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Cortical dendritic spine development and plasticity: insights from *in vivo* imaging

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Dendritic spines are the postsynaptic sites of most excitatory synapses in the cerebral cortex. Their morphology and density change throughout life, reflecting the maturation and reorganization of excitatory circuits. The development of *in vivo* two-photon microscopy has enabled the monitoring of the same dendritic spines over time during different developmental periods. In this review we focus on recent *in vivo* imaging studies in rodents that have revealed cell type-specific and region-specific structural dynamics of dendritic spines. We also discuss how the contributions of local inhibitory neurons and long-distance excitatory and neuromodulatory inputs to the cortex influence dendritic spine development and dynamics. Such studies will facilitate our understanding of how environmental factors and experiences affect cortical synapse development.

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Introduction

Building the complex architecture of the cerebral cortex is a protracted endeavor: the establishment and refinement of synaptic connections between neurons begins prenatally and continues well into postnatal life, and plasticity of such connections persists into adulthood. Rakic and colleagues [1] demonstrated that in various cortical regions of the macaque, synaptogenesis occurs during early postnatal development, following which synaptic density decreases through adolescence until it reaches the adult level and remains relatively stable thereafter. Other mammals, including rodents and humans, also exhibit this developmental pattern [2–4]. Such fixed-tissue studies continue to deepen our understanding of cortical synaptic development, but they provide only single snapshots of the developmental trajectories of synapses. At the turn of the 21st century, two-photon microscopy [5] and fluorescent labeling strategies [6,7] paved the way for observing synapses longitudinally in the living brain [8,9]. Since then, a wealth of *in vivo* imaging studies has revealed that cortical synapses are much more dynamic than can be inferred from fixed-tissue studies. Insight into how cortical synapses are formed, stabilized, and eliminated over the course of development informs how cortical circuits contribute to brain function and behavior across the lifespan. This knowledge also advances our understanding of the pathophysiology of neuropsychiatric disorders, such as autism spectrum disorder and schizophrenia, in which cortical synaptic development is thought to be disrupted [10]. Here, we review recent *in vivo* work on postnatal cortical development, and we discuss how local and long-range circuits affect the development and experience-dependent refinement of excitatory synaptic structures. Specifically, we focus on dendritic spines, the postsynaptic sites of most excitatory synapses in the mature cortex that may serve as structural proxies for excitatory synaptic connectivity [11].

Excitatory synapses are dynamic during development

The first two postnatal weeks in the rodent cortex are associated with a relatively increased presence of filopodia [7]: long, thin structures protruding from the dendritic shaft that differ from dendritic spines morphologically and functionally. *In vivo* imaging reveals that filopodia are highly dynamic. In adolescent mouse somatosensory cortex, most filopodia are short-lived, with fewer than 3% persisting for more than two days. Some surviving filopodia adopt a mushroom-like head and transform into stable dendritic spines, which suggests that they are structural precursors of dendritic spines [12]. Throughout adolescence, the percentage of filopodia among all protrusions (i.e. filopodia and spines) along the apical dendrites of cortical pyramidal neurons (PNs) decreases continuously, until they comprise only a small fraction of protrusions in the adult cortex. At the same time, the presence of dendritic spines with a mature morphology increases, and these spines tend to be highly stable [9,12,13]. Thus, cortical dendritic protrusions exhibit an increasingly mature morphology during postnatal development.

While dendritic spines tend to be more stable than filopodia, they are not static. During adolescence, spine elimination outpaces formation, leading to a net decrease in spine density [12,13]. In adulthood, spine elimination

and formation are balanced, and the overall turnover slows down [8,12,13]. In the mouse visual cortex, around 25% of spines are eliminated over one month during adolescence, while 96% are stable in the adult over the same time interval [8]. Interestingly, presynaptic axonal boutons from different thalamic and cortical neuron populations exhibit diverse dynamics [14,15]. Around 1–2 months of age, most axonal boutons in mouse somatosensory, visual, and auditory cortices are more stable than nearby spines [16]. Together, these studies illustrate that the dynamics of both presynaptic and postsynaptic components of excitatory synapses are developmentally regulated, which impacts excitatory synapse density.

