N-methyl-D-aspartate receptor activity and estradiol: separate regulation of cell proliferation in the dentate gyrus of adult female meadow vole

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Abstract

We have previously found that estradiol increases (within 4 h) but then decreases (within 48 h) cell proliferation in the dentate gyrus of adult female ovariectomized (OVX) rats and of intact meadow voles and that estradiol partially stimulates adrenal activity to suppress cell proliferation in rats. Estradiol enhances N-methyl-D-aspartate receptor (NMDAr) activity and NMDAr activation suppresses cell proliferation in the adult rodent dentate gyrus. Therefore, we tested whether estradiol alters cell proliferation in the dentate gyrus of adult OVX female meadow voles by stimulating NMDAr activity. In experiment 1, OVX females were injected with estradiol (10 µg) or oil and then with NMDA (30 mg/kg) or vehicle 3 h later and bromodeoxyuridine (BrdU; 50 mg/kg). Voles were perfused 1 h after BrdU injection. Relative to oil vehicle, estradiol increased ($P < 0.001$) and NMDA decreased ($P < 0.006$) labeled cell number. Coadministration of estradiol/NMDA increased labeled cell numbers relative to NMDA alone ($P < 0.03$), suggesting that within 4 h estradiol does not influence the effect of NMDA receptors on cell proliferation. In experiment 2, OVX females were injected with either estradiol or oil and then with either MK-801 (1 mg/kg) or vehicle 47 h later and BrdU 48 h later. The animals were perfused 1 h after BrdU was injected. Relative to oil-treated voles, estradiol-treated voles had fewer ($P < 0.006$) and MK-801-treated voles had more labeled cells ($P < 0.0001$) in the dentate gyrus. However, estradiol did not appear to stimulate NMDA receptors to suppress cell proliferation because estradiol (48 h)/MK-801-treated voles had fewer BrdU-labeled cells than oil (48 h)/MK-801-treated voles ($P < 0.06$). The results show that estradiol time-dependently influences cell proliferation but that estradiol does not stimulate NMDAr activity to influence cell proliferation in the dentate gyrus of adult voles.


Introduction

New granule neurons are added to the dentate gyrus of all mammalian species that have been studied, including humans, throughout adulthood (Altman & Das 1965, Cameron et al. 1993, Gould et al. 1997, 1998, 1999, Eriksson et al. 1998, Kornack & Rakic 1999). The number of granule neurons added to the mammalian dentate gyrus appears substantial. In rats, approximately 9000 new cells are produced daily and many of these new cells differentiate into granule neurons (Cameron & McKay 2001). Altering progenitor cell proliferation, the fate of daughter cells or the survival of new granule neurons could increase or decrease neurogenesis in the dentate gyrus. Understanding how different components of adulthood neurogenesis are influenced by a single factor is important because a factor that both increases cell proliferation and decreases the survival of young neurons could produce no net change in new neuron number. Estradiol dynamically influences neurogenesis within the adult rodent dentate gyrus by first increasing and then decreasing cell proliferation (Ormerod & Galea 2001, Ormerod et al. 2003) as well as by enhancing the survival of young neurons (Ormerod et al. 2002). This study was designed to better understand how estradiol dynamically influences cell proliferation in the adult rodent dentate gyrus.

