



## Minireview

# Molecular Mechanisms of Synaptic Specificity: Spotlight on Hippocampal and Cerebellar Synapse Organizers

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Synapses and neural circuits form with exquisite specificity during brain development to allow the precise and appropriate flow of neural information. Although this property of synapses and neural circuits has been extensively investigated for more than a century, molecular mechanisms underlying this property are only recently being unveiled. Recent studies highlight several classes of cell-surface proteins as organizing hubs in building structural and functional architectures of specific synapses and neural circuits. In the present minireview, we discuss recent findings on various synapse organizers that confer the distinct properties of specific synapse types and neural circuit architectures in mammalian brains, with a particular focus on the hippocampus and cerebellum.

**Keywords:** brain disorder, neural circuit, specificity, synapse, synaptic adhesion

## INTRODUCTION

The wiring of the brain occurs during development through a series of precisely orchestrated and complex developmental processes regulated by various mechanisms encompassing astronomical numbers of different neuronal cell types (Shen and Scheiffele, 2010). One of the hallmarks of neuronal cells is that an individual neuron forms a 'specific' network of synaptic connections, and that this circuit specificity

is crucial for most, but not all, functions of the central nervous system (CNS) (Fig. 1). In this manner, each neuron is able to receive various input information from a variety of other neuronal cells, and send processed information to other neuronal cells to rapidly and precisely manage various cognitive tasks. How patterning of specific synaptic connections manifests is complicated, owing to the distinct and diverse structural and functional properties of these connections, which are collectively determined by specific synapse-type properties, such as action potential firing, synaptic plasticity, presynaptic neurotransmitter release probability, and postsynaptic receptor repertoires (Südhof, 2017a).

Although cellular mechanisms that allow specific neuronal connections in early stages of CNS development have been well established, partly thanks to extensive studies on axon guidance pathways, molecular mechanisms underlying events in late stages of CNS development are less clear. For example, how does one neuron accomplish connections within the specific subcellular compartment of other partner neurons, and how are different types of neurons structurally and functionally linked in the final step of neural circuit assembly.

Recent studies have revealed that various cell-surface proteins are instrumental in defining synaptic and circuit specificity, with discrete neuronal cell types exhibiting distinct expression patterns (Foldy et al., 2016; Paul et al., 2017; Shekhar et al., 2016) (Fig. 1). For instance, neurexins and

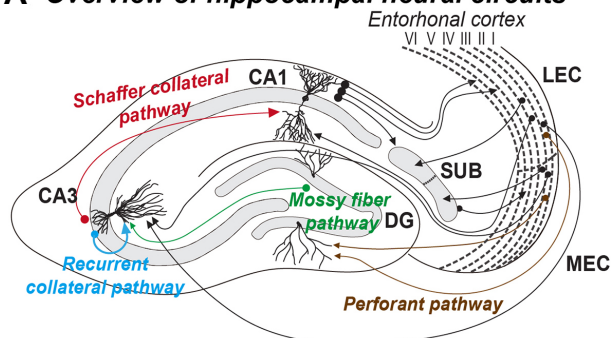
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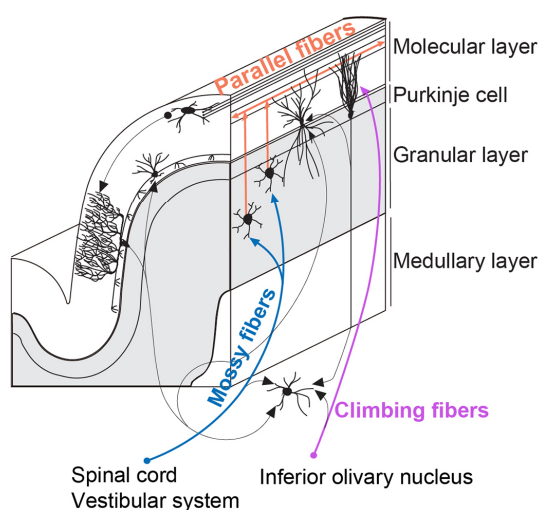
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## A Overview of hippocampal neural circuits



## B Overview of cerebellar neural circuits



**Fig. 1. Overview of the basic neural circuit architectures of the hippocampus and cerebellum.** (A) Illustration of the hippocampal circuitry. The canonical projections involving entorhinal cortical neurons and various hippocampal neurons are depicted by solid arrows. Abbreviations: DG, dentate gyrus; LEC, lateral entorhinal cortex; MEC, medial entorhinal cortex; and SUB, subiculum. (B) Illustration of the cerebellar circuitry. The canonical projections involving various cerebellar neurons are depicted by solid arrows. Abbreviations: CF, climbing fiber; and PF, parallel fiber.

