Astrocytes "Chordinate" Synapse Maturation and Plasticity

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A key step in excitatory synapse maturation is the switch in AMPA receptor subunit composition to GluA2-containing calcium-impermeable receptors. Now, Blanco-Suarez et al. (2018) demonstrate that astrocyte-secreted chordin-like 1 drives this process, enabling synapse maturation and limiting plasticity.

The development and maturation of synaptic connections between neurons is an intricate process that requires the careful orchestration of many molecular players. A key step in the maturation of excitatory glutamatergic synapses in the CNS is a switch in the calcium-permeability of ionotropic AMPA glutamate receptors (AMPARs) that are present at the postsynaptic terminals. The subunit composition of AMPARs governs their permeability, with the presence of the GluA2 subunit rendering the channel impermeable to calcium. AMPARs at immature synapses lack GluA2 and allow calcium into the postsynaptic cell. The switch to GluA2-containing calcium-impermeable AMPARs promotes synapse maturation by decreasing calcium entry into the cell, thereby limiting calcium-dependent plasticity mechanisms and stabilizing the synapse (Diering and Huganir, 2018). In the mouse cortex, this switch happens in a precise regional and temporal fashion, occurring in distinct neuronal layers at specific time points (Brill and Huguenard, 2008). However, the molecular mechanisms that regulate this process, and whether these mechanisms are intrinsic to neurons or can be instructed by extrinsic signals from non-neuronal cells, were previously not known.

A number of studies conducted over the past two decades have demonstrated the importance of astrocyte-secreted signals in regulating the formation of synaptic connections in the brain (reviewed in Allen and Eroglu, 2017). It has been shown that astrocytes secrete several factors, including thrombospondin and Sparcl1 family proteins, that promote the formation of structural/silent synapses, which only contain NMDARs. Previous work by Allen et al. (2012) identified glypican (Gpc) family proteins Gpc4 and Gpc6 as astrocyte-secreted factors that promote the formation of AMPAR-containing, functional synapses by recruiting the GluA1-containing calcium-permeable AMPARs to synapses. However, whether astrocytes also secreted factors to recruit GluA2-containing calcium-impermeable receptors to the synapses to promote functional maturation of synapses was previously unknown.

In this issue of *Neuron*, Blanco-Suarez et al. (2018) identify chordin-like 1 (Chrdl1) as an astrocyte-secreted factor that is necessary and sufficient to induce the formation of mature GluA2-containing synapses. To identify Chrdl1, the authors performed a biochemical screen of astrocyte-secreted factors using purified rat retinal ganglion cell neuron cultures. Purified retinal ganglion cells survive and form extensive arrays of neurites in the absence of astrocytes, but form very few synapses. Addition of an astrocyte-feeder layer or treatment of retinal ganglion cells with astrocyte-conditioned media strongly increases excitatory synapse numbers and function as well as surface AMPAR expression (Allen and Eroglu, 2017). Using this system, Blanco-Suarez et al. (2018) found that application of a biochemical fraction of astrocyte-conditioned media, containing Chrdl1, was sufficient to induce a robust increase in the surface levels of GluA2. Indeed, addition of purified Chrdl1 protein to retinal ganglion cells also significantly increased the number of functionally active synapses and the surface expression of GluA2. Chrdl1 is a member of the chordin family of secreted BMP antagonists; however, the authors found that the mechanism through which Chrdl1 regulates synaptic GluA2 clustering is independent of BMP signaling.

To determine whether astrocyte-secreted Chrdl1 is relevant to the development and maturation of synaptic connections *in vivo*, Blanco-Suarez et al. (2018) used the well-characterized mouse visual cortex as a model system. Using *in situ* hybridization, they examined the developmental and regional expression of Chrdl1 mRNA, finding that Chrdl1 is strongly enriched in astrocytes compared to neurons and is expressed throughout postnatal development and in the adult. In the cortex, Chrdl1 expression peaks between postnatal day 12 (P12) and P14. Interestingly, Chrdl1 displays a heterogeneous expression pattern, with the strongest expression found in the upper cortical layer astrocytes. Notably, the timing and localization of Chrdl1 expression coincides with the GluA2-receptor switch that occurs in layer 2/3 neurons between P12 and P14 (Blanco-Suarez et al., 2018; Brill and Huguenard, 2008).

