Journal Club

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A Novel Mechanism Underlying Activity-Dependent Pruning in Postnatal Prefrontal Cortex

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Review of Mohan et al.

Adolescence is a period of refinement of brain connectivity, particularly within frontal regions, that enables the transition to adult cognitive ability and emotional function. For this reason, adolescence has been thought of as a “critical period” of prefrontal cortex (PFC) development, similar to those observed in sensory cortical regions earlier in life. During these periods, an initial overproduction of synapses is followed by experience-dependent pruning, such that inactive synapses, and the dendritic spines they are located on, are eliminated (Crews et al., 2007; Selemon, 2013). Plasticity such as long-term potentiation (LTP) or long-term depression at particular synapses is thought to be involved in selection of connections to be retained or pruned (Selemon, 2013). Neither the molecular factors that promote spine pruning during adolescence nor those that link synaptic plasticity with structural remodeling during this period are well understood.

Cell adhesion molecules are appealing candidates for regulating structural plasticity because they can trigger cytoskeletal reorganization to promote growth or collapse of neurites via intracellular signaling cascades and are often present at presyn-

aptic or postsynaptic sites, where they may influence or be influenced by synaptic transmission. The L1 family of cell adhesion molecules is of interest in the context of pruning because polymorphisms in several L1 genes have been linked to neuropsychiatric disorders with spine abnormalities, including autism (Salyakina et al., 2011) and schizophrenia (Chen et al., 2005). L1 cell adhesion molecules are also known to interact with class 3 semaphorins (Sema3; Wright et al., 2007; Mohan et al., 2019a), a family of secreted guidance cues that induce repulsion of growing neural processes (Liu and Strittmatter, 2001) and can promote dendritic retraction. In a recent study, Mohan et al. (2019b) examined how Close Homolog of L1 (CHL1) affects dendritic spine dynamics in cortical regions during adolescence, and whether dendritic remodeling at this time is mediated by CHL1-Sema3 interactions.

To begin with, Mohan et al. (2019b) examined spine density in PFC and primary visual cortex of juvenile and adult CHL1-null mice, and found increased density of apical, but not basal, dendritic spines relative to control mice in both regions. This suggests that CHL1 negatively regulates spine number in postnatal development. The CHL1-null mice also had a smaller proportion of more mature mushroom or stubby spines and larger proportions of immature, thin spines compared with controls. Because increases in activity

are thought to drive maturation of thin spines into mushroom spines, that CHL1 deficiency predominately led to an increase in number of thin spines indicates that CHL1 likely plays a role in elimination of less active synapses on apical dendrites.

Next, the authors assayed involvement of Sema3s in CHL1-mediated pruning by treating cultured cortical neurons with Sema3 proteins. In wild-type mice, Sema3B and 3F decreased dendritic spine density, whereas other Sema3s had no effect. Sema3B failed to reduce spine density in cultures from CHL1-null mice, whereas Sema3F reduced spine density to a similar extent as in wild-type mice, suggesting that CHL1 promotes elimination of dendritic spines through interaction with Sema3B. Previous work demonstrated that Sema3F-mediated dendritic spine retraction involves an interaction with NrCAM (Mohan et al., 2019a), another L1 family member, indicating that Sema3B and Sema3F influence dendritic spines through formation of distinct receptor complexes. Strikingly, although most spines expressed either CHL1 or NrCAM, the molecules were rarely expressed in the same spine. Furthermore, Sema3B led to a selective reduction in the density of CHL1-positive spines, whereas Sema3F selectively decreased NrCAM-positive spines. Collectively, these findings show that different Sema3s induce pruning of distinct populations of spines.

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Active, potentiated synapses

Inactive synapses

Figure 1. Potential mechanisms by which L1-Sema3 and β 1-integrin may interact during remodeling of synaptic connectivity in the adolescent PFC. High levels of activity at potentiated synapses are associated with spine stabilization via a β 1-integrin signaling, whereas downregulation of Neuropilin-2 expression via an induction of microRNA-188 may inhibit CHL1-Sema3B signaling. Less-active synapses are subject to elimination by the CHL1-Sema3B mechanism, which also may inhibit β 1-integrin-mediated stabilization.

