Adolescence and "Late Blooming" Synapses of the Prefrontal Cortex

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The maturation of the prefrontal cortex (PFC) during adolescence is thought to be important for cognitive and affective development and mental health risk. Whereas many summaries of adolescent development have focused on dendritic spine pruning and gray matter thinning in the PFC during adolescence, we highlight recent rodent data from our laboratory and others to call attention to continued synapse formation and plasticity in the adolescent period in specific cell types and circuits. In particular, we highlight changes in inhibitory neurotransmission onto intratelencephalic (IT-type) projecting cortical neurons and late expansion of connectivity between the amygdala and PFC and the ventral tegmental area and PFC. Continued work on these "late blooming" synapses in specific cell types and circuits, and their interrelationships, will illuminate new opportunities for understanding and shaping the biology of adolescent development. We also address which aspects of adolescent PFC development are dependent on pubertal processes.

The juvenile to adult transition, referred to as adolescence, is a period of flux from two vantage points: that of the individual who undergoes physical, cognitive, and socioemotional changes and that of the brain that undergoes parallel processes of circuit refinement. Historically, there has been much hand-wringing about the risks associated with adolescence, but adolescence is increasingly recognized as a developmental period that holds great potential for experience-dependent plasticity and learning (Dahl et al. 2018). Although brain maturation during adolescence is most frequently associated with synapse elimination in the prefrontal cortex (PFC) and other association cortices, specific neural projections show unique developmental trajectories, including late growth into the adolescent period (hence, the title's reference to "late blooming" synapses). We propose that understanding the developmental trajectories of distinct cell types and anatomical projections in the PFC can provide insight into windows of vulnerability and opportunity for experience-dependent circuit rewiring. We review existing data about the adolescence-associated changes in PFC structure and function, highlighting cells and circuits with late growth and/or plasticity. We also address which aspects of adolescent PFC development are dependent on pubertal processes.

MATURATION OF DENDRITIC SPINES: BOTH PRUNING AND STABILIZATION

In the human neocortex, longitudinal imaging studies have clearly shown that cortical gray matter thins during the first few decades of life, in a roughly back-to-front pattern, with the frontal associative cortices, including PFC, thinning last (Gogtay et al. 2004). Cortical thinning is thought to be driven in part by the elimination of synapses, a process also referred to as "pruning" (Rakic et al. 1994; Petanjek et al. 2011; Chen et al. 2014; Koss et al. 2014; Drzewiecki et al. 2016). The majority of excitatory glutamatergic synapses on pyramidal neurons in the cortex can be conveniently quantified by counting dendritic spines, postsynaptic structures that protrude ~0.5–3 μm in a twig-like fashion from the dendritic shafts of neuronal arbors. Dendritic spines contain glutamate receptors, scaffolding proteins, and intracellular signaling enzymes (Holtmaat and Svoboda 2009; Sheng and Kim 2011) and also can contain GABA receptors (Chen et al. 2012; van Versendaal et al. 2012) and neuromodulatory receptors (Miner et al. 2003; Yao et al. 2008). The majority of stable dendritic spines are found to make contact with axonal boutons, varicosities on axons that contain an active zone with a collection of synaptic vesicles (Shepherd and Harris 1998).

The advent of two-photon microscopy in the early 2000s made it possible to image the maturation of dendritic spines and other structures within the living brain (Holtmaat and Svoboda 2009). Mouse *Thy1* BAC transgenic lines (Feng et al. 2000), which label a subset of layer 5 pyramidal neurons (primarily pyramidal tract projecting or PT-type) (Table 1), have been the most popular method used in these in vivo imaging studies because the neurons are brightly labeled in a sparse fashion that can be viewed through a thin skull or cranial window (Holtmaat et al. 2009; Porrero et al. 2010). Imaging over hours, days, or weeks it can be seen that although a majority of spines are persistent, new spines may be gained and lost, a process referred to as turnover (Fig. 1). Layer 5 neurons in the somatosensory, visual, and PFC all undergo a reduction

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Table 1. PT and IT neurons in the PFC mature differently during adolescent development