Several studies have shown that estradiol influences cell proliferation in the dentate gyrus of adult rodents. For example, short-term exposure to estradiol (2–4 h) stimulates cell proliferation in the dentate gyrus of adult ovariectomized (OVX) female rats (Tanapat et al. 1999, Banast et al. 2001, Ormerod et al. 2003). Interestingly, we have previously shown that estradiol initially enhances cell proliferation (within 4 h) but subsequently suppresses cell proliferation (within 48 h) in the dentate gyrus of OVX adult female rats, suggesting that estradiol time-dependently influences cell proliferation (Ormerod et al. 2003). In fact, estradiol appears to dynamically regulate...
cell proliferation across rodent species. Ormerod and Galea (2001) found that a 4-h exposure to estradiol tended to increase whereas a 48-h exposure significantly decreased the density of proliferating cells in the dentate gyrus of reproductively inactive female meadow voles (with low circulating estradiol levels). However, in that study all females had intact ovaries and the possibility that other ovarian steroids could also have influenced cell proliferation exists. Therefore, one objective of this study was to investigate whether the dynamic changes in cell proliferation observed in the dentate gyrus of OVX adult female rats and intact female meadow voles following estradiol administration also occur in the dentate gyrus of adult OVX female meadow voles. This finding would verify that the dynamic effects of estradiol are robust across rodent species with diverse reproductive strategies and physiologies.

The mechanisms by which estradiol differentially influences cell proliferation in the adult rodent dentate gyrus are beginning to be explored. Banasr and colleagues (2001) have shown that the estradiol-induced increase in cell proliferation is mediated by serotonin. Specifically, the estradiol-induced increase in cell proliferation in the dentate gyrus of adult OVX female rats is abolished by the administration of the serotonin synthesis inhibitor, PCPA (Banasr et al. 2001). We found that the estradiol-induced suppression in cell proliferation is partially mediated by adrenal steroids because the suppression is eliminated (but not reversed) in adult female rats that are adrenalectomized (Ormerod et al. 2003). Therefore, estradiol must influence another factor, in addition to stimulating adrenal activity, to suppress cell proliferation with longer exposure.

Estradiol exposure for 48 h increases the number and sensitivity of N-methyl-D-aspartate (NMDA) receptors in the hippocampus of adult rats (Weiland 1992, Gazzaley et al. 1996). Furthermore, NMDA receptor activation (via NMDA) decreases and NMDA receptor inactivation (via MK-801 or CGP43487) increases cell proliferation in the dentate gyrus of adult male rats, tree shrews and aged female rats (Cameron & Gould 1994, Cameron et al. 1995, Gould et al. 1997, Bernabau & Sharp 2000, Nacher et al. 2001, 2003 but see Bernabau & Sharp 2000 and Arvidsson et al. 2001). Interestingly, Cameron and colleagues (1998) demonstrated that NMDA receptor activation works downstream of adrenal steroids to suppress cell proliferation, as the effects of low-level or high-level corticosterone can be blocked by NMDA receptor activation or inactivation respectively. Therefore, another objective of the current study was to investigate whether longer exposure to estradiol influences NMDA receptor activity to suppress cell proliferation.

We investigated whether estradiol influences neurogenesis in the dentate gyrus of adult OVX female meadow voles by first increasing (within 4 h) and then decreasing (within 48 h) cell proliferation, and whether estradiol stimulates NMDA receptor activity to suppress cell proliferation. We also examined whether NMDA receptor activation and inactivation influenced cell proliferation as it does in adult male and aged female laboratory rats and tree shrews. Based upon our previous work using adult OVX rats and intact adult female meadow voles, we hypothesized that estradiol would increase cell proliferation within 4 h but decrease cell proliferation within 48 h in the dentate gyrus of OVX adult female meadow voles. We also hypothesized that because estradiol influences NMDA receptor number and sensitivity and NMDA receptor activation influences cell proliferation, administration of the NMDA receptor antagonist, MK-801, could reverse the estradiol-induced suppression in cell proliferation. Discovering how estradiol mediates its diverse effects on neurogenesis in the adult dentate gyrus could promote the development of strategies to control the process in order to replace neurons lost in disease or trauma. Moreover, fully characterizing the time-dependent effects of estradiol on neurogenesis may be important for patients on long-term estrogen replacement therapies, given that neurogenesis has been linked to hippocampus-dependent behavior (Gould et al. 1999, Shors et al. 2001, 2002). For example, estrogen replacement therapy has been purported to reduce the risk and severity of Alzheimer’s disease as well as the associated cognitive impairment (Henderson et al. 2000, Kawas et al. 1997, Sherwin 1997).

