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## Review

# Estrogen and adult neurogenesis in the amygdala and hypothalamus

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### ABSTRACT

In mammals, adult neurogenesis has been extensively studied in the dentate gyrus of the hippocampus and subventricular zone. However, newly proliferated neurons have also been documented in other brain regions, including the amygdala and hypothalamus. In this review, we will examine the evidence for new neurons in the adult amygdala and hypothalamus and then discuss how environmental influences can alter cell proliferation. As some of these environmental effects may be attributed to changes in the levels of circulating hormones, we will provide evidence for estrogen-mediated cell proliferation among different species and between sexes. Finally, we will review recent data suggesting that new neurons may become functionally significant in adulthood.

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## 1. Introduction

Adult neurogenesis has been documented in many brain regions in a variety of species over the past three decades. The presence of new neurons in the adult brain was first

identified in the dentate gyrus of the hippocampus (DG) and the olfactory bulb (Kaplan and Hinds, 1977). Since this discovery, much research has focused on the DG, olfactory bulb, and subventricular zone (SVZ), the region in which progenitors originate and migrate to the olfactory bulb. Among the

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mammalian species that display adult neurogenesis in these brain areas are rats (Kaplan and Hinds, 1977), mice (Kempermann et al., 1997), hamsters (Huang et al., 1998), voles (Ormerod and Galea, 2001; Fowler et al., 2002), tree shrews (Gould et al., 1997), non-human primates (Gould et al., 1999a; Bernier et al., 2002), and even humans (Eriksson et al., 1998). The number of new neurons incorporated in the adult brain is significant; for instance, it has been estimated that adult-born neurons represent around 10–20% of the total neuronal population in the DG (Jacobs et al., 2000).

Similar to the skepticism exhibited when adult neurogenesis was first described in the 1970s, more recent reports of new neurons in brain regions other than the DG and SVZ continue to be met with debate (Kornack and Rakic, 2001; Ming and Song, 2005). However, the most common techniques used to demonstrate new neurons in the DG and SVZ have been employed in these other brain regions with success. Such techniques include colocalization of the cell proliferation marker bromodeoxyuridine (BrdU) with phenotype-specific markers, as well as identification of doublets and triplets. Doublets and triplets are groups of 2–3 cells with small nuclei that are in close proximity to or overlapping one another; this is characteristic of cells that have recently divided or are currently undergoing cell division. While some contend that BrdU may be labeling older, mature neurons undergoing DNA repair, the appearance of BrdU-labeled cells with small nuclei, grouping as doublets or triplets, and co-labeling with immature neuronal (e.g., TuJ1) or immature glial (e.g., NG2) markers indicates that the overwhelming majority of the BrdU labeling is identifying mitotically active cells. However, appropriate controls and caution in interpretation should still be employed in BrdU studies due to limitations of the method (Taupin, 2007). Using these techniques, new neurons have been found in the amygdala and hypothalamus of voles (Fowler et al., 2002), hypothalamus of hamsters and mice (Huang et al., 1998; Kokoeva et al., 2005), amygdala and neocortex of non-human primates (Gould et al., 1999b; Bernier et al., 2002), and amygdala, hypothalamus, striatum and thalamus of rats (Pencea et al., 2001; Keihoff et al., 2006). Furthermore, in humans, multipotent neural precursors have been isolated from the amygdala, temporal and frontal cortex, and subcortical white matter, as well as the hippocampus and ventricular zone (Arsenijevic et al., 2001). Although some reports identify altered cell proliferation in these brain regions following pathological stimulation, such as ischemia, seizures or neurological disease states (Ming and Song, 2005), much of the aforementioned research documents newly proliferated neurons in control subjects and under normal physiological conditions.

While many new neurons are added to the brain during adulthood, the importance of these neurons remains questionable unless their integration within the neural network and involvement in the animal's behavior and/or physiology can be demonstrated. New neurons in the adult brain have been shown to exhibit properties of mature neurons, including the expression of mature neuronal markers (Gould et al., 1999b; Fowler et al., 2002) and phenotype-specific neurochemicals (Benraiss et al., 2001; Kokoeva et al., 2005). In the adult hippocampus, new neurons have also been described as having axons, vesicles and synapses (Kaplan, 2001) and displaying evoked electrical propagation with synaptic transmission (Song et al., 2002b).

Behaviorally, drastically minimizing the proliferation of new cells with systemic or central administration of anti-mitotic drugs results in deficits in the animal's ability to perform tasks dependent on certain mitotic brain regions (Shors et al., 2001; Kokoeva et al., 2005).

