Neuroscience 268 (2014) 10–20

SOCIAL REGULATION OF ADULT NEUROGENESIS IN A EUSOCIAL MAMMAL

D. E. PERAGINE,[a]* J. A. SIMPSON,[a] S. J. MOONEY,[a] M. B. LOVERN[b] AND M. M. HOLMES[a,c]

[a] *Department of Psychology, University of Toronto Mississauga, Mississauga, Ontario L5L 1C6, Canada*

[b] *Department of Zoology, Oklahoma State University, Stillwater, OK, USA*

[c] *Department of Cell and Systems Biology, University of Toronto, Toronto, Ontario M5S 3G5, Canada*

Abstract—The present study examined the effects of social status on adult neurogenesis in an extreme cooperative breeder: the naked mole rat. These animals live in large colonies of up to 300 individuals, with a strict reproductive dominance hierarchy; one female and one to three males breed, and all other members are socially subordinate and reproductively suppressed. We examined the effects of social and gonadal cues on doublecortin (DCX; a marker for immature neurons) immunoreactivity in the dentate gyrus (DG), piriform cortex (PCx) and basolateral amygdala (BLA) by comparing dominant breeding animals to non-breeding subordinates from intact colonies. We also examined DCX expression in subordinate animals that had been removed from their colony and paired with an opposite- or same-sex conspecific for 6 months. Compared to subordinates, dominant breeders had significantly reduced DCX immunoreactivity in all brain areas, with BLA effects confined to females. By contrast, the effects of same- versus opposite-sex housing were region-specific. In the DG and PCx, more DCX immunoreactivity was observed for opposite- than same-sex-paired subordinates. Conversely, same-sex-paired females had more DCX immunoreactivity than opposite-sex-paired females in the BLA. Gonadectomy did not affect DCX expression in opposite-sex-paired animals, and no significant relationships between gonadal steroids and DCX immunoreactivity were detected, suggesting that group differences in neurogenesis are independent of gonadal hormones. The apparent lower neurogenic capacity displayed by breeders contrasts previous reports on neurogenesis and social rank, challenging the conventional view that subordination is stressful and impairs neurogenesis. Future work will clarify whether the present findings can be attributed to status-dependent differences in stress, behavioral plasticity, or life stage.

Key words: basolateral amygdala, doublecortin, eusocial, hippocampus, neurogenesis, piriform cortex.

INTRODUCTION

Social interactions can have striking effects on physiology, behavior, and brain plasticity, including regulation of adult neurogenesis (Lieberwirth and Wang, 2012). Adult neurogenesis is the birth of new neurons in the mature brain and this process occurs predominantly in the dentate gyrus (DG) of the hippocampus and subventricular zone (SVZ) of the forebrain in mammals. Neurons born within these regions integrate into hippocampal and olfactory circuits, respectively, resulting in enhanced synaptic plasticity (Nissant et al., 2009), increased responsiveness to stimuli (Magavi et al., 2005), and improved learning and memory processes (Shors et al., 2001; Rochefort et al., 2002; Snyder et al., 2005; Winocur et al., 2006; Deng et al., 2009; Sultan et al., 2010). Pioneering work on adult neurogenesis centered on song learning in birds (Goldman and Nottebohm, 1983; Paton and Nottebohm, 1984), suggesting a role for adult-born neurons in the support of socially relevant behaviors. Despite this connection, relatively sparse research has addressed the functional and regulatory relationships between adult neurogenesis and social behavior.

Research conducted to date on neurogenesis and social variables has been dominated by two approaches. One line of research highlights how distinct aspects of social life (e.g., sexual encounters, gestation, parenthood, and chemosensory cues conveying the sex, status, and health of conspecifics) regulate the birth, fate, and integration of adult-born neurons. For example, mating encounters or exposure to opposite-sex chemosensory cues up-regulate cell proliferation in the rodent DG and SVZ (Smith et al., 2001; Larsen et al., 2008; Leuner et al., 2010), as well as the survival and differentiation of newborn neurons in the DG and olfactory bulb (OB) (Baudoin et al., 2005; Mak et al., 2007; Larsen et al., 2008; Oboti et al., 2009; Corona et al., 2011). Conversely, aversive interactions with dominant and aggressive conspecifics reduce cell proliferation and survival in the DG, suggesting that

*Corresponding author. Address: University of Toronto Mississauga, 3359 Mississauga Road North, Mississauga, Ontario L5L 1C6, Canada. Tel: +1-905-828 3874.
E-mail address: d.peragine@utoronto.ca (D. E. Peragine).

Abbreviations: ANOVA, analysis of variance; ANCOVA, analysis of covariance; BLA, basolateral amygdala; DAB, diaminobenzidine; DCX, doublecortin; DG, dentate gyrus; E2, 17β-estradiol; LD, light/dark; OB, olfactory bulb; PBS, phosphate-buffered saline; PCx, piriform cortex; P, progesterone; RT, room temperature; T, testosterone; SVZ, subventricular zone.

http://dx.doi.org/10.1016/j.neuroscience.2014.02.044

hippocampal neurogenesis is sensitive to status and social threat cues (Gould et al., 1997, 1998; Czéh et al., 2001; Van der Hart et al., 2002; Kozorovitskiy and Gould, 2004; Simon et al., 2005; Mitra et al., 2006; Yap et al., 2006; Czéh et al., 2007; Thomas et al., 2007; van Bokhoven et al., 2011). A second approach highlights the functional role of adult neurogenesis in social processing and responding. Disrupting neurogenesis within the DG inhibits stress-induced social avoidance (LaGace et al., 2010), while disrupting SVZ/OB neurogenesis impairs the detection and discrimination of opposite-sex odors (Feierstein et al., 2010), and the ability of female mice to discriminate between mating partners and unfamiliar males (Oboti et al., 2011) or between dominant and subordinate mating candidates (Mak et al., 2007).

