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SEXUAL INTERACTIONS WITH UNFAMILIAR FEMALES REDUCE HIPPOCAMPAL NEUROGENESIS AMONG ADULT MALE RATS

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Abstract—Recent experiments have shown that sexual interactions prior to cell proliferation cause an increase in neurogenesis in adult male rats. Because adult neurogenesis is critical for some forms of memory, we hypothesized that sexually induced changes in neurogenesis may be involved in mate recognition. Sexually naive adult male rats were either exposed repeatedly to the same sexual partner (familiar group) or to a series of novel sexual partners (unfamiliar group), while control males never engaged in sexual interactions. Ovariectomized female rats were induced into estrus every four days. Males were given two injections of 5-bromo-2′-deoxyuridine (BrdU) (200 mg/kg) to label proliferating cells, and the first sexual interactions occurred three days later. Males in the familiar and unfamiliar groups engaged in four, 30-min sexual interactions at four-day intervals, and brain tissue was collected the day after the last sexual interaction. Immunohistochemistry followed by microscopy was used to quantify BrdU-labeled cells. Sexual interactions with unfamiliar females caused a significant reduction in neurogenesis in the dentate gyrus compared to males that interacted with familiar females and compared to the control group. The familiar group showed no difference in neurogenesis compared to the control group. Males in the familiar group engaged in significantly more sexual behavior (ejaculations and intromissions) than did males in the unfamiliar group, suggesting that level of sexual activity may influence neurogenesis levels. In a second experiment, we tested whether this effect was unique to sexual interactions by replicating the entire procedure using anestrus females. We found that interactions with unfamiliar anestrus females reduced neurogenesis relative to the other groups, but this effect was not statistically significant. In combination, these results indicate that interactions with unfamiliar females reduce adult neurogenesis and the effect is stronger for sexual interactions than for social interactions.

Key words: adult neurogenesis, sexual behavior, social behavior, familiarity, hippocampus, dentate gyrus.

INTRODUCTION

The study of adult neurogenesis has provided exciting new insights regarding the neural mechanisms by which new memories are formed (Abrous et al., 2005; Kempermann, 2006). Among mammals, neurogenesis occurs throughout adulthood along the sub-granular zone (SGZ) of the dentate gyrus within the hippocampal formation. Newly proliferated neurons from the SGZ migrate a short distance into the granule cell layer (GCL) of the dentate gyrus, where they extend functional axons into the CA3 region of the hippocampus (van Praag et al., 2002; Jessberger and Kempermann, 2003; Zhao et al., 2006). Young hippocampal neurons exhibit enhanced excitability, increased Ca^{2+} conductance, and a lower threshold for inducing long-term potentiation than do mature granule cells (Schmidt-Hieber et al., 2004; Ambrogini et al., 2010). These characteristics make young hippocampal neurons a particularly good substrate for memory formation. Considerable evidence indicates that enhanced hippocampal neurogenesis leads to improved memory (Shors et al., 2001; Snyder et al., 2005; Winocur et al., 2006; Dupret et al., 2007, 2008), and reduced adult neurogenesis has been associated with a variety of neurodegenerative diseases, including age-related dementia (Klempin and Kempermann, 2007; Drapeau and Abrous, 2008). However, there are also numerous contradictory reports indicating that learning actually causes a decrease in adult neurogenesis (Ambrogini et al., 2004; Mohapel et al., 2006; Aztiria et al., 2007), and there is some evidence that elevated neurogenesis leads to increased forgetting (Akers et al., 2014). Novel learning paradigms are needed to clarify why certain types of learning involve increased neurogenesis while others are associated with decreased neurogenesis.

A wide variety of environmental factors induce changes in cell proliferation and/or the survival of new cells in the dentate gyrus, which in turn can cause changes in adult neurogenesis. In general, acute and chronic stress cause a decrease in neurogenesis (Mirescu and Gould, 2006). For example, acute exposure

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Abbreviations: ANOVA, analysis of variance; BrdU, 5-bromo-2′deoxyuridine; GCL, granule cell layer; NeuN, neuronal nuclei; SEM, standard error of the mean; SGZ, sub-granular zone; STFP, social transmission of food preferences; TBS, Tris-buffered saline; WDS, wet dog shakes.

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to aggressive resident males causes decreased neurogenesis among male rats (Thomas et al., 2007), and colony-housed subordinate male rats have reduced neurogenesis compared to dominant males (Kozorovitskiy and Gould, 2004). Social isolation can also be stressful for rodents, and socially isolated male rats have reduced hippocampal neurogenesis relative to group-housed or pair-housed males (Lu et al., 2003; Stranahan et al., 2006; Spritzer et al., 2011).