Increased spine number in CHL1-null mice was accompanied by increased numbers of excitatory synapses detected with electron microscopy, so Mohan et al. (2019b) examined whether corresponding changes in synaptic transmission were observed. Miniature EPSC amplitudes were skewed toward a larger magnitude in CHL1-null mice and there was a greater variance in the total charge which reflects changes in mEPSC amplitude and time course. A uniform change in mEPSC amplitude may not have been observed in CHL1-null mice because the increase in spines was only present in apical dendrites, although the recordings sampled from both apical and basal synaptic sites. Nevertheless, these results raise the possibility that CHL1 regulates excitatory synaptic strength in a subset of PFC pyramidal cells. Decreased paired-pulse facilitation was also observed in CHL1-null mice, suggesting that CHL1 affects presynaptic function through an as-of-yet unknown mechanism.

To better understand how CHL1 modifies synaptic function, coimmunoprecipitation experiments in synaptoneurosomes enriched with both presynaptic and postsynaptic components were conducted to identify proteins associating with CHL1. Interactions with several Plexins and Neuropilins, which have been shown to act as Sema3 coreceptors with L1 cell adhesion molecules (Wright et al., 2007; Mohan et al., 2019a), were probed but CHL1 coimmunoprecipitated only with Neuropilin-2 and PlexinA4 in cortical neurons. Both Neuropilin-2, which can also be a Sema3F coreceptor with NrCAM, and PlexinA4 play a role in AMPA receptor trafficking and could influence synaptic strength when in com-

plex with CHL1 (Yamashita et al., 2014; Wang et al., 2017).

Finally, the authors asked whether CHL1/Sema3B-mediated spine elimination might occur in an activity-dependent manner. Indeed, when cortical neuron cultures were treated with a GABA antagonist to induce an increase in neural activity, elevated Sema3B secretion was observed.

Although it has been speculated that cell adhesion receptors and diffusible molecules that guide connectivity during early development are involved in synaptic remodeling in adolescent PFC, few of the precise molecular players that drive adolescent pruning have been identified. Here, Mohan et al. (2019b) identify interactions between CHL1 and Sema3B as a novel mechanism by which immature or unneeded synapses may be selectively pruned during postnatal remodeling of PFC circuitry. Their findings indicate that CHL1/Sema3B-mediated pruning is likely to occur in an activity-dependent manner, but how spines are selected for elimination by this mechanism, and also the circumstances under which Sema3B is secreted in response to activity require further examination.

Deficiency in CHL1 led to a selective increase in thin dendritic spine number, suggesting that CHL1-Sema3B signaling promotes pruning of immature or unneeded synapses. Neuropilin-2, which was found to be part of CHL1 holoreceptor complex in cortical neurons, may play role in targeting weak connections for removal. Neuropilin-2 is downregulated following induction of LTP through a mechanism involving microRNA-188 (Lee et al., 2012). Because Neuropilin-2 acts as a coreceptor for Sema3B/F, its removal

from the receptor complex might spare potentiated synapses from Sema3-mediated pruning, whereas high Neuropilin-2 expression in spines would mediate elimination of weak synaptic connections (Fig. 1). Thus, one possibility is that induction of LTP protects active connections from Sema3-mediated pruning. Conversely, it will be of interest to determine whether induction of depression in less-active spines enhances expression of Neuropilin-2 and other CHL1 holoreceptor components to promote spine elimination. Changes in synaptic strength are thought to precede spine loss or retention (Selemmon, 2013), so past work showing that Neuropilin-2 and PlexinA4 can promote reduced AMPA receptor surface localization (Yamashita et al., 2014; Wang et al., 2017) could also indicate that CHL1 holoreceptor expression in spines contributes to pruning by downregulating synaptic strength before spine elimination initiated by Sema3B.