Materials and Methods

All experiments were conducted in accordance with the policies established by the University of British Columbia and the Canadian Council on Animal Care regarding the ethical treatment of animals used for research. Every effort was made to minimize the number of animals used per group and their suffering.

Subjects

Forty-one adult female meadow voles (at least >25 g and 60 days old) reared in our breeding colony at The University of British Columbia were used as subjects. The voles were bred and reared in a colony room that was temperature controlled (21 ± 1 °C) with a 16 h light (lights on at 0700 h):8 h dark cycle. All animals were housed in polyurethane paper bedding-lined (Carefresh; Absorption Corporation, Bellingham, WA, USA) cages that contained enrichment supplies (plastic and/or cardboard containers). At 21 days of age, the voles were weaned and housed either with same sex siblings or individually (if the vole was the only female sibling of her litter) until 60 days of age when all voles were housed individually. The voles had free access to tap water and Jamieson Lab Diet #5012 for the duration of the experiment and were given weekly sunflower seed, alfalfa
pellet, carrot, and apple food supplements. All voles were anesthetized with halothane delivered at 2–3% and ovariolectomized (OVX) using sterile surgical techniques. The voles were given one week to recover from surgery prior to each experiment.

Procedure

Experiment 1  Experiment 1 was conducted to determine whether (1) short-term (4 h) exposure to estradiol increases cell proliferation, (2) NMDA receptor activation decreases cell proliferation, and (3) estradiol influences NMDA receptor activity within 4 h to alter cell proliferation in the dentate gyrus of adult OVX female meadow voles. Ovariectomized female meadow voles were injected either with estradiol benzoate (EB; 10 µg) or sesame oil (OIL; 0·05 ml) and then with either vehicle or NMDA (30 mg/kg) 4 h later and BrdU (50 mg/kg) 48 h later. Four hours after the injection of estradiol or sesame oil, the voles were given a single injection of BrdU (50 mg/kg, i.p.) and were perfused 1 h later to assess cell proliferation (see Fig. 1B). Therefore, the effects on cell proliferation were tested in four groups in experiment 1: OIL4+VEH, EB4+VEH, OIL4+MK-801 or EB4+MK-801 (n=5 per group). If estradiol stimulates NMDA receptors to suppress cell proliferation in the dentate gyrus of adult OVX female meadow voles, then we would expect to find that antagonizing NMDA receptor activity with MK-801 would eliminate or reverse the estradiol-induced suppression in cell proliferation.

Drug preparation

Estradiol benzoate (EB; Sigma) solution was prepared by dissolving EB in sesame oil (Sigma) to a concentration of 10 µg EB/0·05 ml sesame oil. The solution was then stored in a light-insensitive container and used for all experiments. All voles were subcutaneously injected with 0·05 ml of the solution (10 µg EB per vole). Although estradiol-induced changes in blood–brain permeability could, in theory, account for the differences in BrdU-labeling between groups, estradiol only alters rat blood–brain barrier permeability after at least 3 weeks of exposure (Ziylan et al. 1990). In addition, previous work has shown that the number of BrdU-labeled cells is elevated in the rostral migratory stream (but not dentate gyrus) of adult female prairie voles with high versus low estradiol levels (Fowler et al. 2002, Smith et al. 2001). N-methyl-D-aspartate (NMAD; Tocris, Ellisville, MO, USA) was prepared just prior to its use in experiment 1. NMAD was dissolved in isotonic saline to a concentration of 30 mg/ml and was injected i.p. in a volume of 0·1 ml/100 g body weight, making the dose of NMAD 30 mg/kg. The non-competitive NMDA receptor antagonist, MK-801 (Tocris) was prepared just prior to its use in experiment 2. MK-801 was dissolved in isotonic saline to a concentration of 1 mg/ml and was injected i.p. in a volume of 0·1 ml/100 g body weight making the dose of MK-801 1 mg/kg. The doses and durations of exposure to NMAD or MK-801 were chosen because they have previously been shown to influence cell proliferation in the dentate gyrus of adult rats (Cameron et al. 1995, 1998). BrdU (Sigma) was prepared just prior to use by dissolving BrdU in freshly prepared saline buffered with 0·7% 2 M NaOH to a concentration of 10 mg BrdU/ml saline. Voles were