Contrasting the effects of social isolation, some recent studies have shown that certain social interactions can enhance adult neurogenesis (Gheusi et al., 2009; Lieberwirth and Wang, 2012). For example, male and female prairie voles exposed to pups for 20 min showed increased hippocampal neurogenesis relative to voles that were not exposed to pups (Ruscio et al., 2008). A similar effect was observed among male mice interacting with pups for two days (Mak and Weiss, 2010), but three weeks of paternal behavior reduced neurogenesis among monogamous California mice (Glasper et al., 2011). Sexual interactions have also been shown to influence adult neurogenesis within the dentate gyrus. Among female mice, both sexual activity and exposure to male pheromones were shown to enhance neurogenesis (Shingo et al., 2003; Mak et al., 2007; Larsen et al., 2008). Among male rats, a single sexual interaction was sufficient to enhance hippocampal cell proliferation, and 14 consecutive days of 30-min sexual interactions caused a significant increase in neurogenesis (Leuner et al., 2010). Repeated sexual interactions (14–28 days) elevated neurogenesis levels among middle-aged male rats to that of young control rats (Glasper and Gould, 2013) and prevented a reduction in neurogenesis caused by chronic restraint stress among male mice (Kim et al., 2013). Thus, there is substantial evidence that the nature of a social interaction influences whether it will increase or decrease adult neurogenesis.

Most past research testing the function of adult neurogenesis has involved spatial memory tasks due to the known role of the hippocampus in spatial cognition. The neurogenesis-enhancing effects of sexual experience are puzzling, in that the hippocampus is not directly involved in regulating sexual behavior (Hull and Dominguez, 2007). A study with hamsters showed that sexual experience had no effect on neurogenesis within regions of the brain specifically known to be involved with mating behavior (i.e., posterior medial amygdala and medial preoptic area) (Antzoulatos et al., 2008). However, chemically blocking neurogenesis throughout the brain was shown to impair sexual behaviors in male rats (Lau et al., 2011). Additionally, the hippocampus is involved in the formation of some types of social memories. For example, lesions of the hippocampus or blocking *c-fos* expression within the hippocampus impaired social transmission of food preferences (STFP) among rats (Clark et al., 2002; Countryman et al., 2005). In addition, training in the STFP task increased hippocampal neurogenesis (Olariu et al., 2005), indicating that hippocampal neurogenesis may play a role in the formation of some social memories. There is also some evidence that the hippocampus plays a role in social recognition. Lesions to the hippocampus disrupted long-term (24 h) and short-term (30 min) social recognition among mice, and control mice were shown to retain social recognition of other individuals for at least 7 days (Kogan et al., 2000). Exposure of hamsters to familiar social partners 24 h after an interaction caused an up-regulation of immediate early gene products in the hippocampus (Lai et al., 2005). Among rats, transection of the fimbria disrupts social memory, suggesting that the hippocampus is involved in forming social memories (Maaswinkel et al., 1996). Thus, it is plausible that hippocampal neurogenesis plays a role in social recognition.

Testing the role of neurogenesis during social interactions provides a new model for determining the function of adult neurogenesis. A functional hippocampus is important for learning a sequence of events (Fortin et al., 2002), and current theory suggests that one of the primary functions of adult neurogenesis may be to facilitate learning spatial or temporal relationships (Aimone et al., 2006). Neurogenesis may, therefore, play a role in learning to distinguish among individuals during future interactions. In support of this hypothesis, exposing female mice to the pheromones of a socially dominant male resulted in increased neurogenesis within the dentate gyrus, and chemically blocking neurogenesis in females eliminated their preference to mate with dominant rather than subordinate males (Mak et al., 2007). We specifically tested whether familiarity with a sexual partner influenced adult neurogenesis by comparing neurogenesis levels in male rats that had been exposed four times to the same estrus female to those that had interacted with four different estrus females. A second experiment involved males interacting with anestrus females to determine if the effects of familiarity on neurogenesis were specific to sexual interactions.

The effects of environmental factors on adult neurogenesis depend upon the age of the newly proliferated cells. For example, hippocampal-dependent learning on the Morris water maze enhanced neurogenesis among cells that were 6–10 days old at the time of training, but not among cells that were 1–5 or 11–15 days old (Epp et al., 2007). This result is supported by a number of other studies with rats showing that training on hippocampus-dependent tasks specifically enhances neurogenesis among relatively young cells (3–11 days old) but not among older or younger cells (Gould et al., 1999; Ambrogini et al., 2000; Dupret et al., 2007; Sisti et al., 2007). Training sessions for STFP also enhanced cell survival when cells were 8 days old during training but not when cells were 16 days old (Olariu et al., 2005). Taken together, these past results indicate that a critical period may exist for environmental factors to influence adult neurogenesis. For rats, this critical period seems to be when cells are approximately 4–10 days old, which corresponds with the final stages of cell migration from the SGZ into the GCL (Brown et al., 2003; McDonald and Wojtowicz, 2005), when axons are rapidly extending and integrating into the existing network (Hastings and Gould, 1999). Based on this information, we used injections of 5-bromo-2′-deoxyuridine (BrdU) to label actively dividing cells 3 days prior to the first