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SEASONAL AND SEX DIFFERENCES IN CELL PROLIFERATION, NEUROGENESIS, AND CELL DEATH WITHIN THE DENTATE GYRUS OF ADULT WILD-CAUGHT MEADOW VOLES

MARK D. SPRITZER,[a,b]* ALYSSA W. PANNING,[a] SHANNON M. ENGELMAN,[a] W. TYLER PRINCE,[b] ALEXANDER E. CASLER,[b] JOANNA E. GEORGAKAS,[b] ELIZA C. B. JAEGER,[b] LAURA R. NELSON,[b] ETHAN A. ROY[b] AND BENJAMIN A. WAGNER[b]

[a] *Department of Biology, Middlebury College, McCardell Bicentennial Hall, Middlebury, VT 05753, USA*

[b] *Program in Neuroscience, Middlebury College, McCardell Bicentennial Hall, Middlebury, VT 05753, USA*

Abstract—Past research indicates that female meadow voles (*Microtus pennsylvanicus*) show decreased neurogenesis within the hippocampus during the breeding season relative to the non-breeding season, whereas male voles show no such seasonal changes. We expanded upon these results by quantifying a variety of endogenous cell proliferation and neurogenesis markers in wild-caught voles. Adult male and female voles were captured in the summer (breeding season) or fall (non-breeding season), and blood samples and brain tissue were collected. Four cellular markers (pHisH3, Ki67, DCX, and pyknosis) were labeled and then quantified using either fluorescent or light microscopy. The volume of the cell layers within the dentate gyrus (hilus and granule cell layer) was significantly larger in males than in females. In both sexes, all the cellular markers decreased significantly in the dentate gyrus during the breeding season relative to the non-breeding season, indicating decreased cell proliferation, neurogenesis, and pyknosis. Only the pHisH3 marker showed a sex difference, with females having a greater density of this cell proliferation marker than males. During the breeding season relative to the non-breeding season, males and females showed the predicted significant increases in testosterone and estradiol, respectively. Overall, these results suggest higher levels of neuronal turn-over during the non-breeding season relative to the breeding season, possibly due to seasonal changes in sex steroids.

Key words: meadow vole, adult neurogenesis, cell proliferation, dentate gyrus, testosterone, estradiol.

INTRODUCTION

The study of adult neurogenesis has addressed fundamental questions regarding how memories are formed (Deng et al., 2010; Yau et al., 2015) and has provided new insights for the treatment of neurodegenerative diseases (Kempermann, 2008; Schoenfeld and Cameron, 2015). Adult neurogenesis involves the proliferation, migration, and differentiation of new neurons within the adult brain. Within the mammalian hippocampus, newly proliferated cells in the dentate gyrus migrate from the sub-granular zone (SGZ) into the granule cell layer (GCL), where they extend functional axons into the CA3 region (van Praag et al., 2002; Jessberger and Kempermann, 2003; Zhao et al., 2006). Newly proliferated neurons within the dentate gyrus exhibit heightened synaptic plasticity relative to mature neurons (Schmidt-Hieber et al., 2004; Ambrogini et al., 2010). This elevated plasticity may make new neurons more responsive to various endogenous and environmental factors that could influence learning and memory. To date, the study of adult neurogenesis has relied heavily on experiments involving captive rodents, but to fully understand how new neurons function, field experiments are needed in which animals are allowed to express their full behavioral repertoire, including foraging, social interactions, reproduction, and predator avoidance (Amrein, 2015).

The diversity of mating systems observed among vole species (*Microtus* spp.) has made them a good model for understanding the evolution of sex differences in spatial memory. Among promiscuous meadow voles (*Microtus pennsylvanicus*), males have larger home ranges than females and expand their ranges during the breeding season to actively search for mates (Gaulin and Fitzgerald, 1989). Sex differences in range size correspond with sex differences in spatial ability: reproductively active male meadow voles outperform females in maze tests, but monogamous vole species do not have a sex difference in range size or spatial ability (Gaulin and

*Correspondence to: Mark Spritzer, Department of Biology, McCardell Bicentennial Hall, Middlebury College, Middlebury, VT 05753, USA. Fax: +1-802-443-2072.

E-mail addresses: mspritze@middlebury.edu (M. D. Spritzer), alyssa.panning@gmail.com (A. W. Panning), sengelman89@gmail.com (S. M. Engelman), wtylerprince@gmail.com (W. T. Prince), aecasler@gmail.com (A. E. Casler), georgakasj10@bcdschool.org (J. E. Georgakas), ej2371@cumc.columbia.edu (E. C. B. Jaeger), lrnelson@middlebury.edu (L. R. Nelson), earoy@middlebury.edu (E. A. Roy), bwagner7591@gmail.com (B. A. Wagner).

Abbreviations: ANOVA, analysis of variance; DCX, doublecortin; ELISA, enzyme-linked immunosorbent assay; GCL, granule cell layer; Ki67, Ki-67 protein; PBS, phosphate-buffered saline; pHisH3, phospho-histone H3; SGZ, sub-granular zone; TBS, Tris-buffered saline.

