Perfusion

Hamsters were deeply anesthetized with sodium pentobarbital (150 mg/kg BW) and perfused intracardially with 150 mL of 0.1 M sodium phosphate buffer (PB, pH=7.4) containing 0.9% NaCl and 0.1% $NaNO_3$, followed by 250 mL 4% paraformaldehyde in PB. The brains were removed, post-fixed in the perfusion fixative for 1 h and cryoprotected for 5 days at 4 °C with 20% sucrose in PB. The brains were rapidly frozen and sectioned coronally at 40 μm. Sections were stored in PB with 0.01% sodium azide at 4 °C until processed for BrdU immunocytochemistry.

Immunocytochemistry for BrdU

Every fourth section was stained for BrdU according to the methods of Fowler et al. (2003). Sections from males in different groups of the same experiment were stained at the same time. To denature the DNA, free-floating brain sections were pre-treated with 2 N HCl for 30 min at 37 °C and then washed to neutralize the acid with 0.1 M borate buffer (pH=8.5) at room temperature (RT) for 20 min. After additional washing with PB, sections were incubated overnight at RT in monoclonal rat anti-BrdU antibody (1:500; AbD Serotec, Raleigh, NC) with 4% normal donkey serum and 0.3% Triton X-100 in PB. The following day, sections were incubated in biotinylated donkey-anti-rat secondary antibody (1:200, Jackson Immunoresearch, West Grove, PA) and the avidin–biotin–horseradish peroxidase complex, each for 1 h at RT with extensive washes in between.