Although most studies of olfactory neurogenesis have focused on the SVZ/OB, there is accumulating evidence that SVZ-derived neuroblasts migrate to brain regions other than the OB. These include the piriform cortex (PCx; Bayer, 1986; Bernier et al., 2002; Shapiro et al., 2007a; Klempin et al., 2011), neocortex (Dayer et al., 2005), amygdala (Bernier et al., 2002; Fowler et al., 2002), and striatum (Bedard et al., 2006). Indeed, neurons are added to the PCx and basolateral amygdala (BLA) at a rate that is concomitant with the production of OB neurons (Bernier et al., 2002). The PCx receives input from the OB and sends efferents to the hippocampus, thereby serving as a relay station for the two canonical neurogenic regions of the adult rodent brain. The survival and differentiation of new PCx neurons is up-regulated by olfactory enrichment (Shapiro et al., 2007b). Furthermore, the BLA has been shown to regulate hippocampal neurogenesis and fear-related activation of newborn neurons (Kirby et al., 2011). Collectively, these findings suggest that new neurons in the PCx and BLA contribute to the stability and plasticity of the limbic system, making them putative targets for social manipulations.

The naked mole-rat (*Heterocephalus glaber*) is an ideal model system for studying the neurobiology of social behavior. They exhibit the most striking example of eusociality among mammals, living in large subterranean colonies characterized by a rigid behavioral and reproductive hierarchy. Colonies typically range in size from 60 to 80 individuals where only a single dominant female (the queen) and one to three dominant males reproduce (Jarvis, 1981). All other colony members are socially subordinate and kept sexually suppressed by the queen (Faulkes et al., 1990). Though less than 1% of subordinate naked mole-rats ever attain breeding status (Jarvis et al., 1994), subordinates that are removed from their natal colonies and paired with an opposite-sex conspecific will often become breeders and start their own colony. Within a week of pairing, subordinates show an increase in gonadal hormones, though pups are usually not produced for several months (Faulkes and Abbott, 1993; Henry et al., 2007; Holmes et al., 2009). Likewise, colony-housed subordinates may become breeders if sexual suppression is lifted by a former breeder's death or removal (Margulis et al., 1995; Clarke and Faulkes, 1997). Though pronounced alterations in neural and endocrine functions accompany the change (Faulkes et al., 1990; Margulis et al., 1995; Clarke and Faulkes, 1997; Seney et al., 2006; Holmes et al., 2008; Holmes et al., 2011; Mooney and Holmes, 2013), many or all subordinates are capable of transitioning to breeding status under the appropriate social conditions. This striking behavioral and physiological plasticity displayed by otherwise mature subordinates makes the naked mole-rat a prime candidate for the study of adult neurogenesis. Therefore, we evaluated neurogenesis in several brain regions of dominant and subordinate naked mole-rats, as well as individuals that had been removed from their colony and given the opportunity to transition social status.

EXPERIMENTAL PROCEDURES

Animals and experimental design

Naked mole-rat colonies were maintained at the University of Toronto Mississauga in polycarbonate tubs of varying sizes (small: 30 cm × 20 cm × 13 cm, medium: 48 cm × 27 cm × 16 cm; large: 65 cm × 45 cm × 23 cm) containing corncob bedding and connected by lengths of acrylic tubing. Animals were fed *ad libitum* on a diet consisting of sweet potato and 19% protein mash, and colonies were maintained on a 12:12 light/dark (LD) cycle in a temperature and humidity controlled room (28–30 °C/50% RH). All animal procedures were approved by the University of Toronto Animal Care Committee and conducted in accordance with federal and institutional guidelines.

Naked mole-rats used in this study were also from a previous report (Mooney and Holmes, 2013). Subjects were dominant breeding adults between 3 and 9 years of age, weighing 36–79 g, and subordinate adults between 2 and 3 years of age, weighing 33–84 g. As we make every effort to match experimental groups within and between colonies, and because breeders are typically the parents of all subordinates within a colony, breeders are consistently older than subordinates. However, naked mole-rats typically reach adult body size within one year, can live for over 30 years in captivity, and do not show signs of aging well into their third decade (O'Riain and Jarvis, 1998; Buffenstein, 2005), thus these experimental animals were all young-aged adults. Subordinates were randomly assigned to four experimental groups: (1) Gonadally intact and colony-housed (SUB; $n = 13$; 8 male, 5 female), (2) gonadally intact and pair-housed with a same-sex unfamiliar subordinate (SS; $n = 14$; 8 male, 6 female), (3) gonadally intact and pair-housed with an opposite-sex unfamiliar subordinate (OS; $n = 13$; 7 male, 6 female), and (4) gonadectomized and pair-housed with an opposite-sex unfamiliar subordinate (GDX; $n = 14$; 7 male, 7 female). All Breeders ($n = 9$; 4 male, 5 female) were gonadally intact, and remained in their natal colonies for the duration of the experiment. Pair-housed animals were maintained in-colony prior to the experiment's initiation, at which time GDX animals were removed for surgery (see Mooney and Holmes, 2013)