Developmental Cognitive Neuroscience 30 (2018) 100–107

Contents lists available at ScienceDirect

Developmental Cognitive Neuroscience

journal homepage: www.elsevier.com/locate/dcn

Adolescent pruning and stabilization of dendritic spines on cortical layer 5 pyramidal neurons do not depend on gonadal hormones

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ARTICLE INFO

Keywords:
Adolescence
Puberty
Gonadal hormones
Dendritic spines
Pruning
Structural plasticity

ABSTRACT

Pyramidal neurons in the neocortex receive a majority of their synapses on dendritic spines, whose growth, gain, and loss regulate the strength and identity of neural connections. Juvenile brains typically show higher spine density and turnover compared to adult brains, potentially enabling greater capacity for experience-dependent circuit 'rewiring'. Although spine pruning and stabilization in frontal cortex overlap with pubertal milestones, it is unclear if gonadal hormones drive these processes. To address this question, we used hormone manipulations and *in vivo* 2-photon microscopy to test for a causal relationship between pubertal hormones and spine pruning and stabilization in layer 5 neurons in the frontal cortex of female mice. We found that spine density, gains, and losses decreased from P27 to P60 and that these measures were not affected by pre-pubertal hormone injections or ovariectomy. Further analyses of spine morphology after manipulation of gonadal hormones suggest that gonadal hormones may play a role in morphological maturation and dynamics. Our data help to segregate hormone-sensitive and hormone-insensitive maturational processes that occur simultaneously in dorsomedial frontal cortex. These data provide more specific insight into adolescent development and may have implications for understanding the neurodevelopmental effects of changes in pubertal timing in humans.

1. Introduction

As the site of most excitatory synapses and some inhibitory and modulatory synapses, dendritic spines are critical mediators of information processing by cortical pyramidal cells (DeFelipe and Farinas, 1992; Holtmaat and Svoboda, 2009; Spruston, 2008). Dendritic spines can be dynamic, with spines being both gained and lost during development (Holtmaat et al., 2005; Johnson et al., 2016a; Zuo et al., 2005a) and as a result of experience (Fu et al., 2012; Munoz-Cuevas et al., 2013; Xu et al., 2009; Yang et al., 2009). Although new and transient spines do not always contain synapses, spine gain and loss are thought to reflect the sampling of new potential synaptic partners and enable the remodeling of connectivity (Berry and Nedivi, 2017; Holtmaat et al., 2005; Trachtenberg et al., 2002; Villa et al., 2016; Zito et al., 2009), particularly in response to learning (Fu et al., 2012; Hayashi-Takagi et al., 2015; Lai et al., 2012; Munoz-Cuevas et al., 2013; Roberts et al., 2010; Xu et al., 2009; Yang et al., 2009). Changes in individual spine morphology are also known to occur with maturation of a new synapse and during development (Berry and Nedivi, 2017).

Spines in the frontal cortex, as well as other cortical regions, are pruned during adolescence in a variety of species, including humans (Petanjek et al., 2011), rats (Koss et al., 2014), and mice (Holtmaat et al., 2005; Johnson et al., 2016a). *In vivo* imaging studies in mice using fluorescently labeled neurons have shown that spines are not only pruned, but also stabilized across adolescence: The fraction of total spines gained and lost per day declines during adolescence, leaving behind a more stable population as animals progress into adulthood (Holtmaat et al., 2005; Johnson et al., 2016a; Zuo et al., 2005a).

Spine pruning and stabilization during adolescence may be associated with a reduction in the capacity for flexible learning and reorganization of neural connectivity. The best evidence for this comes from adolescent zebra finches, in which greater baseline spine turnover predicts greater capacity for flexible song learning (Roberts et al., 2010). Thus, the stabilization of spines across adolescence may relate critically to developmental shifts in the capacity for plasticity and learning.