Cell-type and region-specific development of spines

Many of the studies discussed above focus on cortical layer (L) 5 PNs using transgenic mouse lines in which the *Thy1* promoter drives the expression of cytoplasmic fluorescent proteins (e.g. YFP or GFP) in a subset of these neurons [[17••]]. Taking advantage of the different birthdates of PNs destined to reside in distinct cortical layers, recent studies have used *in utero* electroporation to express fluorescent proteins in cortical L2/3 PNs, allowing comparison of their spine dynamics with those of L5 PNs [17••,18] (Figure 1). Interestingly, spine density is higher on apical dendrites of L2/3 PNs than on L5 PNs, a difference that is apparent from the second postnatal week onward [17••]. Protrusion density along L2/3 PN dendrites in rodent somatosensory cortex rapidly increases during the first postnatal weeks, with spine dynamics (i.e. formation and elimination of spines) decreasing during this time [[17••],18]. However, during adolescence, spine density on L2/3 PN apical dendrites does not change; in contrast, L5 PN spine density decreases by approximately 20% between the second postnatal week and adulthood [12,17••,19]. This discrepancy between L2/3 and L5 PN spine pruning is attributable to differences in their dynamics. Specifically, L2/3 PN spine formation and elimination are comparable in adolescence, while spine elimination outpaces formation on L5 apical dendrites at the same age [17••,20]. By adulthood, spine elimination and formation rates are essentially equal for both neuronal types [17••,19,20]. Across adolescence and adulthood, apical dendritic spines on L2/3 PNs are more dynamic than those on L5 PNs, although turnover rates for both populations decrease with age [17••]. Future studies are needed to determine if such differences arise from intrinsic properties of these neurons or are due to their participation in distinct neuronal circuits.

Spine motility and dynamics also differ among cortical regions. One earlier study shows that spines on L5 PNs change their length rapidly at postnatal day (P) 28, and such changes are significantly larger in somatosensory and auditory cortices than in the visual cortex [16]. Observing spine dynamics in the frontal cortex using an implanted microprism, a recent study reports that spine formation over one day is higher in the prelimbic (PL) region of the medial prefrontal cortex (mPFC) than in frontal association area (FrA) at P30, while spine elimination rates are comparable. This difference in dynamics contributes to a transiently increased spine density in PL at P30 [21••]. Taken together, these studies illustrate the cell type-specific and regional differences in spine dynamics during postnatal development. Furthermore, since many neuropsychiatric disorders (e.g. autism and schizophrenia) are associated with abnormal spine phenotypes and can become symptomatic during early postnatal or adolescent development [10], it is worthwhile to investigate the cell type-specific and region-specific spine dynamics in the context of such disease models.

