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Estrogen-dependent modifications to hippocampal plasticity in paternal California mice (*Peromyscus californicus*)

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ABSTRACT

In many biparental species, mothers and fathers experience similar modifications to circulating hormones. With these modifications come alterations in neural structure and function suggesting that neuroendocrine mechanisms may underlie postpartum plasticity in both males and females. In the biparental California mouse (*Peromyscus californicus*), adult neurogenesis is maintained and anxiety-like behavior is attenuated in fathers during the mid-postpartum period. Given a causal relationship between estrogen and regulation of both adult neurogenesis and anxiety, we aimed to elucidate the role of estrogen-dependent mechanisms in paternal experience-related modifications to hippocampal neuroplasticity in California mice. In Experiment 1, hippocampal estrogen receptor beta (ERβ) mRNA expression, along with circulating estradiol concentrations, were determined throughout the postpartum period. An upregulation in ERβ expression was observed in postnatal day 16 males compared to virgins. Additionally, a rise in circulating estradiol concentrations was detected on postnatal day 2 compared to virgins; levels began to decline toward virgin levels on postnatal day 16 and postnatal day 30. In Experiment 2, we determined the role of estrogen-dependent mechanisms in adult neurogenesis and anxiety-like behavior by treating virgin and paternal males with saline or the selective estrogen receptor modulator, tamoxifen (TMX), during the time of axon extension (i.e., one week after bromodeoxyuridine injection). While TMX failed to alter elevated plus maze performance, TMX treatment inhibited survival of adult born neurons but only in paternal mice. These findings highlight the potential for estrogen-dependent pathways to mediate hippocampal adult neurogenesis in paternal mice.

1. Introduction

In males and females, the postpartum period is accompanied by numerous alterations to both the structure and function of the hippocampus. This region plays a key role in the mediation of anxiety and feedback of the stress response. Anxiety-like behavior, for example, is modulated by the dentate gyrus (DG) of the hippocampus (Kheirbek et al., 2013), a brain region that exhibits extensive adult neurogenesis that is altered during the postpartum period of males and females (reviewed in Leuner et al., 2010). Adult neurogenesis can be modulated by many hormones, including those of pregnancy, parturition, and lactation (Pereira, 2016). While males do not undergo the same physiological alterations that accompany reproduction in females, some of the neuroendocrine changes that accompany parenthood are comparable (Sundström Poromaa et al., 2017) and have similar effects on neural circuitry regulating parental behavior in both males and females (Numan and Insel, 2003). However, the extent to which paternal experience-related neuroendocrine modifications underlie hippocampal plasticity remains unclear.

The California mouse (*Peromyscus californicus*) is a biparental, monogamous species, in which males exhibit all aspects of parental care aside from nursing (Dudley, 1974; Gubernick, 1988; Gubernick and Alberts, 1987). Throughout the mid-postpartum period (postnatal day [PND] 15-21), California mouse fathers exhibit reduced anxiety-like behavior (Glasper et al., 2015; Hyer et al., 2016) while maintaining the survival of adult born neurons in the DG (Hyer et al., 2016). During this time, increased offspring mobility (Vieira and Brown, 2002) and thermoregulation (Rosenfeld et al., 2013) promotes a shift in parental care strategy, from more passive behaviors (e.g., huddling and grooming) to more active behaviors (e.g., retrievals; Bester-Meredith et al., 1999; Marler et al., 2003). Given that this shift in parental care strategy coincides with enhancements in hippocampal neuroplasticity in California

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mouse fathers, it is likely that paternal engagement with offspring contributes to experience-induced hippocampal plasticity.

Few studies have examined the neuroendocrine mechanisms underlying paternal experience-related hippocampal plasticity. Since California mouse mothers and fathers exhibit similar alterations to hippocampal structure (i.e., adult neurogenesis; Glasper et al., 2011), the neuroendocrine mechanisms that mediate hippocampal plasticity in maternal females provide a promising avenue to begin parallel investigations in paternal males. During the early postpartum period, estradiol promotes paternal care in California mice (Trainor and Marler, 2002). Given that the hippocampus is a potent site of action for estradiol, estradiol is a potential neuroendocrine mechanism by which paternal experience may alter hippocampal plasticity in California mice.

Estradiol augments both cell proliferation and cell survival in the hippocampus of male and female rodents. In non-lactating rats, cell proliferation peaks during proestrus, when estradiol levels are highest, compared to the estrus and diestrus stages of the estrous cycle (Tanapat et al., 1999). Moreover, peripheral administration of estradiol increases the number of newborn cells in the DG of ovariectomized (OVX) female rats (Ormerod et al., 2003; Tanapat et al., 2005). Similarly, chronic peripheral estradiol injections increase cell survival in gonadectomized (GDX) male meadow voles if administered during a period of axon extension (Ormerod et al., 2004). Though the mechanism(s) by which estradiol regulates adult neurogenesis remains unclear, the localization of estradiol receptors on adult-born cells suggests that receptor subtypes may have differential effects on cell proliferation and adult neurogenesis (Isgor and Watson, 2005; Shughrue et al., 1997). As the effects of estradiol on adult neurogenesis in male meadow voles is specific to cell survival (Ormerod et al., 2004), and estrogen receptor beta (ERβ) extensively colocalizes with DG granule cells, it is possible that the mechanism(s) by which estradiol alters survival of new neurons in the DG is via activation of estrogen receptors (Galea et al., 2006).