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Figure 1 Timelines for experiments 1 and 2. (A) In experiment 1, OVX voles were injected with oil (0·05 ml) or estradiol (10 µg) and then with either vehicle or NMDA (30 mg/kg) 3 h later and BrdU (50 mg/kg) 47 h later. Forty-eight hours after the injection of estradiol or sesame oil vehicle, the voles were given a single injection of BrdU (50 mg/kg, i.p.) and were perfused 1 h later to assess cell proliferation (see Fig. 1B). Therefore, the effects on cell proliferation were tested in four groups in experiment 2: OIL48+VEH, EB48+VEH, OIL48+MK-801 or EB48+MK-801 (n=5 per group). If estradiol stimulates NMDA receptors to suppress cell proliferation in the dentate gyrus of adult OVX female meadow voles, then we would expect to find that antagonizing NMDA receptor activity with MK-801 would eliminate or reverse the estradiol-induced suppression in cell proliferation.

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injected i.p. with 0.5 ml/100 g of the solution (50 mg/kg). This dose has been used to label cells dividing in the dentate gyrus of mice (Kempermann et al. 1997) and voles (Ormerod & Galea 2001, Smith et al. 2001, Fowler et al. 2002).

**Histology**

At the end of each experiment, voles were anesthetized with sodium pentobarbital and then perfused with a solution of 4% paraformaldehyde (Sigma) in PBS, 1 h after BrdU was injected. Following perfusion, brains were extracted and refrigerated overnight in perfusate at 4°C. The following day, the brains were sectioned (40 µm thick sections) through the entire dentate gyrus using an oscillating tissue slicer (Leica VT1000S) in a bath of 0.1 M phosphate buffer (PB). Sections were pre-treated in a solution of 0.2% H2O2 in PB for 20 min and then rinsed in PB before being mounted on slides treated with 3% 3-aminopropyltriethoxy-silane in acetone (Sigma) to enhance slide adherence.

**BrdU peroxidase immunohistochemistry**

Tissue was processed to reveal BrdU labeling by applying solutions directly to the slide-mounted sections as described previously (Ormerod & Galea 2001, Ormerod et al. 2003). The sections were rinsed repeatedly between steps in PBS (0.1 M sodium phosphate heptahydrate in 0.9% saline; pH 7.4) unless stated otherwise. Sections were incubated in 0.05% trypsin (Sigma) in 0.1% CaCl2 Tris–HCl buffer (pH 7.5) for 10 min to permeabilize cells. Then, DNA was denatured by applying 2 M HCl for 30 min and then the sections were repeatedly rinsed in PBS (pH 6.0). Sections were blocked with 5% normal horse serum (NHS) for 30 min and then incubated overnight in mouse monoclonal antibody against BrdU (1:100 + 3% NHS + 0.5% Tween 20; Boehringer Mannheim, Laval, Quebec, Canada) at room temperature. The following day, sections were incubated in mouse secondary antisera (1:29 + 3% NHS; Vector Laboratories, Burlington, ON, Canada) for 4 h and then in avidin-biotin horseradish peroxidase complex (ABC Elite Kit; 1:50; Vector Laboratories) for 60 min. Sections were reacted for about 10 min in 0.02% diaminobenzidine (DAB; Sigma) and 0.003% H2O2 in Tris–buffered saline and then counterstained with cresyl violet acetate, dehydrated and coverslipped with Permount (Fisher Scientific, Nepean, ON, Canada).