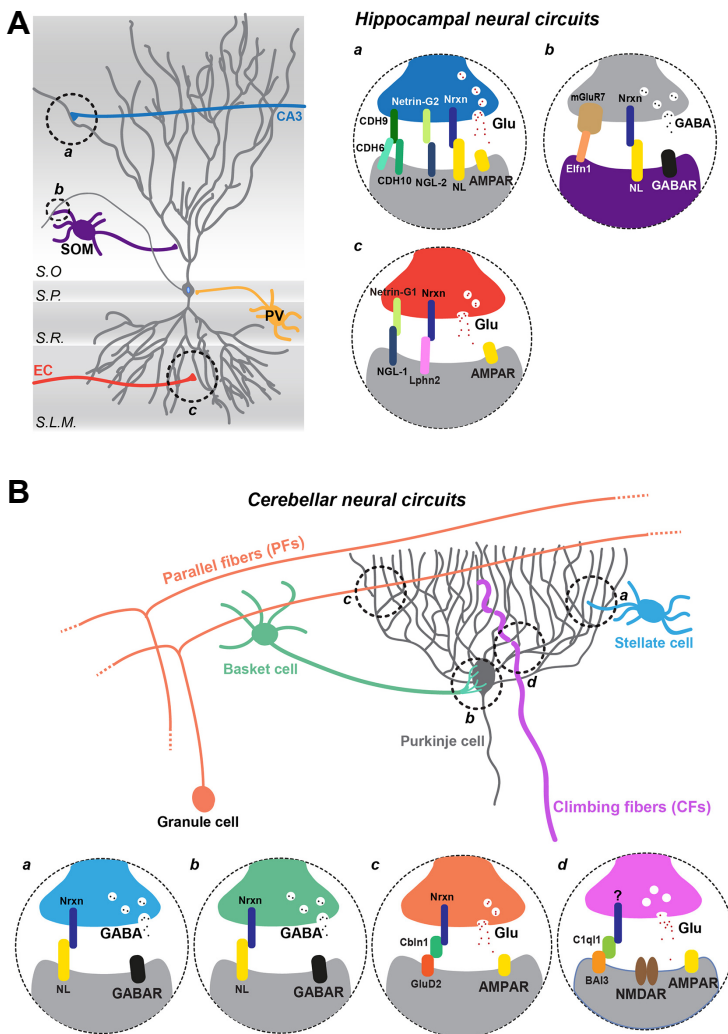
neuroligins are prototypical pairs of synaptic cell-surface proteins that determine key synaptic parameters in distinct neural circuits (Anderson et al., 2015; Aoto et al., 2013; 2015; Foldy et al., 2013; Zhang et al., 2015). In addition, leucine-rich repeat (LRR)-containing cell-surface proteins have emerged as key building blocks for synaptic and circuit specificity by virtue of their specific connectivity with other cell-surface proteins (de Wit and Ghosh, 2016; Ko, 2012; Um and Ko, 2013; 2017). However, given the vast number of synapse and circuit types in the mammalian brain, how the variety of cell-surface proteins orchestrates discrete aspects of synapse and circuit development in a context-dependent manner remains unknown. Moreover, it is still unclear how glial cells contribute to subcellular specificity across various brain areas (Allen and Eroglu, 2017; Williams et al., 2010).

In the present minireview, we discuss the roles of different synaptic cell-surface proteins in regulating specific synaptic connectivity and properties across various brain areas, focusing on those in the hippocampus and cerebellum. Because several excellent recent reviews have addressed the role of LRR proteins in synaptic and circuit specificity (de Wit and Ghosh, 2014; 2016), we mainly discuss the roles of other classes of synaptic adhesion molecules in specifying the properties of distinct synapse types.