In the current paper, we will review recent developments in the field of adult mammalian neurogenesis by focusing on the amygdala and hypothalamus – two brain regions important for social and reproductive behaviors. First, we will examine the evidence for new neuronal proliferation in these two brain regions. We will then focus on the effects of social environment in regulating neurogenesis. Since social environment may alter hormonal levels which, in turn, may underlie the changes in cell proliferation, we will examine the potential involvement of estrogen and other gonadal steroid hormones in mediating neuronal proliferation. Finally, we will conclude with a discussion of the putative functional significance of these new cells and importance within the neural network.

## 2. Adult neurogenesis in the amygdala and hypothalamus

The amygdala has been implicated in a variety of social and reproductive-associated behaviors, including olfactory and pheromonal processing (Meredith, 1991), copulatory actions (Harris and Sachs, 1975), aggression (Wang et al., 1997), and social learning and memory (Kirkpatrick et al., 1994; Cahill et al., 1996). In addition to its well-known functions in homeostasis, endocrine regulation, and reproductive behaviors (Brooks, 1988), the hypothalamus also appears to be involved in pheromonal processing (Dudley et al., 1996) and social affiliation (Albert and Walsh, 1984; Gobrogge et al., 2007). Given their similarity in function, it is not surprising that the amygdala and hypothalamus are interconnected (Aizawa et al., 2004).

Among the first reports of neurogenesis in the adult amygdala, Bernier and colleagues (2002) identified new cells in the rostral temporal lobe of adult New World (*Saimiri sciureus*) and Old World (*Macaca fascicularis*) monkeys. BrdU-labeled cells were documented in the amygdala, piriform cortex, and adjoining inferior temporal cortex. Many of these new cells expressed the immature neuronal marker, TuJ1, or the mature neuronal markers, NeuN and MAP-2. Further reports have also demonstrated amygdaloid neurogenesis in brain injury models. For example, in adult rats, seizures induced by  $\gamma$ -aminobutyric acid (GABA)-A receptor antagonism result in cell death, as well as increased neurogenesis, in the amygdala (Park et al., 2006), and removal of the olfactory bulbs increases the number of new cells in the basolateral amygdala (Keihoff et al., 2006).

In the hypothalamus, the presence of adult neuronal proliferation has been examined in several rodent species. In hamsters, photoperiod appears to influence new cell number; animals housed in a short-day photoperiod exhibit more BrdU-labeled cells than those housed in a long-day photoperiod (Huang et al., 1998). In rats, intraventricular administration of brain-derived neurotrophic factor (BDNF) increases the number of new neurons in the hypothalamus (Pencea et al., 2001). More recently, Kokoeva et al. (2005) have found that administration of ciliary neurotrophic factor (CNTF) enhances cell proliferation in the arcuate, ventromedial and dorsomedial nuclei of the

hypothalamus. These cells express neuronal markers, as well as markers found in other mature hypothalamic neurons, such as neuropeptide Y (NPY), phosphorylated signal transducer and activator of transcription (pSTAT3), and pro-opiomelanocortin (POMC). The new neurons appeared to have complex morphologies with many arborized projections or a single process extending from the soma. In addition to its effects on neurogenesis, CNTF induced an expected decrease in body weight. Interestingly, coadministration of CNTF with the anti-mitotic drug Ara-C centrally into the lateral ventricles eliminated the proliferation of cells in the hypothalamus and prevented the long-term effects on body weight, suggesting a potential role of these new hypothalamic neurons in homeostatic function. It is important to note that for both of these studies with neurotrophic factors, the control groups receiving vehicle or saline infusions also exhibited BrdU-labeled cells in the hypothalamus, indicating that cell proliferation still occurred under the comparative "normal" conditions.