Both CHL1 and NrCAM form complexes with Neuropilin-2 but appear to be affected by different Sema3s, so it will be important to determine how certain spines come to express CHL1 versus NrCAM and are thus subject to different forms of Sema3-mediated elimination. It is possible that different types of L1/Sema3-mediated spine elimination play a role in activity-dependent competition between different local excitatory and/or afferent connections, such as inputs from the amygdala and hippocampus (Cunningham et al., 2002; Caballero et al., 2016), which are also refined during adolescence. To test this hypothesis, experiments that determine whether select afferent or local

populations of neurons express *Sema3B* versus *3F* will be needed.

L1-Sema3 signaling is likely one of several mechanisms involved in activity-dependent synaptic refinement in adolescent PFC. Indeed, another paper recently published in *JNeurosci* found that β 1-integrin, an extracellular matrix adhesion molecule, is involved in spine stabilization or retention in the adolescent orbitofrontal cortex (DePoy et al., 2019). Selective knock-down of β 1-integrin before adolescence led to a reduction in dendritic spine number and deficits in expectancy updating, a key cognitive function mediated by the OFC. β 1-integrin, which is expressed throughout postnatal frontal cortex (Shapiro et al., 2017), contributes to induction of LTP (Chan et al., 2006; Babayan et al., 2012), suggesting a parallel link between dendritic spine dynamics and synaptic plasticity with β 1-integrin supporting retention of active, potentiated connections, and the L1-Sema3 interactions promoting removal of less active or depressed synapses. Notably, in earlier work, Mohan et al. (2019a) found that *NrCAM-Sema3F*-mediated activation of *Neuropilin-2* led to decreased β 1-integrin expression at the synapse via *Plexin* activity, suggesting that these two mechanisms could work together to determine which connections are stabilized and which undergo pruning (Fig. 1).

Although Mohan et al. (2019b) found that *Sema3B* was secreted in response to a widespread, experimentally induced increase in neural activity in culture, it will be important to determine in what situations *Sema3s* are secreted in intact circuits and in response to experience. One possibility is that release of *Sema3B* by recently potentiated synapses drives elimination of less active neighboring spines expressing *CHL1* receptor complexes. Alternatively, *CHL1* holoreceptor expression at inactive or depressed synapses could render them vulnerable to *Sema3B*-mediated removal upon more general increases in network activity. Activity-dependent *Sema3B* secretion may play an on-demand role in refining excitatory connectivity in adoles-

cent PFC, such that increased activity initiates *CHL1/Sema3B*-mediated pruning to limit unneeded inputs. This would not only prevent hyperexcitability, but also promote efficient and precise neural communication in mature PFC networks. Given that this type of functional refinement is thought to support cognitive maturation during adolescence, and that deficiency in *CHL1* impairs cognitive functions mediated by PFC (Kolata et al., 2008), *CHL1/Sema3B*-mediated pruning of spines may be a novel mechanism supporting PFC-dependent adolescent cognitive development. Thus, studies by Mohan et al. (2019a,b) are a valuable step toward understanding the molecular specifiers that link synaptic remodeling with development of adult PFC function.