## HIPPOCAMPAL CIRCUITS

### Entorhinal cortex to hippocampal CA1 circuit

The entorhinal cortex (EC) constitutes a fraction of the medial temporal lobe, and acts as the main interface between the hippocampus and neocortex. The EC is divided into two subdivisions—lateral and medial—that exhibit distinct anatomical features and input-output connectivity (Witter et al., 2000a; 2000b). For example, the lateral EC is strongly connected to the perirhinal cortex, olfactory and insular cortex, and the amygdala. In contrast, the medial EC preferentially connects with the postrhinal cortex, the subiculum, and occipital and retrosplenial cortices (Witter et al., 2000a). Two EC subdivisions target the same neurons in the dentate gyrus and CA3, while they reciprocally connect to different groups of neurons in the CA1 and subiculum. Similar to the case in other cortices, neurons in the EC are grouped into six different layers, each of which features a dominant cell type. In particular, layer II neurons mainly project to the dentate gyrus and hippocampal CA3 subfield, and axons of layer III neurons project largely to the hippocampal CA1 subfield and subiculum (Witter et al., 2000a). Notably, EC-hippocampal neural circuits play a critical role in spatial and temporal dimensions of episodic memory by combining cortical exteroceptive information with interoceptive representations to translate higher-order complex cognitive functions (Eichenbaum et al., 2007; Kitamura et al., 2015b). Medial EC neurons mediate spatial navigation through grid cells, head-direction cells, and border cells (Sasaki et al., 2015). In contrast, lateral EC neurons preferentially mediate olfactory, visual, or tactile responses (Eichenbaum et al., 2007). Recent studies have directly mapped the medial EC-hippocampal CA1 and lateral EC-hippocampal CA1 connectivities that are distinctively required for olfactory associative learning (Li et al., 2017), episodic memory tasks involving a temporal delay and/or a behavioral switch (Suh et al., 2011), and temporal gating of intrahippocampal information flow (Basu et al., 2016). In addition, island cells in the medial EC provide a direct feedforward inhibitory circuit to distal apical dendrites of the stratum lacunosum-moleculare (SLM) of hippocampal CA1 pyramidal neurons (Kitamura et al., 2015a; 2015b). To date, only a few molecular mechanisms that mediate development of selective EC-hippocampal neural circuits have been identified. Latrophilin-2, a member of the  $\alpha$ -latrotoxin-binding adhesion G protein-coupled receptor (GPCR) family, was recently found to be expressed in excitatory synapses in distal dendrites of the SLM (Anderson et al., 2017) (Fig. 2A). Deletion of latrophilin-2 from the mouse hippocampal CA1 selectively induced a loss of synaptic inputs from layer III EC



**Fig. 2. Key molecular determinants that regulate specific properties of hippocampal and cerebellar neural circuits.**

(A) Simplified neural circuit schematic showing projections from the entorhinal cortex (EC) to the hippocampal CA1 subfield (red), from the hippocampal CA3 subfield to the CA1 subfield (blue), from the hippocampal CA1 pyramidal neuron to somatostatin-positive inhibitory neuron (purple). A subset of the indicated cell-surface proteins confers the synaptic specificity between hippocampal CA1 pyramidal neurons and other brain area neurons: (a) connections of the CA3 with distal dendrites of hippocampal CA1 in the SO layer; (b) connections of somatostatin (SOM)-positive interneurons with hippocampal CA1 in the SO layer; and (c) connections of the EC with hippocampal CA1 in the SLM layer. Abbreviations: AMPAR,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; CDH, cadherin; EC, entorhinal cortex; Efn, extracellular leucine-rich repeat and fibronectin type III domain containing; Lphn, latrophilin; mGluR, metabotropic glutamate receptor; NGL, netrin G-ligand; NL, neuroligin; Nrnx, neurexin; S.L.M., stratum lacunosum moleculare; S.O., stratum oriens; SOM, somatostatin; S.R., stratum radiatum; and PV, parvalbumin. (B) Simplified schematic highlighting the diversity of molecular players that confer synaptic specificity in cerebellar neural circuits. Abbreviations: AMPAR,  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor; BC, basket cell; BAI, brain-specific angiogenesis inhibitor; Cbln, cerebellin precursor; C1ql, complement component 1 (q subcomponent-like); CF, climbing fiber; GABA,  $\gamma$ -aminobutyric acid; GABAR, GABA-type ionotropic receptor; GC, granule cell; Glu, glutamate; Glu $\delta$ , glutamate receptor  $\delta$  family; MF, mossy fiber; NMDAR, N-methyl-D-aspartate receptor; Nrnx, neurexin; NL, neuroligin; PC, purkinje cell; PF, parallel fiber; and SC, stellate cell.