Given the links between spine dynamics and learning, it is of great interest to understand the mechanisms driving the pruning and stabilization of dendritic spines during adolescence. We have previously hypothesized that exposure to gonadal steroids during puberty may

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https://doi.org/10.1016/j.dcn.2018.01.007
Received 18 August 2017; Received in revised form 12 January 2018; Accepted 19 January 2018
Available online 31 January 2018

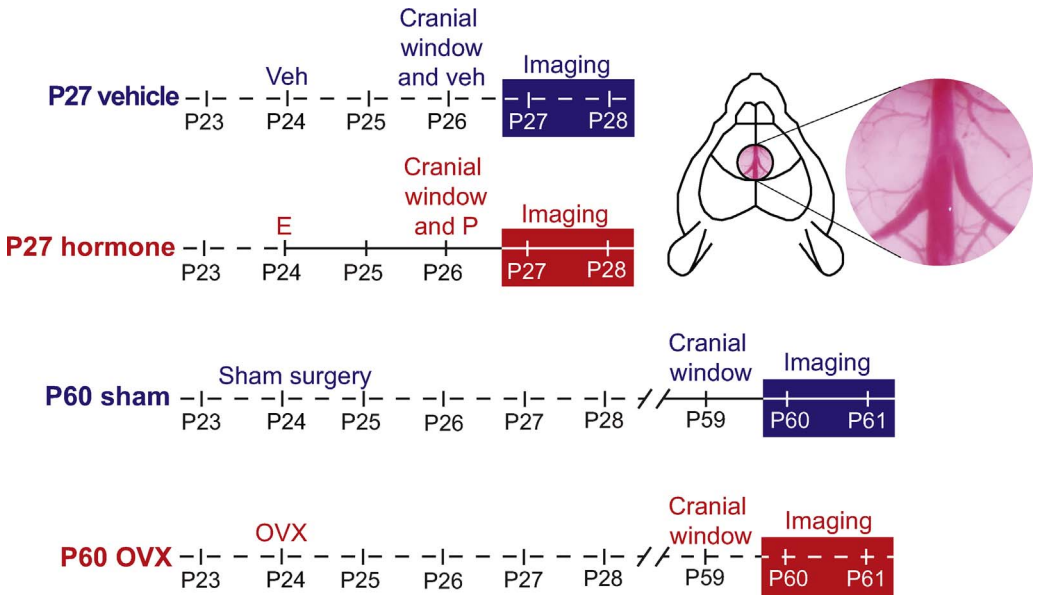

Fig. 1. Experimental timeline. To model early puberty, intact female mice were injected with estradiol (E) at P24 and progesterone (P) at P26 (N = 12 mice), while controls were injected with vehicle at both ages (N = 12 mice). To prevent pubertal exposure to gonadal hormones, female mice were ovariectomized (OVX) or received sham surgery at P24 (N = 8 OVX mice; N = 8 sham mice). On the timelines, solid lines indicate exposure to pubertal gonadal hormones, while dashed lines indicate pre-pubertal status or absence of gonads. A cranial window was placed over the dorsomedial frontal cortex anterior to bregma as shown in the schematic. The inset is a photograph of a representative window taken at the end of cranial window surgery. The diameter of the craniotomy is 2.5 mm. Apical dendrites of layer 5 pyramidal cells were imaged in layer 1 of dorsomedial frontal cortex at P27–P28 in vehicle- and hormone-treated mice and in young adulthood (age range P55–65, labeled as P60) in OVX and sham mice.

decrease capacity for plasticity and circuit remodeling in frontal cortex (Piekarski et al., 2017b), which may be mediated, in part, by reduced spine turnover. Correlational evidence in humans and mice supports this possibility: The onset of spine pruning in human frontal cortex coincides with the average age at puberty onset (Petanjek et al., 2011), and measures of cortical thinning in human structural MRI data correlate with pubertal development (Herting et al., 2015; Herting et al., 2014; Peper et al., 2009). Spine pruning and stabilization in layer 5 pyramidal neurons in mouse frontal cortex also occur during pubertal development (Johnson et al., 2016a), sex-specific changes in cortical synapse density occur during adolescence in rats (Drzewiecki et al., 2016), and hormone treatment can alter spine density and turnover in cortical pyramidal cells of adult mice (Tan et al., 2012; Wang et al., 2017). Although these data suggest a potential role for pubertal hormones in the maturation of spine dynamics, establishing a causal relationship requires experimental manipulation of gonadal hormone exposure during adolescence. It is therefore still unknown whether pubertal hormones influence the maturation of spine dynamics during adolescence.