Pyramidal cell type	PT type (L5)	IT type (L5 and 2/3)
Morphology	 Thick-tufted (apical dendrites)	 Simple or thin-tufted (apical dendrites)
Projection targets	Pyramidal tract, pons, ipsi-striatum	Telencephalon, contra and ipsi-cortex, contra and ipsi-striatum
Firing properties	Adapting	Non adapting
Labeled in *Thy 1* YFP-H line?	Yes, YFP^{+}	No, YFP^{-}
PFC spine pruning	Density decreases P25 to P60 (Johnson et al. 2016a) Change in density not sensitive to prepubertal ovarian hormone injection or ovariectomy (Boivin et al. 2018)	Spine density decreases P29 juvenile to P60 adult (unpublished data)
PFC spine stabilization	24-h spine gains and losses decrease P25 to P60 (Johnson et al. 2016a) Turnover not sensitive to prepubertal ovarian hormone injection or ovariectomy (Boivin et al. 2018)	Unknown in PFC
Inhibitory neurotransmission onto PFC pyramidal neurons	mIPSCs and mEPSCs stable P25 to P45 (Vandenberg et al. 2015)	L5: mIPSC amplitude increases P25 to P45 (Vandenberg et al. 2015) L2/3: mIPSC frequency and amplitude increase P25 to P45 Change in mIPSC frequency sensitive to ovarian hormones (Piekarski et al. 2017a)

in spine density and stabilization in daily spine turnover during the juvenile (~postnatal day[P]20–30) to young adult (~P60–180) transition period (Figs. 1 and 2; Grutzendler et al. 2002; Holtmaat et al. 2005; Zuo et al. 2005; Johnson et al. 2016a; Boivin et al. 2018).

This stabilization process may be just as important as pruning for neural plasticity. It is hypothesized that spine turnover allows for the sampling of new synaptic partners and reorganization of connectivity (Trachtenberg et al. 2002; Holtmaat et al. 2005; Stepanyants and Chklovskii 2005)—processes that are important during stages of learning (Xu et al. 2009; Roberts et al. 2010; Fu et al. 2012; Muñoz-Cuevas et al. 2013; Johnson et al. 2016b). Increasingly lower levels of gains and losses during

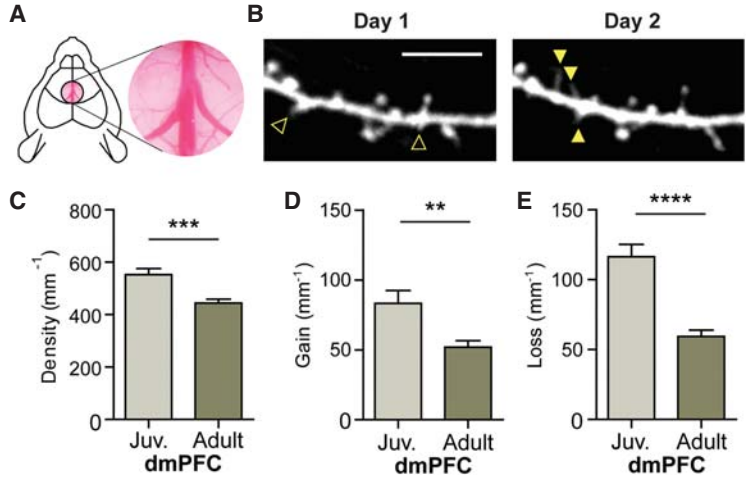

Figure 1. Juvenile mice have higher spine density and turnover on PT-type layer 5 pyramidal cells in dmPFC compared to adults. (*A*) Schematic of a cranial window in mouse dmPFC. (*B*) Longitudinal imaging of the same layer 5 apical dendrite across 24 h in the *Thy1* YFP-H line that labels the layer 5b pyramidal tract (PT) projecting-type neurons. Open arrows indicate spines that were lost from day 1 to day 2. Closed arrows indicate new spines that were gained from day 1 to day 2. Scale bar, 5 μm. (*C*) Juvenile mice have higher spine density compared to adult mice ($P < 0.001$). (*D*) Juvenile mice show greater 24 h spine gains compared to adult mice ($P < 0.01$). (*E*) Juvenile mice show greater 24 h spine loss compared to adults ($P < 0.0001$). Bars represent mean ± SEM. (**) $P < 0.01$, (***) $P < 0.001$, (****) $P < 0.0001$. These data are from male mice (Johnson et al. 2016a). Note that data from females from the same mouse line (Boivin et al. 2018) show comparable changes. (*A*,*B*, Reprinted from Boivin et al. 2018; *C*–*E*, Reprinted from Johnson et al. 2016a.)