**Data analyses**

Prior to analysis, slides were coded to blind the experimenter to the treatment conditions. Total BrdU-labeled (intensely stained medium round or oval cells; Fig. 2A) and pyknotic cells (with pale or absent cytoplasm, dark spherical chromatin and no nuclear membrane; Fig 2B) throughout the granule cell layer and subgranular zone of the dentate gyrus were stereologically estimated as described previously (Cameron et al. 1993, Ormerod & Galea 2001, Ormerod et al. 2003). To stereologically

**Figure 2** Photomicrographs of BrdU-labeled cells and a pyknotic cell. (A) Photomicrograph of BrdU-labeled cells located in the subgranular zone of an OVX female vole exposed to estradiol for 4 h before BrdU was injected. These cells are representative of those counted in the dentate gyrus of all groups. (B) Photomicrograph of a representative pyknotic or dying cell in the subgranular zone between the granule cell layer (GCL) and the hilus. Scale bar represents 10 µm.
estimate cell numbers, total BrdU-labeled and pyknotic cells were counted on every 10th section (8 sections per vole; \(P=1.00\)) throughout the rostral-caudal extent of the dentate gyrus per vole using a 100 × objective under a Nikon Elipse (E600) light microscope. The counts were then applied to a modified version of the optical fractionator formula (West et al. 1991, described in Ormerod et al. 2003) to project what was counted on every 10th section to that which would be counted on the entire dentate gyrus. Areas were measured using the digitizing software, Analytical Software Imaging Station (Imaging Research, Brock University, Ontario, Canada) and the dentate gyrus volume was estimated using Cavalieri’s principle (Gunderson et al. 1988). Because we have previously reported BrdU-labeled cell densities (Galea & McEwen 1999, Ormerod & Galea 2001) we also calculated BrdU-labeled cell densities (number of cells/area) on 5 anatomically matched sections per vole (where the dentate gyrus lies just beneath the corpus callosum and the infrapyramidal and suprapyramidal blades are joined at the crest; between A −3.3 and A −4.8 in rats) in order to compare the density of BrdU-labeled cells with stereological estimates of total BrdU-labeled cells in the dentate gyrus. Our relative densities and stereologically estimated total BrdU-labeled cell numbers were similar to those reported by Cameron and McKay (2001).

**Statistical analyses**

In experiment 1, the dependent variables (total BrdU-labeled cells, BrdU-labeled cell density, and total pyknotic cells) were analyzed using ANOVA, with hormone (EB4, OIL4) and drug (NMDA, VEH) as the independent variables. In experiment 2, the dependent variables (total BrdU-labeled cells, BrdU-labeled cell density, and total pyknotic cells) were analyzed using an ANOVA, with hormone (EB48, OIL48) and drug (MK801, VEH) as the independent variables. For both experiments, Pearson product-moment correlations were run between dependent variables, and the Newman–Keuls procedure was used as the post-hoc analysis. All statistical procedures were set at \(P=0.05\).

**Results**

**Experiment 1. Relative to vehicle, estradiol increased and NMDA decreased cell proliferation but estradiol did not appear to stimulate NMDA receptor activity to influence cell proliferation after a 4-h exposure**