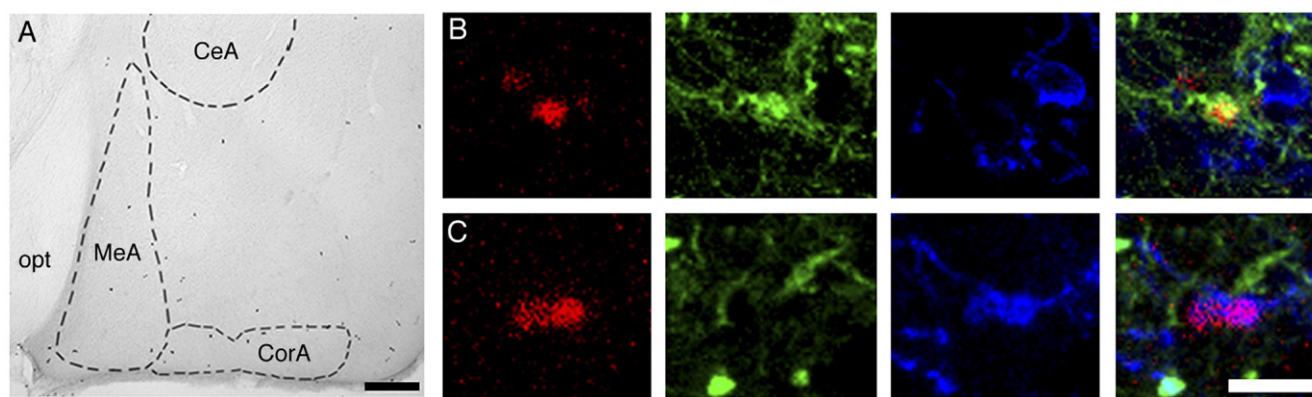
In our studies (Fowler et al., 2002, 2003, 2005), new neurons have been found in the cortical, medial and central nuclei of the amygdala and in the arcuate and ventromedial nuclei of the hypothalamus in prairie (*Microtus ochrogaster*) and meadow (*Microtus pennsylvanicus*) voles under normal physiological conditions, as well as with hormonal treatments. In addition, BrdU cells co-label with the neuronal markers TuJ1, NeuN or MAP-2, indicating neuronal phenotypes (Fig. 1). We also provide evidence that these new cells appear to proliferate locally within the amygdala (Fowler et al., 2003); BrdU cells co-labeled with the early neuronal (TuJ1) or glial (NG2) markers can be identified in the amygdala and hypothalamus as early as 30 min following an acute intraperitoneal injection of BrdU. In referring to the previously established rate of cellular migration in the adult brain (Luskin and Boone, 1994), it is most probable that these neuronal and glial progenitors divided locally. This presence of TuJ1 in proliferating cells is consistent with other evidence that neuron-restricted progenitor cells can express cell-type-specific markers, such as TuJ1 and doublecortin, continue to undergo self-renewal, and can differentiate into multiple neuronal phenotypes (Mayer-Proschel et al., 1997; Memberg and Hall, 1994; Brown et al., 2003; Mo et al., 2007).

The DG does contain a larger number of proliferating cells than the amygdala and hypothalamus, which may be why it has been more accepted as a mitotically active region in adulthood. As a comparison, the DG contains approximately 2.6 times more cells than the amygdala and approximately 136 times more cells than the ventromedial hypothalamus in prairie voles (Fowler et al., 2005). However, the number of cells in each brain area may not be as important as the function of each individual new cell within the neural network.

### 3. Effects of social environment

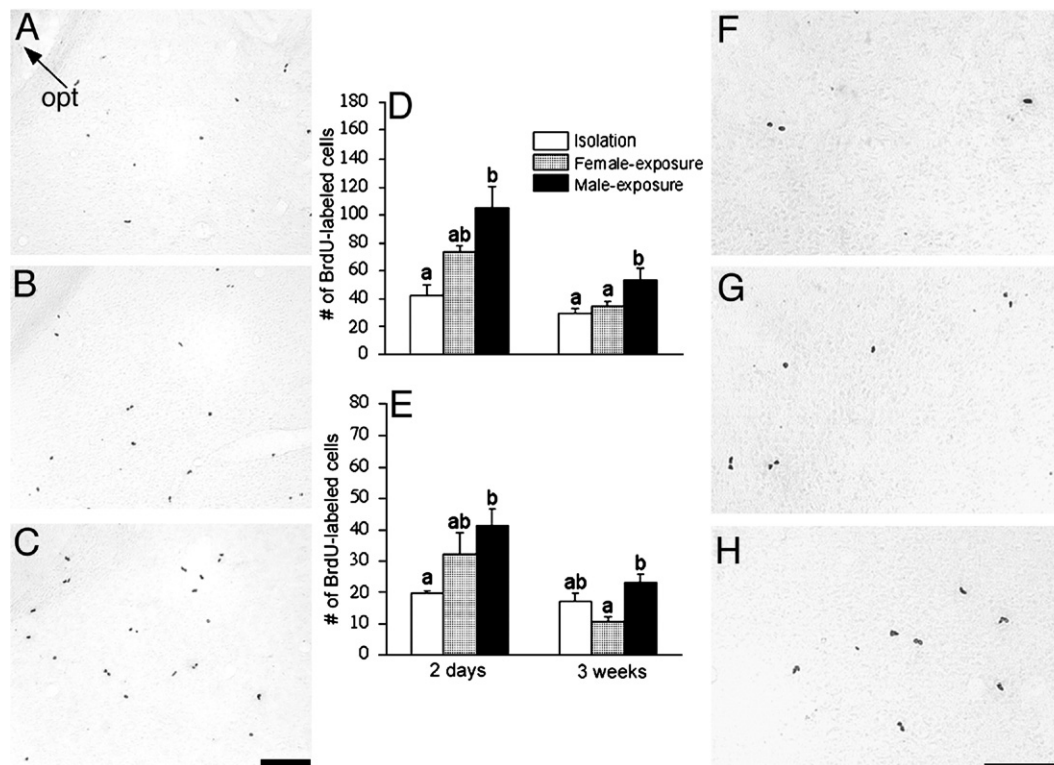
It has been well documented that the external environment influences adult neurogenesis. In an early study, Gerd Kempermann et al. (1997) found that mice housed in an enriched environment displayed enhanced survival, but not proliferation, of new neurons in the DG. The enriched animals also performed better on a spatial learning task than did controls, elucidating the possible functional significance of these cells. Using similar housing conditions, these findings were then replicated in rats (Nilsson et al., 1999). In a subsequent study, mice given access to only a running wheel exhibited similar levels of neurogenesis in the DG as those in the enriched environment, suggesting that voluntary running wheel activity contributed to the enhanced neurogenesis (van Praag et al., 1999).