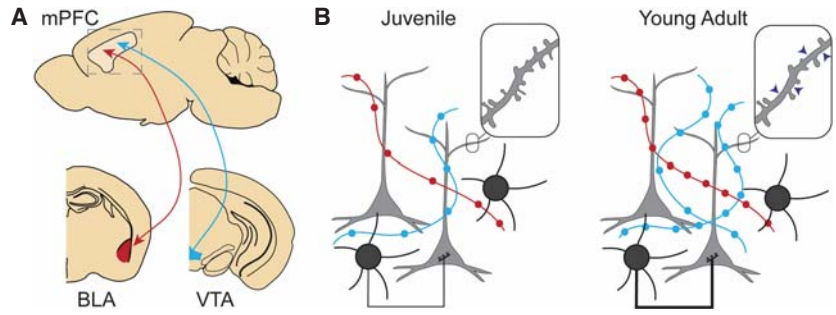

Figure 2. Late blooming synapses in adolescence. (*A*) Cross-sectional view of long-range projections that show late growth into adolescence. Both the BLA (red) and VTA (cyan) are reciprocally connected to the mPFC, and arrows indicate sites of increasing innervation during the adolescent transition (Benes et al. 2000; Cunningham et al. 2002; Naneix et al. 2012; Reynolds et al. 2018). In addition, studies have shown that the top-down projection from the mPFC to BLA shows growth during adolescence (Arruda-Carvalho et al. 2017). (*B*) Schematic of mPFC circuit rewiring processes during the adolescent transition. Layer 5 pyramidal neurons in mPFC (IT and PT types) undergo both spine pruning and stabilization (reduction in daily spine losses and gains). Blue arrowheads indicate spines that have been pruned. Axons from BLA (red) show increases in bouton density as well as bouton gains in young adult mice compared to juveniles (Johnson et al. 2016a). Axons from VTA (cyan) increasingly innervate the mPFC (Benes et al. 2000; Naneix et al. 2012), show increased dopamine synapses onto pyramidal neurons (Lambe et al. 2000), and show enhanced activity-dependent plasticity (Mastwal et al. 2014) during the adolescent transition. Local interneurons are shown (black cells) to indicate complex changes in inhibitory neurotransmission during adolescence onto specific pyramidal neurons (Vandenberg et al. 2015; Piekarski et al. 2017a). See main text above and Table 1 for more details.

development leads to stabilization of connectivity and reduced potential for rewiring. However, on the upside, stabilization in adulthood is thought to be critical for maintaining established connections, consolidating learning, and promoting efficiency (Blakemore and Choudhury 2006; Bourne and Harris 2007).

Notably, adolescent maturation of spine density and turnover may differ by region and cell type (Holtmaat et al. 2005; Pattwell et al. 2016) and can also show nonlinear patterns of change, such as an inverted U trajectory (Koss et al. 2014). Cortical layer 2/3 cells, which are typically intratelencephalic projecting (IT)-type neurons (Shepherd 2013), show different maturation and experience dependent plasticity profiles (Tjia et al. 2017) compared to PT neurons or neurons in other layers (Table 1).

SPINE DYNAMICS AND ADOLESCENCE—A ROLE FOR PUBERTY?

Changes in spine density and turnover in the PFC of humans and rodents overlaps with pubertal milestones (Giedd et al. 1999), raising the question of whether the pubertal increase in steroid hormones drives synaptic pruning or stabilization during adolescence. Recently, human neuroimaging studies have focused on parsing out differences in the effects of age and pubertal status on brain development (Neufang et al. 2009; Peper et al. 2009; Paus et al. 2010; Bramen et al. 2011). Some studies find that testosterone is associated with decreases in cortical thickness in postpubertal males (Nguyen et al. 2013) and gray matter volume in the frontal lobes (Koolschijn et al. 2014). Another study found that pubertal tempo (the rate of change in Tanner staging) predicted the rate of change in cortical thickness in some regions (Herting et al. 2015). Similarly, rats show a decrease in overall synaptic density in the medial PFC across adolescence, and male rats that are matched by age but differ in pubertal status show significantly different synaptic density in this region (Drzewiecki et al. 2016). Drzewiecki examined the density of synaptophysin puncta, so there was no information regarding cell type. Together these data support the possibility that pubertal testosterone drives pruning in the frontal lobes during adolescence, but more work needs to be done to isolate the cell type.

In a recent study, we manipulated ovarian hormones and measured spine density and dynamics in layer 5 *Thy1* YFP-H expressing neurons in the mouse dorsomedial prefrontal cortex (dmPFC). We found that in these cells spine pruning and stabilization were not sensitive to ovarian hormone manipulation (either by prepubertal hormone injection or ovariectomy), but spine motility and morphology showed some significant effects of manipulation (Boivin et al. 2018). In ongoing work, we are examining the effect of prepubertal gonadectomy in males and females on IT-type cells of the dmPFC to determine if this cell type shows puberty-dependent maturation of spine density (Table 1).