The role of pubertal hormones in brain maturation is particularly important in light of recent advancement in the age of puberty onset in girls and boys (Herman-Giddens, 2006) and the negative educational and mental health outcomes associated with early-onset puberty in girls (Graber, 2013). We have previously shown that in female mice, pubertal hormones drive maturation of inhibitory neurotransmission in layer 2/3 of the dorsomedial frontal cortex (Piekarski et al., 2017a), a region implicated in a variety of cognitive and affective behaviors in rodents and humans (Blakemore and Robbins, 2012; Felix-Ortiz et al., 2016; Johnson and Wilbrecht, 2011). However, it is unknown whether pubertal hormones drive other aspects of frontal circuit maturation, such as spine pruning and stabilization, or maturation of average spine morphology. Revealing which aspects of frontal circuit maturation are hormone-dependent and which are not is critical for understanding normative adolescent development and the implications of the advancing age of puberty onset in humans.

Here, we show the results of *in vivo* 2-photon microscopy experiments designed to test the role of pubertal hormones in the maturation of spine density and turnover on layer 5 pyramidal neurons in the female mouse frontal cortex. The experiments were initially designed to answer three questions: 1) Do female mice show spine pruning and stabilization in the dorsomedial frontal cortex across adolescence, as has been shown in males (Johnson et al., 2016a); 2) Does pre-pubertal hormone exposure induce an early reduction in spine density and turnover; and 3) Are gonadal hormones necessary for the maturation of spine density and turnover across adolescence. We found that females, like males, show spine stabilization and pruning across adolescence on layer 5 pyramidal cells in frontal cortex, but these processes are unaffected by pre-pubertal hormone exposure or gonadectomy. These negative data led us to pursue opportunistic follow-up analyses of the morphology of spines to ask, 4) Does pre-pubertal hormone treatment or gonadectomy alter the maturation of spine morphology across adolescence and/or morphological dynamics over 24 h? In these analyses, we did find subtle but significant effects that suggest ovarian hormones do contribute to the morphological maturation of dendritic spines on layer V neurons and the dynamic remodeling of their morphology during early adulthood.

2. Methods

2.1. Animals

Female C57BL/6J mice from the Thy-1-YFP-H line (Jackson Laboratory, Bar Harbor, Maine), in which a subset of layer 5 pyramidal cells is fluorescently labeled (Feng et al., 2000; Porrero et al., 2010), were bred in our animal facility. We chose to use females in this study due to public health issues surrounding the advancing age of puberty in girls (Graber, 2013; Herman-Giddens, 2006), and based on previous data showing that pubertal hormones in females drive maturation of inhibitory neurotransmission in the dorsomedial region of frontal cortex imaged in this study (Piekarski et al., 2017a). All mice were weaned on postnatal day (P)21 and housed in groups of 2–3 same-sex siblings on a 12:12hr reverse light:dark cycle (lights on at 10PM). All procedures were approved by the Animal Care and Use Committee of the University of California, Berkeley and conformed to principles enunciated in the NIH Guide for the Care and Use of Laboratory Animals.

2.2. Pre-pubertal hormone exposure to induce early-onset puberty

To advance age at puberty onset, gonadally intact females were injected with 17 beta-estradiol benzoate (0.01 mg/kg subcutaneous) at P24 and progesterone (20 mg/kg subcutaneous) at P26 (Fig. 1; (Piekarski et al., 2017a)). This treatment advances first peripubertal exposure to gonadal steroids and is sufficient to induce endogenous puberty (Ramirez and Sawyer, 1965; Smith and Davidson, 1968). A vehicle control group was injected with equivalent volumes of oil