Circuit-specific cortical spine development and plasticity

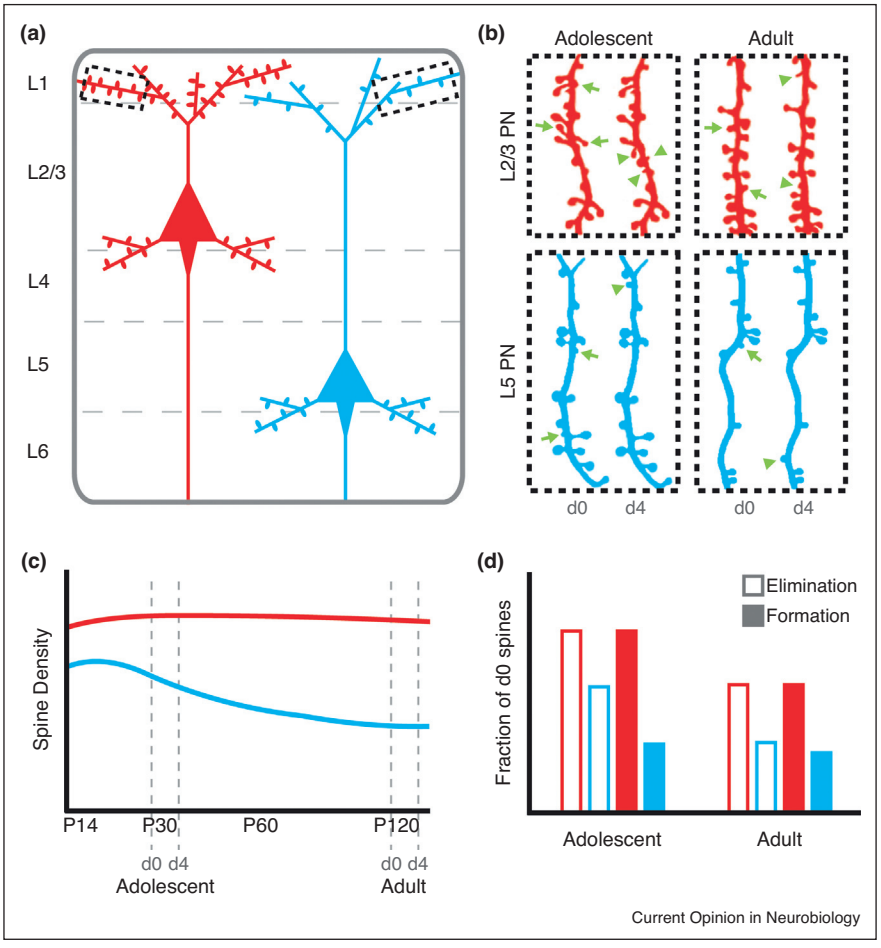
Influence of local inhibitory circuits

Within the cortex, local inhibitory interneurons (INs) releasing gamma-aminobutyric acid (GABA) play an important role in the development and plasticity of dendritic spines. Recent work shows that GABA can facilitate spine formation on L2/3 PNs of 1–2 week-old mice [22], and the expression of α4βδ GABA receptors at dendritic spines is associated with spine pruning in hippocampal CA1 PNs during adolescence [23]. These studies highlight the diverse effects of GABA signaling on spine dynamics; future work is needed to resolve whether the influence of GABA on spines (i.e. participating in spine formation or elimination) is regulated by factors such as cell type, receptor subtype, or developmental stage. Similar to dendritic spines, cortical inhibitory synapses are dynamic, and their formation and elimination can be influenced by experience [24–26]. Furthermore, structural and functional changes in INs are associated with experience-dependent spine plasticity. A recent study shows that motor skill learning induces loss of axonal boutons of somatostatin-expressing (SOM) INs, and optogenetically activating SOM axonal boutons during motor training blocks the stabilization of newly formed spines [27]. Additionally, restraint stress, a paradigm that immobilizes the mouse in a tube for two hours daily, reduces the activity of parvalbumin-expressing (PV) INs, and pharmacogenetically increasing their activity prevents stress-induced spine elimination [28••]. Together, these studies provide evidence that experience-induced IN activity changes may be permissive for dendritic spine structural plasticity; although, how this relationship is developmentally regulated warrants further investigation.

Long-range cortical circuits

Thalamocortical (TC) projections transmit information from subcortical and peripheral structures to the cortex. In the somatosensory (barrel) cortex, TC axons travel to the cortex during late prenatal and perinatal development and are

Figure 1



Schematic illustrating differences between L2/3 and L5 PN dendritic spine density and dynamics in adolescence and adulthood. **(a, b)** Repeated *in vivo* imaging of L2/3 (red) and L5 (blue) PN apical dendritic branches in adolescent and adult mice can reveal spine formation (arrowheads) and elimination (arrows). **(c, d)** Time course of spine density (c) and dynamics (d) of L2/3 (red) and L5 (blue) PNs from adolescence to adulthood. In contrast to L5 PN spines, L2/3 PN apical dendritic spines are more dynamic and are not pruned during adolescence.
Based on data from [17••].

concentrated within L4 barrels [29]. Dynamic extension and retraction of TC axonal branch tips are observed during the first postnatal week and gradually decrease by P14; the addition of new branches decreases by the third postnatal week [15]. On the postsynaptic side, barrel cortex L4 neuron dendrites undergo refinement between P4 and P6, which biases their orientation toward the TC axons within the barrel [30]. Deletion of the obligatory N-methyl-D-aspartate receptor (NMDAR) subunit, NR1, from barrel cortex L4 cells disrupts this orientation bias and decreases spine density by P16, suggesting the importance of glutamatergic signaling in TC circuit development [30]. In adulthood, presumptive TC axonal boutons in the visual cortex are highly stable, and bouton elimination and formation are balanced [14,31•,32]. However, focal stroke [32] and prenatal exposure to altered maternal thyroid hormone levels [31•] may change TC axonal dynamics in adults. Optogenetic stimulation increases the dynamics of TC axonal boutons during recovery from stroke by enhancing the formation and stabilization of new *en passant* boutons [32]. The presumptive targets of TC projections also respond to experience-driven plasticity, since barrel cortex L4 neuron spine elimination transiently increases following whisker trimming [33]. These findings suggest that TC circuits undergo experience-dependent structural plasticity throughout life.