CHANGES IN INHIBITORY NEUROTRANSMISSION ONTO PYRAMIDAL NEURONS IN THE ADOLESCENT PFC

Inhibitory neurotransmission onto pyramidal neurons in the PFC is remodeled during the adolescent period (Le Magueresse and Monyer 2013; Lewis and Melchitsky 2013; Rinetti-Vargas et al. 2017). Recent work in rodents is illuminating the cell type specificity of these changes and the potential role of pubertal processes in their regulation. The maturation of excitatory synaptic input and intrinsic properties of inhibitory interneurons has also been shown to be cell type–specific (Miyamae et al. 2017), but we do not review this work here.

As mentioned previously, in the *Thy1* YFP-H transgenic mouse line, YFP^+ cells are predominantly PT type, where-

as YFP cells are mostly IT type (Miller et al. 2008; Porrero et al. 2010). We previously used *Thy1* YFP-H mice to target layer 5 PT and IT neurons of the dmPFC for in vitro patch clamp recordings. We observed a developmental increase in miniature inhibitory postsynaptic current (mIPSC) amplitude from juvenile (P21–25) to the late adolescent period (P40–50) in YFP^- neurons (IT) but not YFP^+ neurons (PT) (Vandenberg et al. 2015). These data suggest that IT-type neurons in the dmPFC may show a more protracted maturation compared to PT-type neurons.

In layer 2/3 pyramidal neurons of the dmPFC, which are of the IT type, we have also observed significant maturation of inhibitory neurotransmission across the adolescent transition. Recording from L2/3, we observed a significant increase in mIPSC frequency and amplitude and a decrease in tonic inhibitory currents from P25 to P40–45 (Piekarski et al. 2017a). Using gonadal hormone manipulation experiments we found that these changes in mIPSC frequency and tonic inhibitory current could be accelerated by prepubertal injection of ovarian hormones in females (Piekarski et al. 2017a). Changes in mIPSC frequency in L2/3 could also be blocked by prepubertal ovariectomy and rescued by later hormone injection (Piekarski et al. 2017a). These data suggest that alterations to inhibitory inputs onto IT-type cells in the PFC occur in adolescence through several mechanisms. It is not yet known how changes in inhibition on IT-type cells affect spine pruning or stabilization across development. Interestingly, recent studies have shown that parvalbumin interneuron activity can counteract stress-induced spine loss in somatosensory cortex (Chen et al. 2018) and frontal cortex (Ng et al. 2018). Given the role of inhibitory neurotransmission in the regulation of sensory cortex sensitive period plasticity (Hensch 2005), preliminary data suggest a potential parallel in the adolescent PFC (Piekarski et al. 2017a,b).

AXONAL BOUTONS IN THE PFC ACROSS ADOLESCENT DEVELOPMENT: PATHWAY-SPECIFIC CHANGES

The pyramidal neurons of the PFC integrate long-range inputs from a number of different cortical and limbic regions, including the contralateral cortex, orbitofrontal cortex (OFC), basolateral amygdala (BLA), ventral hippocampus (vHPC), and thalamus (Gabbott et al. 2005; Hoover and Vertes 2007). These inputs impinge on pyramidal cell dendritic spines and are largely glutamatergic, but they may also drive feed-forward inhibition through synaptic contacts on local inhibitory neurons (Delevich et al. 2015; McGarry and Carter 2016; Anastasiades et al. 2018). Neuromodulatory inputs also innervate the PFC from subcortical areas (Dembrow and Johnston 2014; Baker et al. 2018). The connectivity patterns of these projections undergo changes during adolescence, but they do not all show a pruning maturation pattern and are not time locked to changes in overall spine density.

Reciprocal connections between the PFC and amygdala are thought to underlie emotion regulation (Kim et al. 2003; Phelps et al. 2004; Lai et al. 2012; Cho et al. 2013; Janak and Tye 2015; Pattwell et al. 2016) with greater recruitment of the frontal cortex and enhanced negative functional connectivity between frontal cortex and amygdala accompanying better emotion regulation with age (McRae et al. 2012; Silvers et al. 2015). Studies in both humans and rodents suggest that the connections between the amygdala and PFC undergo protracted maturation into adolescence (Bouwmeester et al. 2002b; Gee et al. 2013; Gabard-Durnam et al. 2014; Johnson et al. 2016a; Arruda-Carvalho et al. 2017). In rodents, BLA axons first reach the frontal cortex during the first postnatal week, but increasingly innervate the frontal cortex during adolescence and young adulthood (Cunningham et al. 2002; Johnson et al. 2016a; Pattwell et al. 2016), increasing synapse density onto both excitatory and inhibitory neurons (Cunningham et al. 2002, 2008). In vivo imaging of axonal bouton turnover in mice shows BLA axons in the dmPFC show a late "bloom" in plasticity with greater bouton gains and losses in young adulthood compared to the juvenile postweaning period (Fig. 2). Notably, this pattern was not observed in another long-range axonal pathway that innervates the dmPFC from OFC (Johnson et al. 2016a).