Social interaction with conspecifics significantly influences adult neurogenesis as well. These findings have been best demonstrated in voles. The female prairie vole is highly social, can be induced into behavioral estrus by male exposure, and forms selective social attachment after mating (Carter and Getz, 1993; DeVries et al., 1996; Wang et al., 1998). Therefore, the vole model provides an excellent opportunity to study the effects of environmental and endocrine changes on physiology and behavior. In our study (Fowler et al., 2002), female prairie voles that were exposed to a male for 48 h with mating had more BrdU-labeled cells in the amygdala and hypothalamus than did females housed in social isolation (Fig. 2). The difference within the amygdala persisted 3 weeks later, indicating positive effects on both the proliferation and survival of the new cells. The



**Fig. 1** – [A] Photomicrograph of newly proliferated cells in the medial (MeA) and cortical (CorA) nuclei of the amygdala in the vole brain. CeA: central nucleus of the amygdala, opt: optic tract. Scale bars = 100  $\mu\text{m}$ . [B & C] Confocal laser microscope images of cells stained for BrdU (red), TuJ1 (green), NG2 (blue), and all three markers in the amygdala. BrdU and TuJ1 colocalized cells display a yellow image [B], and BrdU and NG2 colocalized cells display a purple image [C]. Scale bar = 10  $\mu\text{m}$ .





**Fig. 2** – Photomicrographs of BrdU-labeled cells in the amygdala [A–C] and hypothalamus [F–H]. Female prairie voles were isolated [A & F], exposed to a female [B & G], or mated and exposed to a male [C & H] for 2 days. Scale bars = 100  $\mu$ m. opt: optic tract. In the amygdala [D], 2 days of the male-exposure increased the number of BrdU-labeled cells and this effect was sustained 3 weeks later. A similar effect was also found in the hypothalamus [E]. Cell counts are presented as number of cells per section, and brain sections were anatomically matched across subjects. Letters represent the results of the post hoc test; bars with non-shared letters differed statistically from each other at  $p < 0.05$ . Error bars indicate standard error of the mean.

influence on cell proliferation was site specific, as group differences were not found in the DG or caudate/putamen. In a separate study, 48 h of mating significantly increased the number of new cells in the SVZ in female prairie voles (Smith et al., 2001). Together, these data suggest that experience with a male enhances the proliferation and/or survival of new neurons in a site-specific manner in the brain of adult female prairie voles.

Since the amygdala receives direct input from the olfactory bulb (Meredith, 1991), pheromonal and/or olfactory input may have contributed to cell proliferation within the amygdala. Indeed, in female prairie voles, exposure to male bedding alone for 48 h increased the density of BrdU-labeled cells in the amygdala, particularly in the medial and cortical nuclei, in comparison to control animals exposed to their own bedding (Liu et al., 2001a). Such effects were not found in other brain areas or when females were exposed to the bedding from other conspecific females. In addition, no such effect was found in males (Liu et al., 2007). These data indicate a stimulus-, brain region- and sex-specific effect of chemosensory cues on cell proliferation in female prairie voles.