Estradiol significantly increased (main effect of hormone: \(F_{(1,17)}=14.3, P≤0.001\)) and NMDA significantly decreased (main effect of drug: \(F_{(1,17)}=9.6, P≤0.006\)) the total number of BrdU-labeled cells in the dentate gyrus of adult female meadow voles (Fig. 3A). Within 4 h, estradiol did not stimulate NMDA receptors to alter cell proliferation because hormone treatment did not interact with drug treatment to influence labeled cell number (\(P=0.8\); Fig. 3A). In fact, Fig. 3A shows that estradiol enhanced cell proliferation regardless of drug condition. BrdU-labeled cell density was strongly and positively correlated with total BrdU-labeled cell number (\(r_{(21)}=0.93; P≤0.001\)). Neither the total number of pyknotic cells (\(P=0.7\); see Fig. 3B) nor pyknotic cell density (\(P=0.8\)
significantly differed between groups. The mean (±S.E.M) total area on which BrdU-labeled cells were counted was similar between groups (F\((1,17)=0.70, P≤0.05\); OIL\(48\)+VEH=2.5±0.2 mm\(^2\); EB4+VEH=2.6±0.1 mm\(^2\); OIL\(48\)+NMDA=2.4±0.1 mm\(^3\); EB4+NMDA=2.4±0.2 mm\(^3\)), verifying that differences observed between groups in BrdU-labeled cell numbers were not related to volumetric differences.

Experiment 2. Relative to vehicle, estradiol significantly decreased and MK-801 significantly increased cell proliferation but estradiol did not interact with NMDA receptors to alter cell proliferation within 48 h

Estradiol significantly decreased (main effect of hormone: F\((1,16)=10.1, P≤0.006\) and MK-801 significantly increased (main effect of drug: F\((1,16)=36.4, P≤0.0001\) the number of BrdU-labeled cells. Similar to experiment 1, estradiol did not stimulate NMDA receptors within 48 h to influence cell proliferation because the hormone \times drug interaction effect was not significant (P≤0.8; Fig. 4A). In fact, Fig. 4A shows that estradiol suppressed cell proliferation, both in the absence and presence of MK-801. BrdU-labeled cell density was strongly and positively correlated with total BrdU-labeled cell number (t\((20)=0.94; P≤0.001\)). Neither the total number of pyknotic cells (P=0.9; see Fig. 4B) nor pyknotic cell density (P=0.9) differed significantly between groups. The mean (±S.E.M) total area on which BrdU-labeled cells were counted did not differ between groups (F\((1,16)=1.17, P=0.3\); OIL\(48\)+VEH=2.8±0.2 mm\(^2\); EB4+VEH=2.5±0.2 mm\(^2\); OIL\(48\)+MK-801=2.5±0.2 mm\(^2\); EB4+MK-801=2.5±0.1 mm\(^3\)), verifying that differences observed between groups in BrdU-labeled cell numbers were not related to volumetric differences.

Discussion

These data demonstrate that estradiol dynamically influences cell proliferation in the dentate gyrus of adult female meadow voles but does not interact with NMDA receptors to mediate its effects on cell proliferation. Estradiol increased cell proliferation within 4 h but decreased cell proliferation within 48 h in the dentate gyrus of OVX adult female meadow voles, consistent with what we have reported previously in the dentate gyrus of adult female OVX rats and intact meadow voles (Ormerod & Galea 2001, Ormerod et al. 2003). In addition, NMDA receptor activation (via NMDA) suppressed and NMDA receptor blockade (via MK-801) enhanced cell proliferation, which extends previous findings showing that NMDA receptor activity regulates cell proliferation in the dentate gyrus of adult rats and tree shrews (Cameron & Gould 1994, Cameron et al. 1995, Gould et al. 1997, Bernabau & Sharp 2000, Nacher et al. 2001, 2003). Estradiol did not appear to influence NMDA receptors to alter cell proliferation at either the 4 h or 48 h time point because estradiol increased proliferation in the presence or absence of NMDA (within 4 h) and decreased proliferation in the presence or absence of MK-801 (within 48 h; see Figs 3A and 4A). Pyknotic cell number did not differ between groups and total pyknotic cell number was similar to that which we
have reported previously (Ormerod & Galea 2001, Ormerod et al. 2003).