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FitzGerald, 1986; Kavaliers et al., 1993; Galea et al., 1994). Furthermore, the hippocampus is the primary brain region responsible for the processing of spatial information (Packard and McGaugh, 1996; McDonald and White, 1993; Devan et al., 1996; Pearce et al., 1998), and hippocampal volume is sexually dimorphic among meadow voles but not among monogamous pine voles (Jacobs et al., 1990). Home range size is positively correlated with spatial ability in male meadow voles, and males with better spatial memory locate the nests of more females in the wild (Spritzer et al., 2005). Thus, evidence suggests that sexual selection has shaped sex differences in spatial memory and ranging behavior in meadow voles, but the underlying neuroendocrine control of these sex differences remains unclear.

In their pioneering work, Galea and McEwen (1999) investigated the seasonal and sex differences in cell proliferation and cell death in wild meadow voles. They caught voles during the breeding and non-breeding seasons and injected them with ^{3}H-thymidine to label actively dividing cells. The density of ^{3}H-thymidine-labeled cells along the GCL of the dentate gyrus was significantly higher in females captured during the non-breeding season compared to females captured during the breeding season and compared to males captured during either season. Similarly, non-breeding females had higher densities of pyknotic cells (indicative of apoptosis) along the GCL than did any of the other groups. No seasonal differences in cell proliferation or pyknosis were found among the male voles, which suggests that seasonal changes in estradiol levels may regulate seasonal changes in the dentate gyrus of female voles. In support of this idea, the density of newly proliferated cells in female voles was inversely related to both serum corticosterone and estradiol levels. Subsequent experiments with captive animals showed that reproductively active female voles had lower levels of cell proliferation compared to reproductively inactive females and this effect was induced by prolonged exposure to elevated estradiol (Ormerod and Galea, 2001). Additional experiments showed that reproductive status had no effect on cell proliferation among male voles, but reproductively active males did have higher cell survival rates within the dentate gyrus than did reproductively inactive males (Ormerod and Galea, 2003). Finally, experiments with rats suggest that seasonal changes in testosterone may be responsible for this change in cell survival (Spritzer and Galea, 2007). In summary, female voles show seasonal changes in both cell proliferation and cell death within the dentate gyrus, which seem to be regulated by estradiol, while males show a seasonal change in cell survival, which might be regulated by testosterone.

Some studies with other wild rodent species have assessed sex differences and seasonal changes in neurogenesis. Analysis of two vole species (*Clethrionomys glareolus* and *Microtus subterraneus*) and wood mice (*Apodemus flavicollis*) captured during the breeding season revealed no sex differences in cell proliferation or cell death (Amrein et al., 2004). No sex differences in cell proliferation or neurogenesis within the dentate gyrus were found among wild-caught chipmunks (*Tamias amoenus*), gray squirrels (*Sciurus carolinensis*), or red squirrels (*Tamiasciurus hudsonicus*) (Barker et al., 2005; Johnson et al., 2010). Thus, sex differences in hippocampal neurogenesis seem to be fairly rare in wild rodents, but male Richardson's ground squirrels (*Urocitellus richardsonii*) have more neurogenesis than do females during the breeding season (Burger et al., 2014). This species is noteworthy in that males range widely during the breeding season in search of mates (Michener and McLean, 1996), similar to the situation in meadow voles (Spritzer et al., 2005). Other researchers have suggested that elevated neurogenesis may enhance the fitness of rodents living in more physiologically challenging environments (Cavegn et al., 2013). This hypothesis may explain the observation that both meadow voles and ground squirrels show higher levels of neurogenesis in the fall and winter than during the spring and summer (Burger et al., 2014; Galea and McEwen, 1999).

The goal of this study was to determine the effects of sex and season (breeding vs. non-breeding) upon cell proliferation, neurogenesis, and cell death within the hippocampus of wild-caught meadow voles. Galea and McEwen (1999) captured voles and brought them into captivity for 24 h before assessing cell proliferation using ^{3}H-thymidine. In an attempt to reduce any artifacts of captive housing, we collected brain tissue immediately after capturing voles in the field and used endogenous protein markers of cell phenotypes. Specifically, we quantified two markers of cell proliferation (phospho-histone H3 and Ki67), one marker of newly generated neurons (doublecortin), and a morphological marker of pyknosis. Expression of histone 3 phosphorylated at the serine-10 residue (pHisH3) occurs exclusively during late G_2 and mitosis of the cell cycle (Hendzel et al., 1997). Ki67 is expressed more broadly during all active stages of the cell cycle (G_1, S, G_2, and mitosis) but not during cell cycle arrest (G_0) and it plays a role in chromatin remodeling (Scholzen and Gerdes, 2000; Takagi et al., 2014). Doublecortin (DCX) is a microtubule-associated protein expressed in actively dividing neuronal precursor cells and in newly generated neurons during the first 14 days after cell division (Brown et al., 2003; Couillard-Despres et al., 2005). Pyknotic cells were detected using a Nissl stain and characterized by condensed chromatin near the center of the cell (Galea and McEwen, 1999; Barker and Galea, 2008).

EXPERIMENTAL PROCEDURES

Subjects

Meadow voles were trapped in a grassy wetland adjacent to the Middlebury College campus in Middlebury, Vermont. An 18×10 grid of Sherman live-traps ($7.5 \times 9 \times 23$ cm) was spaced at 5-m intervals, with each trap marked by a red pin flag. Traps were baited with cracked corn near dusk (1700–1800 h), and the traps were insulated with cotton balls in the fall. Traps were checked at dawn (0700–0800 h) the following morning. Voles were trapped during the non-breeding season (September 7-November 7, 2008) and breeding season (June 10-July 30, 2009). A total of 552 trap