The amygdala sends excitatory projections to various regions of the cerebral cortex. These connections are dynamic and may influence cortical circuit development. Basolateral amygdala (BLA) axonal bouton density in the dorsomedial PFC increases between adolescence and adulthood, despite overall spine pruning during this time, suggesting a unique developmental trajectory of amygdalocortical circuits [34•]. In mouse PL during early adolescence, spine density and formation rate are transiently increased before decreasing to adult levels by late

adolescence, and this pattern is mirrored by changes in connectivity between PL and BLA, as well as between PL and ventral hippocampus [21••]. This suggests that developmental regulation of frontal cortex spine dynamics is driven by contributions from long-range circuits. Inputs from the lateral amygdala to the primary auditory cortex are important for fear memory. A recent study shows that there are pathway-specific increases in spine formation on auditory cortex L5 PNs and formation of axonal boutons arising from lateral amygdala following fear conditioning, suggesting the formation of new synapses [35••]. These findings reveal how the amygdala can exert developmental and experience-dependent influences on cortical circuits.

Neuromodulation

Neuromodulatory inputs, such as those arising from catecholaminergic and cholinergic neurons, are present throughout the cortex. The cortex receives catecholaminergic inputs from locus coeruleus and the ventral tegmental area, the former releasing norepinephrine and the latter releasing dopamine. Morimoto *et al.* recently examined catecholaminergic bouton turnover in mice expressing GFP under control of the tyrosine hydroxylase promoter. They found that these boutons in the frontal and somatosensory cortex are more dynamic than axonal boutons of L2/3 PNs, suggesting that catecholaminergic circuits in cortex have the capacity for substantial structural plasticity [36]. Catecholaminergic signaling in the cortex may influence spine development. Dopaminergic signaling is mediated through dopamine receptors, which are broadly classified into D1-like and D2-like receptors (D1Rs and D2Rs, respectively). Several studies suggest that D1R activation increases spine density [37,38], while D2R does the opposite [39]. Consistent with this, a recent study has shown that dopamine depletion in young adult motor cortex enhances spine dynamics; specifically, antagonizing D1Rs and D2Rs promotes spine elimination and formation, respectively [40•]. The cerebral cortex receives cholinergic inputs from the basal forebrain. Cholinergic signaling through the nicotinic class of acetylcholine receptors (nAChRs) is important for dendritic spine development and cortical circuit plasticity in mice (reviewed in [41]). Deletion of Lynx1, an endogenous inhibitor of nAChRs, enhances cholinergic signaling [42] and increases spine turnover in the visual cortex of adult mice [43]. Together, these findings reveal the contributions of dopaminergic and cholinergic neuromodulatory circuits to developmental and experience-dependent plasticity of cortical spines, an area of research deserving more attention in the future.

Molecular mechanisms contributing to activity-dependent spine dynamics

Several *in vivo* imaging studies have identified molecular mediators that may link experience-dependent or developmental changes in synaptic activity to structural plasticity of dendritic spines. One such factor is Ca^{2+}/calmodulin dependent kinase II (CaMKII), which is known to play a role in long term potentiation within spines [44] and is important for the stabilization of newly formed spines induced by experience *in vivo* [45]. Rho GTPases also contribute to actin rearrangement within spines, and thus are poised to act as mediators of structural and functional spine plasticity (reviewed in [46]). Interestingly, prolonged activation of a photoactivatable form of the Rho GTPase Rac1 *in vivo* leads to spine shrinkage and impairs performance on a learned motor task [47]. Knock-out of the *Fmr1* gene and the subsequent loss of its protein product, Fragile X Mental Retardation Protein (FMRP), leads to greater spine elimination and formation in the mouse somatosensory cortex during adolescence and prevents changes in spine turnover normally induced by whisker trimming [48]. Further, experience-dependent spine remodeling in adolescent *ephrin-A2* knockout mice is impaired [49]. Both FMRP and Eph receptor-ephrin signaling are known to contribute to actin-binding protein signaling and spine structural development and plasticity [50,51]. Additionally, ablation of the retinoic acid receptor RARα in PNs increases somatosensory cortex spine elimination in adolescence. This increased elimination depends on whisker-mediated sensory input [52], suggesting a role for retinoic acid signaling in activity-dependent spine plasticity, potentially through AMPA receptor trafficking [53]. Future work will determine how molecular mechanisms underlying spine structural plasticity differ among cell types and circuits, how these mechanisms change across the lifespan, and will provide a more complete understanding of dendritic spine development and plasticity.