#### 4. Effects of gonadal steroid hormones

The structure and function of certain brain areas depend on the levels of circulating hormones during adulthood (Garcia-

Segura et al., 1994; McEwen and Alves, 1999); therefore, the influence of hormones on adult neurogenesis has become a fascinating research area. In addition to regulating reproductive and other behaviors, estrogen has been implicated in brain development, neuroprotection, and cognition (Dohler et al., 1983; McEwen et al., 1997). On the cellular level, estrogen plays a role in the proliferation (Fowler et al., 2005), survival (Leranth et al., 2000) and activation (Insel, 1990) of neurons.

The effects of estrogen on adult neurogenesis were first examined in the DG and SVZ. Fluctuations in cell proliferation across the estrus cycle have been shown in the DG of the adult female rat; the number of new cells is highest during proestrus, and ovariectomy decreases, whereas estrogen replacement restores, new cell number relative to intact controls (Tanapat et al., 1999). In the DG of female meadow voles, exposure to an acute treatment of estradiol produces a transient increase, followed by a decrease, in the number of new neurons (Ormerod and Galea, 2001). Female voles are induced ovulators and display an elevated level of estrogen following exposure to a conspecific male (Dluzen and Carter, 1979; Seabloom, 1985). In female prairie voles (Smith et al., 2001), male exposure induces an increase in the number of BrdU-labeled cells in the anterior division of the SVZ. This effect can be prevented by ovariectomy and reinstated with estradiol treatment, indicating that the effects of male exposure/mating on cell proliferation are, in part, attributable to circulating levels of estrogen.

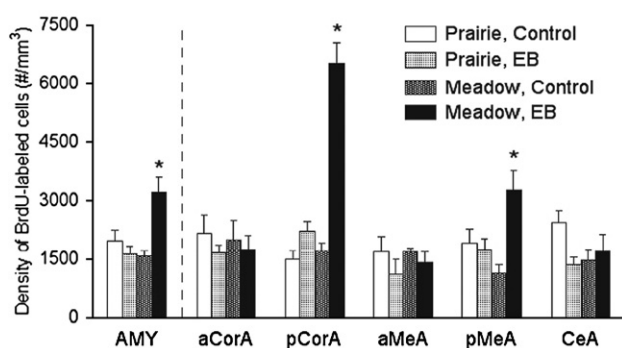
The amygdala and hypothalamus of many species, including rats (Pfaff and Keiner, 1973), hamsters (Wood et al., 1992) and voles (Hnatzuk et al., 1994), contain an abundance of estrogen receptors and are responsive to gonadal hormones (Insel, 1990; Coolen and Wood, 1999). *In vitro*, estrogen has been shown to regulate the proliferation and/or survival of new neurons in the fetal rat amygdala and hypothalamus (Arimatsu and Hatanaka, 1986; Chowen et al., 1992). As male exposure elevates serum estrogen in female voles, we tested the hypotheses that estrogen up-regulates cell proliferation in the amygdala and hypothalamus in female prairie voles and that responsiveness to estrogen varies between species with different life strategies.

Adult female prairie and meadow voles were ovariectomized and then treated with saline vehicle or estradiol, which produced species-specific effects (Fowler et al., 2005) (Fig. 3). Within the amygdala, estradiol treatment significantly increased the density of new neurons in the posterior cortical and posterior medial nuclei of the amygdala in meadow, but not prairie, voles. These differences in cell proliferation were site-specific, as treatment effects were not found in the DG or ventromedial hypothalamus (VMH) (Fowler et al., 2005). The ability of estradiol to differentially influence cell proliferation in meadow, but not prairie, voles could be partially explained by species differences in regional densities of estrogen receptor alpha (ER $\alpha$ ) (Fig. 4). Female meadow voles displayed higher densities of ER $\alpha$ -labeled cells in the posterior cortical amygdala than did female prairie voles. Interestingly, a high percentage of BrdU-labeled cells in both species coexpressed ER $\alpha$  (Fig. 4G), indicating that estrogen may exert a direct effect on cell proliferation. This conclusion appears to be further supported by data from other labs. ER $\alpha$  and ER $\beta$  have been localized on neural stem cells in embryonic and adult rats (Brannvall et al., 2002), and the estrogen receptor antagonist ICI 182,780 can partially block the estradiol-induced enhancement of cell

proliferation in the adult female rat DG (Nagy et al., 2006). This still leaves the question of why male-exposure and mating increased cell proliferation in the amygdala of female prairie voles (Fowler et al., 2002), but estrogen administration alone did not exert a similar effect (Fowler et al., 2005). Several other factors could be responsible, including chemosensory cues (as mentioned above), social interaction, and other hormones such as prolactin. Alternatively, estradiol was administered via implanted pellet in our study (Fowler et al., 2005), so the natural temporal pattern of estrogen release with male-induced estrus may have been important.