References

- Babayan AH, Kramár EA, Barrett RM, Jafari M, Häettig J, Chen LY, Rex CS, Lauterborn JC, Wood MA, Gall CM, Lynch G (2012) Integrin dynamics produce a delayed stage of long-term potentiation and memory consolidation. *J Neurosci* 32:12854–12861.
- Caballero A, Granberg R, Tseng KY (2016) Mechanisms contributing to prefrontal cortex maturation during adolescence. *Neurosci Biobehav Rev* 70:4–12.
- Chan CS, Weeber EJ, Zong L, Fuchs E, Sweatt JD, Davis RL (2006) β 1-Integrins are required for hippocampal AMPA receptor-dependent synaptic transmission, synaptic plasticity, and working memory. *J Neurosci* 26:223–232.
- Chen QY, Chen Q, Feng GY, Lindpaintner K, Chen Y, Sun X, Chen Z, Gao Z, Tang J, He L (2005) Case-control association study of the close homologue of L1 (*CHL1*) gene and schizophrenia in the Chinese population. *Schizophr Res* 73:269–274.
- Crews F, He J, Hodge C (2007) Adolescent cortical development: a critical period of vulnerability for addiction. *Pharmacol Biochem Behav* 86:189–199.
- Cunningham MG, Bhattacharyya S, Benes FM (2002) Amygdalo-cortical sprouting continues into early adulthood: implications for the development of normal and abnormal function during adolescence. *J Comp Neurol* 453:116–130.
- DePoy LM, Shapiro LP, Kietzman HW, Roman KM, Gourley SL (2019) β 1-Integrins in the developing orbitofrontal cortex are necessary for expectancy updating in mice. *J Neurosci* 39:6644–6655.
- Kolata S, Wu J, Light K, Schachner M, Matzel LD (2008) Impaired working memory duration but normal learning abilities found in mice that are conditionally deficient in the close homolog of L1. *J Neurosci* 28:13505–13510.
- Lee K, Kim JH, Kwon OB, An K, Ryu J, Cho K, Suh YH, Kim HS (2012) An activity-regulated microRNA, miR-188, controls dendritic plasticity and synaptic transmission by downregulating *neuropilin-2*. *J Neurosci* 32:5678–5687.
- Liu BP, Strittmatter SM (2001) Semaphorin-mediated axonal guidance via rho-related G proteins. *Curr Opin Cell Biol* 13:619–626.
- Mohan V, Sullivan CS, Guo J, Wade SD, Majumder S, Agarwal A, Anton ES, Temple BS, Maness PF (2019a) Temporal regulation of dendritic spines through *NrCAM-Semaphorin3F* receptor signaling in developing cortical pyramidal neurons. *Cereb Cortex* 29:963–977.
- Mohan V, Wade SD, Sullivan CS, Kasten MR, Sweetman C, Stewart R, Truong Y, Schachner M, Manis PB, Maness PF (2019b) Close homolog of L1 regulates dendritic spine density in the mouse cerebral cortex through *semaphorin 3B*. *J Neurosci* 39:6233–6250.
- Salyakina D, Cukier HN, Lee JM, Sacharow S, Nations LD, Ma D, Jaworski JM, Konidari I, Whitehead PL, Wright HH, Abramson RK, Williams SM, Menon R, Haines JL, Gilbert JR, Cuccaro ML, Pericak-Vance MA (2011) Copy number variants in extended autism spectrum disorder families reveal candidates potentially involved in autism risk. *PLoS One* 6:e26049.
- Selemon LD (2013) A role for synaptic plasticity in the adolescent development of executive function. *Transl Psychiatry* 3:e238.
- Shapiro LP, Parsons RG, Koleske AJ, Gourley SL (2017) Differential expression of cytoskeletal regulatory factors in the adolescent prefrontal cortex: implications for cortical development. *J Neurosci Res* 95:1123–1143.
- Wang Q, Chiu SL, Koropoulis E, Hong I, Mitchell S, Easwaran TP, Hamilton NR, Gustina AS, Zhu Q, Ginty DD, Hagan RL, Kolodkin AL (2017) *Neuropilin-2/PlexinA3* receptors associate with *GluA1* and mediate *Sema3F*-dependent homeostatic scaling in cortical neurons. *Neuron* 96:1084–1098.e7.
- Wright AG, Demyanenko GP, Powell A, Schachner M, Enriquez-Barreto L, Tran TS, Polleux F, Maness PF (2007) Close homolog of L1 and *neuropilin 1* mediate guidance of thalamo-cortical axons at the ventral telencephalon. *J Neurosci* 27:13667–13679.
- Yamashita N, Usui H, Nakamura F, Chen S, Sasaki Y, Hida T, Suto F, Taniguchi M, Takei K, Goshima Y (2014) *Plexin-A4*-dependent retrograde semaphorin 3A signalling regulates the dendritic localization of *GluA2*-containing AMPA receptors. *Nat Commun* 5:3424.