Conclusion

Cortical dendritic spines develop and respond to experiences, and their dynamics are modulated by local and long-range circuit components (Table 1). However, many factors influencing cortical spine development and plasticity remain to be determined, particularly the contributions of presynaptic inputs and structural dynamism in other cell types. Technological advances in co-imaging presynaptic and postsynaptic structures [35••,54], fluorescent labeling of specific neuronal and non-neuronal populations [55,56], and novel *in vivo* microscopy techniques that can image synaptic structures in deeper areas of the brain [57–59], will enable further dissection of cell type-specific circuit contributions to cortical spine development. In addition, future studies combining structural imaging with functional readouts and manipulations (e.g. calcium imaging and optogenetic circuit mapping) will reveal how developmental synaptic and circuit reorganization relate to brain function and behavior. Using these techniques in conjunction with various animal models will illustrate how risk factors for neuropsychiatric and neurological disorders impact dendritic spine development, and also how

Table 1

Region-specific and circuit-specific synaptic dynamics *in vivo*. *Abbreviations*: Cortical layer (L); pyramidal neuron (PN); somatostatin (SOM); parvalbumin (PV); thalamocortical (TC); medial prefrontal cortex (mPFC); dorsomedial prefrontal cortex (dmPFC); basolateral amygdala (BLA); ventral CA1 (vCA1); orbitofrontal cortex (OFC)

Cortical region	Circuit investigated	Imaged structure	Age	Result	Ref.
Auditory, somatosensory, visual	—	Excitatory axonal boutons and L5 PN spines	1–2 months	Boutons are more stable than spines	[16]
Frontal, motor, somatosensory, visual	—	L5 PN spines	Various	Spine elimination outpaces formation during adolescence, but balances in adulthood	[8,12,13]
Motor, somatosensory	—	L2/3 and L5 PN spines	Various	L2/3 PN spine density higher than L5 PN, absence of adolescent pruning, balanced formation/elimination	[17••,20,13]
Motor	Local inhibition	Inhibitory neuron axons and L2/3 PN spines	6+ weeks	SOM neuron activation blocks motor training-induced stabilization of new spines	[27]
Somatosensory	Local inhibition	L5 PN spines	1 month	PV neuron activation blocks stress-induced spine elimination	[28••]
Somatosensory	TC	TC axons	1-3 weeks	Axonal branch addition and length changes decrease by 2–3 weeks	[15]
Somatosensory, visual	TC	TC axons	2.5+ months	Boutons highly stable, balanced formation and elimination	[14,31•,32]
Visual	TC	TC axons	2–4 months	Increased TC axon dynamics in adult offspring following maternal hypothyroidism and hyperthyroidism	[31•]
Somatosensory	TC	L4 neuron	5 months	Whisker trimming transiently increases spine elimination	[33]
Somatosensory	TC	TC axons	2–5 months	Stimulation of TC axons increases formation and stabilization of new *en passant* boutons	[32]
Prelimbic mPFC	Amygdalocortical; hippocampus-cortical	L5 PN spines	1 and 2 months	Transient increases in spine density and formation in parallel with increased connectivity with BLA and vCA1	[21••]
dmPFC	Amygdalocortical; cortico-cortical	L5 PN spines; BLA and OFC axons	1 and 2 months	Like spines, OFC bouton dynamics decrease with age, but unlike spines, their density does not decrease; BLA bouton formation and density increase	[34•]
Auditory	Amygdalocortical	Amygdalo-auditory and fronto-auditory axons, L2/3 and L5 PN spines	7–10 weeks	Fear conditioning increases lateral amygdala bouton formation and cortical spine formation	[35••]
Frontal, somatosensory	Catecholaminergic	Catecholaminergic boutons, L2/3 PN boutons	2–4 months	Catecholaminergic boutons are more dynamic than L2/3 PN axon boutons	[36]
Motor	Dopaminergic	L5 PN spines	1–3 months	Dopamine depletion enhances spine dynamics; activation of different dopamine receptor types exerts different effects on spine dynamics	[40•]
Visual	Cholinergic	L2/3 and L5 PN spines	3–7 months	Enhancement of cholinergic signaling by Lynx1 deletion increases spine turnover and spine elimination following monocular deprivation	[43]

synapse and circuit development are perturbed by extrinsic factors, such as environmental toxins and stress [28••,60–62]. Thus, a better understanding of cortical circuit development will likely facilitate the establishment of new interventions and treatments for human disease.

Conflict of interest statement

Nothing declared.

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