Much less work has been done to investigate the effects of hormonal status on neurogenesis in adult males. In the DG, estradiol treatment enhances the survival of new neurons in castrated male meadow voles (Ormerod et al., 2004). Interestingly, these effects of estradiol on neuronal survival appear to only occur during a period coincident with axonal extension, suggesting a hormonal influence on the process of cellular maturation. In castrated male hamsters, testosterone administration increases the volume of the medial amygdala (Cooke et al., 2002), a finding which could not be attributed to changes in soma size and suggesting the possibility of increased neurogenesis in response to testosterone. Since testosterone may be converted to dihydrotestosterone (DHT) or estrogen by aromatase, an enzyme that is found in high levels in the amygdala (Roselli et al., 1984), this finding may be due to either androgenic or estrogenic influences.

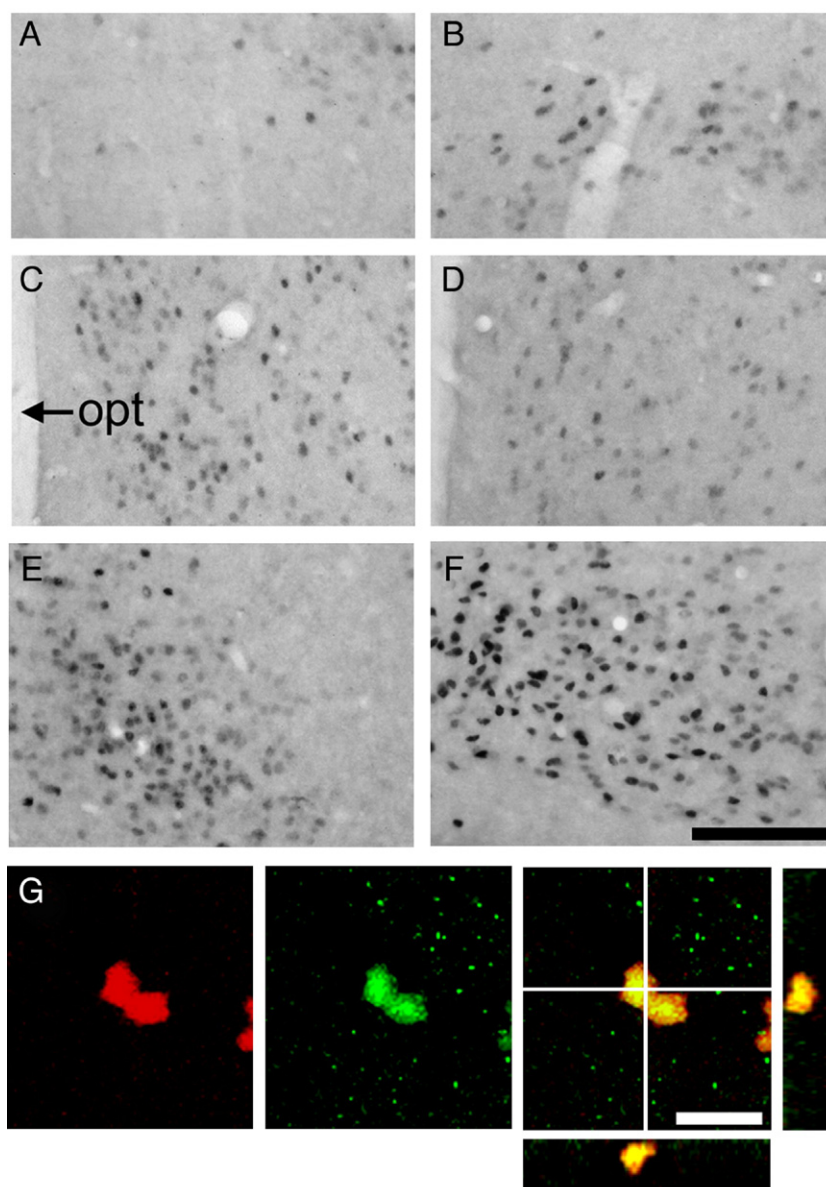
We performed a study in which castrated male meadow voles were treated with oil (control), testosterone, estradiol, or DHT (Fowler et al., 2003). Testosterone treatment increased the number of proliferating neurons in the cortical and medial nuclei of the amygdala, but not in the DG or VMH (Fig. 5). Treatment with estradiol had a similar effect, whereas DHT was ineffective. These data indicate that gonadal steroid hormones most likely act through an estrogenic-mediated mechanism to regulate adult neurogenesis in the amygdala of male meadow voles. Given the facts that new cells can be identified in the amygdala as early as 30 min following an acute BrdU injection (Fowler et al., 2003), proliferating cells contain estrogen receptors in the amygdala (Fowler et al., 2005), and a high level of aromatase is present within this brain region (Roselli et al., 1984), we may infer that the effects of both estrogen and testosterone on cell proliferation occurred locally within the amygdala.