Histological studies have also shown striking late growth of dopaminergic terminals into the primate and rodent PFC extending from the juvenile period well into the young adult period (Kalsbeek et al. 1988; Benes et al. 2000; Naneix et al. 2012; Willing et al. 2017; Hoops et al. 2018). In mice, dopaminergic boutons in the PFC show greater activity-dependent plasticity in mid adolescence compared to adulthood (Mastwal et al. 2014). Furthermore, data suggest that late-arriving dopamine axons can influence the structure of layer 5 pyramidal neurons in PFC. Expanding on previous findings (Manitt et al. 2011, 2013), a recent study from the Flores laboratory showed that ectopic growth of VTA dopamine axons into the mPFC during adolescence was associated with reduced dendritic complexity and spine density onto layer 5 pyramidal neurons (Reynolds et al. 2018). These studies highlight an important potential interaction between late-developing dopamine axons, which may be particularly sensitive to environmental inputs, and adolescent spine pruning in mPFC.

Efferents from the PFC also undergo pathway-specific late growth during the adolescent period, at the time when dendritic spine density is pruning. Rodent studies show that mPFC axons begin to innervate the BLA between the second and third postnatal week (Bouwmeester et al. 2002a). This is consistent with findings in humans, in which mPFC-amygdala connectivity (positive coupling) first appears in preadolescence, around age 10 (Gabard-Durnam et al. 2014). A recent study in mice provides a more detailed description of the development of mPFC axons that target the BLA. Using anterograde labeling, Arruda-Carvalho et al. (2017) found that mPFC fibers show late innervation of the BLA compared to other target regions (including the striatum, thalamus, and claustrum), first reaching the amygdala at P15 and increasing its innervation until P30. Importantly, Arruda-Carvalho et al. determined that this connection undergoes synaptic strengthening during adolescence, reaching peak levels at

P30, with a transient increase in feed-forward inhibition at P30. Notably, another study suggests that mPFC inputs to the BLA in rats undergo late pruning between P45 and P60 (Cressman et al. 2010).

CONCLUSION

In summary, new imaging and labeling methods and attention to cell and circuit specificity have enhanced our understanding of PFC development. It has been repeatedly confirmed that dendritic spines on layer 5 cortical pyramidal neurons across the cortical sheet are more numerous and more plastic in early life, with high levels of turnover, but become stable in adulthood, with a large population of spines estimated to persist for the lifetime of the animal (Zuo et al. 2005; Holtmaat and Svoboda 2009; Bloss et al. 2011; Pattwell et al. 2016). This pruning and stabilization process occurs in the PFC during adolescence and is likely to contribute to changes in behavior, learning and memory, and executive function. Axons that target the PFC or emerge from the PFC can also show synaptogenesis followed by pruning and stabilization during development, but this is not always the rule, and the timing may be shifted relative to PFC dendritic spines or other afferent projections (Benes et al. 2000; Cunningham et al. 2002; Johnson et al. 2016a). Inhibition onto pyramidal neurons in the PFC also changes with development in a cell type–specific manner (Le Magueresse and Monyer 2013; Vandenberg et al. 2015).

Stabilization of connectivity in the PFC and cortex more generally may stabilize neuron function and learned information in order to prepare the brain for adulthood. Yet there are later developing, hence "late blooming," synapses on specific cells and circuit projections that show elevated levels of plasticity when the majority of synapses are being pruned or becoming more stable. These exciting "late blooming" changes may be particularly important for learning that occurs during adolescence or the expression of new behaviors appropriate for gaining independence or the transition to adulthood (Dahl et al. 2018). In future experiments, it will be important to test if these "late blooming" synapses show special sensitivity to experience, either concurrent to their development or in response to earlier life events. Given that some of these synapses are sensitive to the timing of puberty onset, these data also raise concern about the earlier shift in the timing of puberty onset that is occurring in developed nations (Piekarski et al. 2017b).

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