neurons, and acted in a homeostatic manner to trigger an increase in synaptic connections formed by Schaffer collaterals emanating from hippocampal CA3 pyramidal neurons (Anderson et al., 2017). Moreover, loss of latrophilin-2 in the mouse SLM was found to impair learning and memory tasks involving sequential temporal switching, but not spatial learning (Anderson et al., 2017). These results suggest that a specific cell-surface protein (latrophilin-2) determines the property of a specific neural circuit (lateral EC to hippocampal SLM) to enable cognitive flexibility. Given the multivalent structural domains of latrophilin-2, it will be interesting to understand how the *trans*-synaptic ligands of latrophilin-2 (e.g., neurexins, teneurins, and fibronectin leucine-rich transmembrane proteins [FLRTs]) are involved in recognizing layer III EC inputs onto the hippocampal SLM (Boucard et al., 2012; Lu et al., 2015; O'Sullivan et al., 2012; Silva et al., 2011) (Fig. 2A). An additional provocative question is whether signaling by the adhesion GPCR involving latrophilin-2 and its ligands orchestrates the engagement of G protein-mediated signaling. Furthermore, it is possible that oth-

er latrophilins (latrophilin-1 or -3) may also act in the same neural circuit, or specifically function in other hippocampal neural circuits.

### Hippocampal CA3 to CA1 circuit

The Schaffer collateral pathway involves axon projections emanating from hippocampal CA3 pyramidal neurons that target proximal dendrites of hippocampal CA1 dendrites. This neural circuit is a crucial part of memory formation and has been a main locus for investigation of mechanisms involved in hippocampal synaptic plasticity. Numerous synaptic molecules have been shown to be critical for hippocampal long-term synaptic plasticity, which is dependent on postsynaptic N-methyl-D-aspartate (NMDA)-type glutamate receptors (Huganir and Nicoll, 2013). However, how this neural circuit is molecularly specified has been largely unknown. Among the few known molecular components is netrin-G ligand-2 (NGL-2), an LRR-containing synaptic membrane protein that is strongly expressed in hippocampal CA1 pyramidal neurons. Intriguingly, loss of NGL-2 selective-

ly decreases excitatory synapse development in the stratum radiatum (SR) layer, but not in the SLM, of hippocampal CA1 pyramidal neurons (DeNardo et al., 2012). Moreover, NGL-2 contributes to the formation of this specific neural circuit through interactions with its presynaptic ligand netrin-G2, which is specifically expressed by CA3 Schaffer collateral axons, but not by EC axons (DeNardo et al., 2012; Nishimura-Akiyoshi et al., 2007) (Fig. 2A). In contrast, it is possible that netrin-G ligand-1 (NGL-1) and its presynaptic ligand netrin-G1 specifically regulate the properties of neural circuits that link the EC to the SLM of hippocampal CA1 pyramidal neurons. Although this has not been experimentally demonstrated, it is plausible considering that netrin-G1 is specifically expressed in EC axons, but not in CA3 Schaffer collateral axons (Nishimura-Akiyoshi et al., 2007). CA3 Schaffer collateral axons are also connected to the stratum oriens (SO), which has functionally distinct properties (Meigas et al., 2001). A recent study reported the involvement of heterophilic interactions of type II cadherins in the CA3-CA1 SO neural circuitry (Basu et al., 2017). Cadherin-9 is strongly expressed in CA3 pyramidal neurons (Williams et al., 2011), and cadherin-6 and cadherin-10 are specifically expressed in CA1 pyramidal neurons (Basu et al., 2017). Strikingly, cadherin-9 is specifically required for excitatory synapse development and high-magnitude synaptic potentiation (Basu et al., 2017). Moreover, cadherin-6 and cadherin-10 function as postsynaptic binding partners for cadherin-9 to mediate the high-magnitude, but not normal-magnitude, long-term potentiation that is uniquely observed in the CA1 SO layer (Basu et al., 2017) (Fig. 2A). Given the diversity of cadherins that are widely expressed in the brain, an analysis of the contributions of other cadherins to the building of input-specific excitatory and inhibitory synapses in different neural circuit contexts is warranted.