**Fig. 3 – Species–treatment interactions in the density of BrdU-labeled cells in the amygdala (AMY) of voles. In the posterior cortical (pCorA) and medial (pMeA) nuclei of the amygdala, EB treatment elicited a significant increase in the density of BrdU-labeled cells only in meadow voles. No species–treatment interactions were found in the anterior cortical (aCorA), anterior medial (aMeA), or central (CeA) nuclei of the amygdala. Cell counts were obtained using stereological methods and represent density counts for the entire brain area (mm<sup>3</sup>). \* $p < 0.05$ ; error bars indicate standard error of the mean.**

## 5. Effects of neurotransmitters and growth factors

Estrogen may also influence neurotransmitter and/or growth factor systems to mediate adult neurogenesis. For example, estrogen increases serotonin receptor mRNA expression in the brain (Zhou et al., 2002; Shingo and Kito, 2003). In the DG, an increase in serotonin or chronic antidepressant treatment enhances cell proliferation, whereas depletion of serotonin reduces cell proliferation (Brezun and Daszuta, 2000; Jacobs et al., 2000; Malberg et al., 2000), and a serotonin antagonist can block the estradiol-induced enhancement of cell proliferation (Banar et al., 2001). BDNF-positive cells are found in many mitotic brain areas (Castren et al., 1995; Conner et al., 1997), and in



**Fig. 4** – [A–F] Photomicrographs displaying ER $\alpha$ -labeling in the amygdala and ventromedial hypothalamus (VMH) in prairie (left) and meadow (right) voles. In the posterior cortical nucleus of the amygdala [pCorA; A & B] and VMH [E & F], meadow voles [B & F] had more ER $\alpha$ -labeled cells than did the prairie voles [A & E]. However, no species differences were found in the posterior medial nucleus of the amygdala [pMeA; C & D]. opt: optic tract. Scale bar=200  $\mu$ m. [G] Confocal laser microscope images display labeling for BrdU (red), ER $\alpha$  (green), and both markers in the pCorA in voles. In the right panels, the BrdU and ER $\alpha$  colocalized cells display a yellow image, and cross marks on the larger image indicate the location of views along the y–z axis (right) and x–z axis (below) to demonstrate 3D colocalization of BrdU and ER $\alpha$ . Scale bar=5  $\mu$ m.

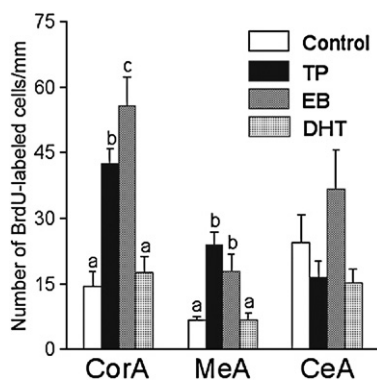
the prairie vole brain, BDNF mRNA and protein are expressed in the amygdala and hypothalamus (Liu et al., 2001b). *In vitro*, BDNF enhances the number and survival of new neurons derived from the SVZ (Goldman, 1998), and *in vivo*, BDNF infusions into the ventricles increase the number of new cells in several brain areas, including the olfactory bulb, striatum, and hypothalamus of rats (Zigova et al., 1998; Pencea et al., 2001). It has also been reported that astrocytes may secrete BDNF and induce neurogenesis in adult neural stem cells (Ikeda et al., 2001; Song et al., 2002a). Furthermore, endothelial cells can secrete BDNF and clusters of proliferating cells are found around vasculature (Leventhal et al., 1999; Palmer et al., 2000). Finally, BDNF also increases serotonin

activity (Siuciak et al., 1996), and alternatively, serotonin reuptake inhibitors increase BDNF expression (Duman et al., 1997) in the rat, suggesting that BDNF and serotonin may act synergistically to regulate cellular proliferation in the adult brain.