Estradiol not only affects hippocampal plasticity in male and female rodents, but also alters anxiety-like behavior mediated by the hippocampus. Compellingly, anxiety-like behavior, measured by performance on the elevated plus maze (EPM), is increased in OVX female rats compared to gonadally intact females; exogenous estradiol administration rescues this anxious phenotype (Frye and Walf, 2004). Direct administration of 17β-E_2, or a selective estrogen receptor modulator (SERM) with greater affinity for ERβ vs. ERα (e.g., coumestrol or diarylpropionitrile), into the hippocampus of OVX female rats reduces anxiety-like behavior (Walf and Frye, 2007). Likewise, both genomic (Filova et al., 2015) and non-genomic (Carrier et al., 2015) effects of estradiol have been associated with reduced anxiety-like behavior in GDX male rats during performance in the open field task. Importantly, this estradiol-related decrease in anxiety-like behavior appears dependent on activation of ERβ in female mice (Walf et al., 2009). Indeed, estradiol does not attenuate anxiety-like behavior in male ERβ knockout mice, suggesting that ERβ is necessary for the anxiolytic effects of estradiol in males as well (Frye et al., 2008). Taken together, these findings indicate that estradiol is anxiolytic in both male and female rodents and this reduction in anxiety-like behavior is likely driven by ERβ-dependent mechanisms within the hippocampus.

We performed a series of experiments to determine to what extent postpartum structural and functional plasticity in California mouse fathers is dependent on estrogen-related mechanisms in the hippocampus. First, we measured relative gene expression of ERβ, and other parenting-related hormones (i.e., prolactin, vasopressin, and oxytocin) in virgin males and fathers during the early-, mid-, and late-postpartum period. Second, we quantified circulating estradiol concentrations in virgin males and fathers across the postpartum period. Last, we determined to what extent paternal experience and modulation of estrogen receptors altered anxiety-like behavior and adult hippocampal neurogenesis in California mice.

2. Methods

2.1. Animals

California mice were born in our colony, weaned on PND30, and housed in same-sex dyads until 60–90 days of age. Ad libitum access to food and water was provided. The colony was maintained on a 16:8 reversed light:dark cycle (lights off at 1000 h). Experiments were approved by the University of Maryland Institutional Animal Care and Use Committee and conformed to guidelines provided by the National Institutes of Health for the care and use of animals.

2.2. Experiment 1

2.2.1. Experimental design

Gonadally intact male and female California mice were randomly placed into one of two experimental groups: virgins (male/male dyad) and fathers (male/female dyad). All non-sibling pairs were housed undisturbed (male/male, 42.33 ± 1.84 d; male/female, 45.73 ± 2.04 d), except for routine cage changes. Male/female pairs gave birth to 1.67 ± 0.12 offspring.

2.2.2. Tissue collection

To determine the extent to which reproductively-relevant hormone receptor expression is altered in the hippocampus of California mouse males, whole hippocampi were dissected from the brains of age-matched virgins and paternal males on PND2, PND16, or PND30. Following cervical dislocation, brains were rapidly extracted from the skull, hemispheres were separated, hippocampi were dissected and placed into RNAse-free centrifuge tubes, flash frozen in liquid nitrogen, and stored at − 80 °C until processing. Total time from cervical dislocation to liquid nitrogen was 370.42 ± 8.88 s.

2.2.3. Quantification of mRNA expression

Prior to nucleic acid isolation, hippocampi were homogenized in TRIzol reagent (Life Technologies, Grand Island, NY, USA) using a glass Dounce homogenizer. Total RNA was isolated with TRIzol reagent following manufacturer's instructions and quantified via spectrophotometry. One μg of total RNA was reverse transcribed into cDNA using the High Capacity cDNA Reverse Transcription Kit (Applied Biosystems, Foster City, CA, USA; cat. no. 4368814). Real-time qPCR was used to assess mRNA expression of hormones and neuropeptides associated with parental care (Table 1). Primer and probe sets were

Table 1
Quantitative reverse-transcription polymerase chain reaction primer and probe sequences.

Target	Forward primer (5′-3′)	Reverse primer (3′-5′)	Probe (5′-3′)	Accession ID
Esr2	GCTGATGTGGCGCTCGAT	CCCTCATCCCTGTCCAGAAC	ACCACCCTGGCAAGCTCATCTTT	XM_015992015.1
Prlr	CGACATTTGTGGATCTCAGGTT	CTGCCCTTGCTTTCATCCTA	AGGTGGTATTGTCCATTCAGAAGACC	XM_006991365.2
Avpr1a	CGCCTCTTGGGTGCTGAGT	CGATTTCGATCATAGAGAAGATGAAGT	CTACTGAGCACACCGCA	XM_006973335.2
Oxtr	TTCCTTGGGCGCATTGAC	GTGCTGGACGCCTTTCTTC	CGTGCAGATGTGGAGCGTCTGG	XM_006993911.2
ActB	GTACGACCAGAGGCATACAG	CTGAACCCTAAGGCCAACC	AGACCTTCAACACCCCAGCCATG	XM_006992672.2
Tbp	CAAGTTTACAGCCAAGATTCACG	TTCACCAATGACTCCTATGACC	CACTCCTGCCACACCAGCTTCT	XM_015994899.1

Esr2, estrogen receptor 2; *Prlr*, prolactin receptor; *Avpr1a*, Vasopressin 1a receptor; *Oxtr*, oxytocin receptor; *ActB*, beta actin; *Tbp*, TATA-box binding protein.