Does Chrdl1 regulate synapse maturation by initiating a GluA1-to-GluA2-subunit-containing AMPAR switch *in vivo*? To answer this question, Blanco-Suarez et al. (2018) generated a Chrdl1 knockout (KO) mouse. Immunohistochemical analyses showed that in the upper layers of the visual cortex at P14, Chrdl1 KO mice display a significant decrease in GluA2 expression, but no change in GluA1. Furthermore, they found that GluA2 is

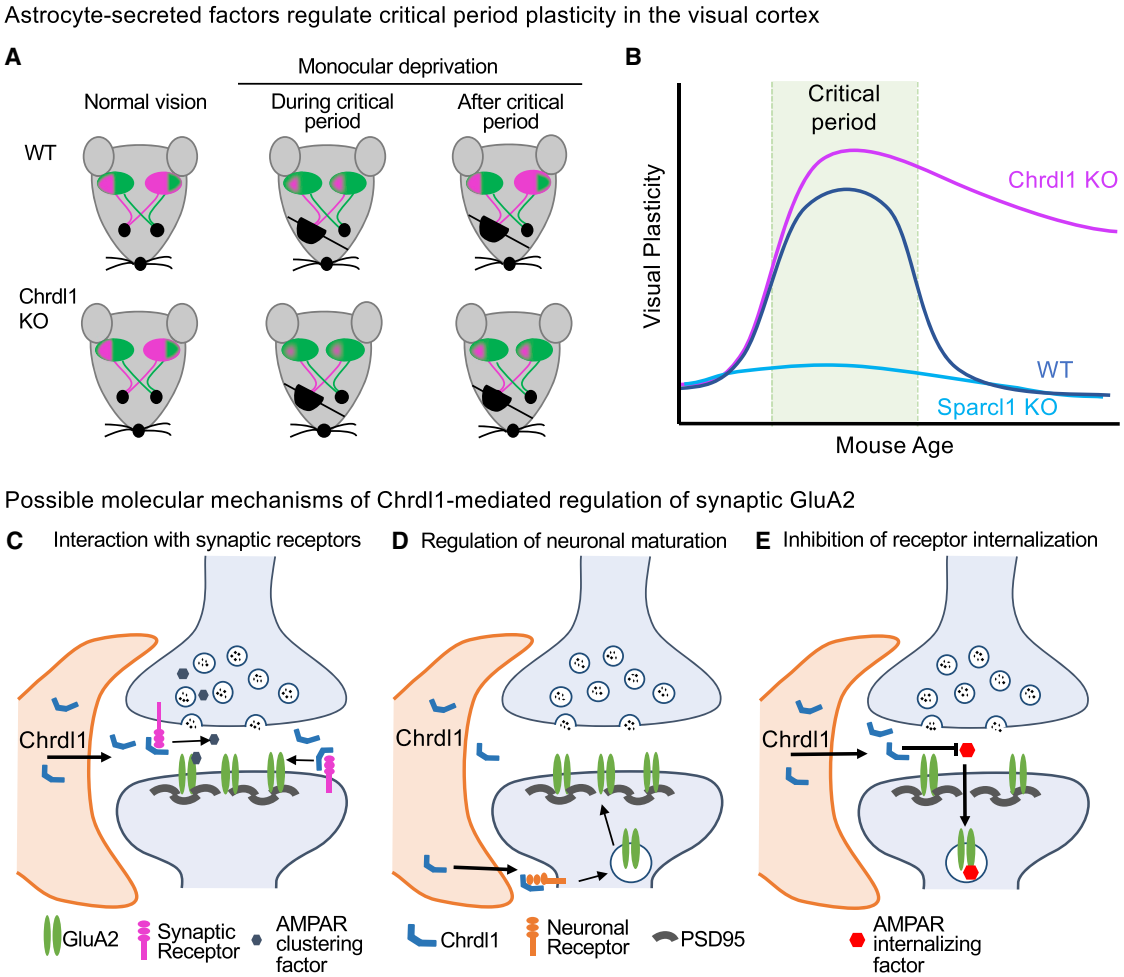

Figure 1. Astrocyte-Secreted Chrdl1 Controls Critical Period Plasticity via Inducing AMPAR-Subunit Switch
(A) Chrdl1 is required for restricting ocular dominance plasticity during and after critical periods.
(B) Astrocyte-secreted factors Chrdl1 and Sparcl1 differentially control ocular dominance plasticity.
(C–E) Possible molecular mechanisms of Chrdl1-mediated regulation of synaptic GluA2 levels. (C) Chrdl1 might act directly at synapses by engaging a pre- and/or post-synaptic receptor. (D) Chrdl1 could initiate a neuronal maturation program that controls the expression and/or surface trafficking of GluA2. (E) Chrdl1 might inhibit a mechanism that prevents the retention of GluA2-containing AMPARs at synapses.

lost from both intracortical and thalamocortical excitatory synapses in the Chrdl1 KO mice. These mice also showed a significant decrease in the number of thalamocortical synapses. In physiological analyses, Chrdl1 KO mice displayed altered kinetics of AMPA-mediated excitatory synaptic events in layer 2/3 pyramidal neurons at P14. Importantly, deletion of Chrdl1 did not cause any overt deficiencies in astrocyte or neuron development, indicating the phenotypes observed in Chrdl1 KO mice are due to a loss of the specific functions of astrocyte-secreted Chrdl1 at the synapse.