We believe that the differential effects of estradiol on cell proliferation observed within 4 versus 48 h of its administration are time- rather than dose-dependent. Serum estradiol levels are high 4 h after an estradiol injection, intermediate 48 h after an estradiol injection and low or undetectable following a vehicle injection but the number of labeled cells is elevated 4 h after an estradiol injection and suppressed 48 h after an estradiol injection relative to a vehicle injection (Ormerod & Galea 2001, Ormerod et al. 2003). In addition, cell proliferation is suppressed 4 h after an estradiol injection (same dose used as in the present study) in the dentate gyrus of adult female rats exposed to low dose estradiol (via silastic implant) for 1 week (E M Falconer and L A M Galea, personal communication). Future work could verify whether the differential effect of estradiol on cell proliferation is time-dependent by keeping the dose constant over 48 h.

**Estradiol suppresses cell proliferation by stimulating adrenal activity but not NMDA receptor activity**

Previous work has shown that estradiol increases cell proliferation in the dentate gyrus of adult rats by stimulating serotonin synthesis (Banasr et al. 2001), and suppresses cell proliferation partially by stimulating adrenal steroids in the adult rodent dentate gyrus (Ormerod et al. 2003). However, removing estradiol’s stimulatory effect on the hypothalamic–pituitary–adrenal axis via adrenalectomy eliminates but does not reverse the suppression in cell proliferation observed to occur in the dentate gyrus of adult female rats 48 h after an estradiol injection (Ormerod et al. 2003), suggesting that estradiol stimulates a factor, perhaps in addition to adrenal steroids, to suppress cell proliferation. Similar to effects shown previously in the dentate gyrus of adult male and female rats (Cameron & Gould 1994, Cameron et al. 1995, Gould et al. 1997, Bernabau & Sharp 2000, Nacher et al. 2001, 2003), we found that NMDA receptor activation decreased and NMDA receptor blockade increased cell proliferation in adult female meadow voles. However, the NMDA receptor-mediated effects on cell proliferation observed in the current study occurred despite the presence of estradiol and did not alter estradiol’s effects on cell proliferation.

Of course, we cannot completely dismiss the possibility that estradiol interacts with NMDA receptors. Perhaps the use of CGP43487, a longer-lasting NMDA receptor antagonist than MK–801 (Schmutz et al. 1990, Cameron et al. 1995) would have yielded different results. However, our finding that similar relative estradiol-induced increases (within 4 h) in cell proliferation in the presence or absence of NMDA and similar relative estradiol-induced decreases (within 48 h) in the presence or absence of MK–801 exist, suggests that the effects we observed are straightforward. Thus, within our experimental parameters estradiol did not work through NMDA receptors to influence cell proliferation.

Although we found that estradiol and NMDA receptors influenced proliferation independently, the effects of co-administering estradiol+NMDA or estradiol+MK-801 on cell proliferation were additive. Remembering that the effects of stress (Gould et al. 1997, 1998, Tanapat et al. 2001, Holmes & Galea 2002), some forms of learning (Gould et al. 1999), exercise (van Praag et al. 1999a,b) and hormones (Cameron & Gould 1994, Cameron et al. 1998, Cameron & McKay 1999, Tanapat et al. 1999, Banasr et al. 2001, Ormerod et al. 2003) can all influence cell proliferation, perhaps independently, is important as most non-laboratory-reared mammals are likely to experience all these phenomena daily.

**Estrogen could time-dependently influence cell proliferation in the dentate gyrus of adult rodents through numerous pathways**