## 6. Functional significance of the new cells

The amygdala, hypothalamus and olfactory bulb have been implicated in olfactory/pheromonal processing (Meredith, 1991; Dudley et al., 1996), social learning and memory (Albert and Walsh, 1984; Kirkpatrick et al., 1994; Cahill et al., 1996;





**Fig. 5 – The effects of testosterone propionate (TP), estrogen benzoate (EB), or 5 $\alpha$ -dihydrotestosterone (DHT) on the density of BrdU-labeled cells in the brain of male meadow voles. TP or EB treatment induced an increase in the density of BrdU-labeled cells in the cortical (CorA) and medial (MeA), but not central (CeA), nuclei of the amygdala. Cell counts represent the number of cells per mm<sup>2</sup>. Letters represent the results of the post hoc test; bars with non-shared letters differed statistically from each other at  $p < 0.05$ . Error bars indicate standard error of the mean.**

Brennan and Keverne, 1997), and sexual and social behaviors (Harris and Sachs, 1975; Brooks, 1988; Williams et al., 1992), while the DG plays an important role in spatial learning and memory (Shors et al., 2001). Since new cells are being incorporated into these areas during adulthood, one is led to question the functional significance of the adult-born neurons. An early study demonstrated that cells produced in adulthood exhibit properties of functional neurons (Kaplan, 2001) and *in vitro* cultures from adult songbirds show that new neurons can become synaptically competent and develop stimulus-evoked and spontaneous action potentials (Goldman and Nedergaard, 1992). More recent *in vitro* studies have also shown that new cells from the adult rat hippocampus can become electrically active neurons and exhibit functional synaptic transmission (Song et al., 2002b). It remains to be determined whether new neurons in the amygdala and hypothalamus exhibit these physiological and synaptic properties of mature neurons, and until this is demonstrated, one may question whether these neurons become functionally competent. However, given the evidence from other brain areas, we have no reason to suspect that these new cells in the amygdala and hypothalamus do not mature in a similar manner.

The ability of the new neurons to contribute to adult neural processing has also been demonstrated *in vivo*. In mice and golden hamsters, new cells in the olfactory bulb may become activated following odor exposure (Carlen et al., 2002; Huang and Bittman, 2002). In addition, adult mice that have deficits in the migration of olfactory bulb neuronal precursors display impaired discrimination between odors (Gheusi et al., 2000), and an increase in the number of olfactory bulb neurons following odor enrichment is associated with enhanced short-term odor memory (Rochefort et al., 2002). A few studies have begun to elucidate the functional significance of estrogen-mediated neurogenesis as well. ER $\alpha$  and ER $\beta$  receptor knockout mice display deficiencies in social recognition (Choleris et al., 2003), possibly due to dysfunction

in the amygdala. In male meadow voles, the estradiol-induced survival of new DG neurons correlates with enhanced spatial memory (Ormerod et al., 2004). Finally, treatment with an anti-mitotic drug prevents adult cell proliferation in the hypothalamus of mice and results in deficits in body weight regulation (Kokoeva et al., 2005). Similar anti-mitotic drug treatment also induces deficits in hippocampal-dependent memory formation in rats; importantly, after recovery from the drug treatment, new neurons can be produced, and trace memory acquisition is restored (Shors et al., 2001). In our pilot experiment in prairie voles, treatment with an anti-mitotic drug resulted in a reduction in the number of BrdU-labeled cells in the amygdala and an inhibition of mating-induced pair bonding, indicating a correlation between new cells in the amygdala and social behavior (C.D. Fowler, unpublished observation). One caveat of all the aforementioned studies using the anti-mitotic drug is that the Ara-C was administered systemically or centrally. Although the resulting effects of administration have been documented to involve the brain regions examined (e.g., body weight regulation involves the hypothalamus and pair bonding involves the amygdala), site specificity needs to be addressed in further studies.

## 7. Conclusions

In summary, neurogenesis in the adult brain has been identified in many mammalian species. The rate of proliferation and the fate of new neurons may be influenced by a variety of factors. Aspects of an animal's external environment may induce changes in its internal physiology, which, in turn, can act on neurochemical and/or neurotransmitter systems to affect cellular proliferation and/or survival. Although many studies have characterized the factors that increase or decrease new cell numbers, the cellular mechanisms that directly act on the proliferation and/or survival have yet to be fully elucidated. Furthermore, several studies have established relationships between the presence of newly proliferated cells and behavioral/cognitive functions, but more research needs to be done to determine the exact contribution of these new neurons.

## Acknowledgments

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