Previous studies suggested that the switch from GluA1- to GluA2-containing AMPARs drives the closing of developmental periods of plasticity in the cortex (Diering and Huganir, 2018). Therefore, the authors next tested whether the loss of Chrdl1 affects the visual experience-dependent critical period of plasticity in the mouse visual cortex. Eliminating vision from one eye (e.g., via monocular enucleation) during a critical period corresponding to postnatal days P28 to P32 in mice drives the remodeling of synaptic connections in the binocular zone of the visual cortex. The inputs from the intact eye take over the territories of the axons that relayed the inputs from the lost eye (Figure 1A). This phenomenon is known as ocular dominance plasticity. However, if the eye is lost after the closure of the critical period, then monocular enucleation no longer induces remodeling of eye-specific inputs (Tropea et al., 2009). Blanco-Suarez et al. (2018) found that the critical period plasticity following monocular enucleation is enhanced in Chrdl1 KO mice compared to WT mice. Remarkably, loss of vision in one eye also induced significant plasticity and remodeling in the binocular zone of the adult Chrdl1 KO mice, but not in WT mice (Figures 1A and 1B). This result demonstrates that the loss of Chrdl1 results in extended critical period plasticity that remains open well into adulthood. Taken together, Blanco-Suarez et al. (2018) demonstrate

that astrocyte-secreted Chrdl1 drives the AMPAR switch at synapses during brain development, and show that astrocytes play a vital role in regulating the closure of critical period plasticity in the maturing brain.

The findings of Blanco-Suarez et al. (2018) raise many intriguing questions and open the door for several new lines of future investigation. For example, while the authors demonstrated that Chrdl1 regulates synaptic GluA2 levels and synapse development independently of BMP signaling, the molecular mechanisms through which Chrdl1 regulates synapse maturation remain to be determined. Previously, Farhy-Tselnicker et al. (2017) found that astrocyte-secreted Gpc4 promotes synaptic GluA1 AMPAR clustering by binding to presynaptic receptor protein tyrosine phosphatase delta (RPTPδ) and stimulating the release of AMPAR-clustering factor neuronal pentraxin 1. Chrdl1, like Gpc4, might act directly at synapses by engaging a neuronal receptor at the pre- and/or post-synapse (Figure 1C). Alternatively, Chrdl1 could initiate a neuronal maturation program that controls the expression and/or surface trafficking of GluA2 (Figure 1D). Chrdl1 might also inhibit a mechanism that prevents the retention of GluA2-containing AMPARs at synapses (Figure 1E).

A curious observation from Blanco-Suarez et al. (2018) is that Chrdl1 expression is highest in the upper cortical layers of the visual cortex between P12 and P14. This time point coincides not only with the AMPAR receptor subtype switch, but also with sensory input that is initiated by the opening of eyelids and onset of vision in mice. Could vision-induced activity in the cortex control the regional and temporal specificity of Chrdl1 expression? Examining the expression of Chrdl1 in dark-reared mice could provide insights into the role that sensory input plays in regulating Chrdl1 expression in astrocytes. Astrocytes between and within different brain regions show molecular and functional heterogeneity (Ben Haim and Rowitch, 2017). Thus, the possibility also exists that intrinsic properties of upper cortical layer astrocytes, or in their cross-talk with layer 2/3 neurons, could be controlling the timing and location of Chrdl1 expression.

Blanco-Suarez et al. (2018) found that Chrdl1 expression is necessary to limit ocular dominance plasticity in the binocular zone of the visual cortex following monocular enucleation (Figure 1A). These findings provide additional insights into the roles of astrocytes as key regulators of formation, maturation, and plasticity of synaptic circuits both during development and in the adult. Previous studies have shown that mice lacking astrocyte-secreted synaptogenic protein Sparcl1/Hevin display the opposite phenotype, where no ocular dominance plasticity is observed in Sparcl1/Hevin KO mice (Singh et al., 2016) (Figure 1B). Of note, both Chrdl1 and Sparcl1 are particularly important for the formation and plasticity of thalamocortical synapses in the visual cortex, as loss of either one leads to a significant decrease in the number of thalamocortical synapses in the upper layers of the visual cortex (Risher et al., 2014; Blanco-Suarez et al., 2018). The opposing roles of Chrdl1 and Sparcl1 in regulating plasticity further highlight the importance of astrocytes in establishing and maintaining plasticity and reveal the complexity of the underlying molecular mechanisms. Further detailed molecular studies will be necessary to understand how multiple astrocyte-secreted cues function together to regulate formation, maturation, and plasticity of other circuits and other types of synapses.

In summary, the study by Blanco-Suarez et al. (2018) defines a novel role for astrocytes in synapse maturation and plasticity and opens the door for many future lines of investigation into the role of astrocytes in the establishment and maintenance of synapses and circuits in the CNS.

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