Both known estrogen receptors (ER), subtypes ERα and ERβ, are expressed in the hippocampus, including the subgranular zone (Shughrue et al. 1997, Weiland et al. 1997, Milner et al. 2001) and can activate numerous second messenger pathways as well as stimulate gene expression (Kawata 1995, Beyer 1999 for review). Therefore, estradiol could influence a number of factors to first increase and then decrease cell proliferation within the adult rodent hippocampus. A recent study has shown that progenitor cells derived from the adult rat ventricular lining express ERβ and to a lesser extent ERα (Brännvall et al. 2002). Estradiol regulates the expression of both estrogen receptor isoforms in the hippocampus (Prange-Kiel et al. 2003 for example) and time-dependent estradiol-induced alterations in the expression of either ERα or ERβ could influence cell proliferation. Interestingly, in progenitor cells, estradiol reverses an epidermal growth factor-factor-stimulated increase in proliferation (Brännvall et al. 2002) suggesting that estradiol mediates differential effects upon adult-derived progenitor cells in vitro depending upon the presence or absence of other factors. Of course, the properties of progenitor cells derived from the ventricular subependymal and subgranular zone may differ (Seaberg & van der Kooiy 2002).

In addition to influencing cell proliferation in the adult rodent dentate gyrus (Ormerod & Galea 2001, Ormerod et al. 2003, current study), estradiol has also been shown to enhance the survival of young neurons. Estradiol enhances the survival of neurons migrating in the adult avian songbird forebrain (Burek et al. 1995) by up-regulating the production of brain-derived growth factor in endothelial cells (Loissant et al. 2003 for example) and time-dependent estradiol effects (Gould et al. 1997, 1998, Tanapat et al. 1999, Banasr et al. 2001, Ormerod et al. 2003) can all influence cell proliferation, perhaps independently, is important as most non-laboratory-reared mammals are likely to experience all these phenomena daily.
time-dependently influences cell proliferation as well as enhances the survival of young neurons could complicate the interpretation of results from studies utilizing intact female animals to investigate the effect of estradiol, or other factors, on neurogenesis.

**New neurons appear functional and influence hippocampus-dependent behavior**

Approximately 270,000 new cells are produced monthly within the dentate gyrus of adult rats and many of these cells differentiate into neurons (Cameron & McKay 2001). These new granule neurons extend axons to the CA3 region within 4–10 days after birth (Hastings & Gould 1999) and resemble mature granule neurons electrophysiologically by 4 weeks after birth (van Praag et al. 2002). Therefore, young granule neurons could contribute rapidly to the influence that the dentate gyrus has over hippocampal activity and, therefore, hippocampus-dependent behavior. In fact, research has shown that adult neurogenesis is related to some forms of hippocampus-dependent behavior (Gould et al. 1999, Shors et al. 2001, 2002). Because estradiol does not appear to alter the differentiation of new dentate neurons (Tanapat et al. 1999, Ormerod et al. 2003), estradiol-induced changes in cell proliferation likely alter the number of new granule neurons that are integrated into existing hippocampal circuitry and could influence hippocampus-dependent behavior.

**Implications**

Estradiol diversely influences neurogenesis in the adult rodent dentate gyrus by first increasing and then decreasing progenitor cell proliferation as well as by enhancing the survival of young granule neurons. Estradiol can increase cell proliferation by stimulating serotonin activity and can suppress cell proliferation by stimulating adrenal steroids (but not via an NMDAr influence). Discovering the mechanisms by which estradiol mediates its diverse effects over the production and survival of new granule neurons may facilitate our understanding of how to control the process so as to restore the neuronal loss associated with disease or trauma in the hippocampus and other areas of the adult central nervous system. Hippocampus-dependent behavior enhances the survival of young granule neurons and young granule neurons appear necessary for successful performance of some hippocampus-dependent tasks. Clearly understanding estradiol’s role over adult neurogenesis could improve neuronal replacement strategies and assist the development of strategies that manipulate new neurons in situ. This study demonstrates that estradiol influences cell proliferation in a similar manner in the dentate gyrus of species with diverse reproductive strategies and provides an insight into the mechanism of this effect.

**Funding**

This research was funded by an Alzheimer Society of Canada Operating Grant to LAMG and an NSERC Post Graduate Scholarship and Killam predoctoral fellowship to BKO. There is no conflict of interest.

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Received 17 June 2003
Accepted 12 August 2003
Made available online as an Accepted Preprint 22 August 2003