### Hippocampal dentate gyrus to CA3 circuit

CA3 pyramidal neurons receive various types of inputs from dentate gyrus granule neurons, other CA3 neurons, and EC neurons with distinct synaptic properties. In particular, dentate gyrus-CA3 mossy fiber synapses connect glutamatergic dentate granule neurons to both glutamatergic CA3 pyramidal neurons and GABAergic interneurons. The former synapses are morphologically special and unique, with multiple active zones and multi-headed spines called thorny excrescences (TEs), whereas the latter synapses are formed between filopodia projecting from the presynaptic bouton and nearby GABAergic interneurons. Moreover, filopodial mossy fiber synapses mediate feed-forward inhibition of CA3 pyramidal neurons to fine tune the main dentate gyrus-CA3 neural circuits in performing cognitive tasks (Ruediger et al., 2011; Torborg et al., 2010). Although both main dentate gyrus-CA3 and filopodial mossy fiber synapses are anatomically linked, the identity of the target-specific cues that construct these different synapse types has been largely unknown. Recent studies demonstrated that Kirrel3 (also called Neph2), an evolutionarily conserved homophilic immunoglobulin superfamily member, is specifically required for filopodial mossy fiber synapses (Martin et al., 2015; 2017). Kirrel3 is highly expressed in dentate gyrus granule neurons

and a subset of GABAergic interneurons (Martin et al., 2015). Functionally, Kirrel3 is required to maintain feed-forward inhibition and constrain CA3 neuron excitability in juvenile mice (Martin et al., 2015) (Fig. 2A). In support of this observation, loss of Kirrel3 causes a significant increase in excitatory synaptic transmission in dentate gyrus granule neurons during a specific developmental time window (Roh et al., 2017). Future studies should address how molecular complexes involving Kirrel3, its extracellular ligands, and intramolecular components [e.g. PDZ-containing excitatory postsynaptic scaffolds; see (Choi et al., 2015)] act in concert to shape filopodial mossy fiber synapses in the hippocampal CA3. In addition, it would be interesting to determine whether Kirrel3 is also involved in specific filopodial mossy fiber synapses of adult-born dentate gyrus granule neurons.

### Hippocampal CA1 pyramidal neuron-interneuron circuit

CA1 pyramidal neurons receive inputs from neurons of various brain areas, including the EC, thalamus, and CA3 (Spruston, 2008). Intriguingly, distinct populations of GABAergic interneurons form specific synapses with specific subcellular compartments of CA1 pyramidal neurons to achieve targeted inhibition in the hippocampal network (McBain and Fisahn, 2001; Spruston, 2008). Two types of feedback inhibition that limit sustained CA1 pyramidal neuron firing have been identified: onset-transient inhibition mediated by parvalbumin (PV)-positive interneurons (Basket cells) and late-persistent inhibition mediated by neocortical Martinotti cells and somatostatin (SOM)-positive oriens-lacunosum moleculare (OLM) interneurons (Spruston, 2008). These two types of neural circuits exhibit distinct short-term plasticity properties. PV-positive interneurons receive depressing input with a high release probability through targeting of the perisomatic region of CA1 pyramidal neurons and other interneurons, whereas OLM interneurons receive facilitating input with a low release probability through targeting of apical dendrites of CA1 pyramidal neurons. Recent studies have shown that Elfn1 (extracellular leucine-rich repeat fibronectin containing 1) is selectively expressed in postsynaptic sites of SOM-positive interneurons in the hippocampal CA1 SO and regulates presynaptic release probability to direct the formation of highly facilitating pyramidal OLM synapses (Sylwestrak and Ghosh, 2012; Tomioka et al., 2014) (Fig. 2A). Importantly, loss of Elfn1 in mice induces alteration of metabotropic glutamate receptor 7 (mGluR7) expression, selectively in the SLM layer of the CA1 and the hilus of the dentate gyrus (Tomioka et al., 2014). In support of these electrophysiological and anatomical observations, seizure susceptibility is increased and impulsivity is heightened in Elfn1-knockout mice (Dolan and Mitchell, 2013; Tomioka et al., 2014).

Neurexins and neuroligins function as context-dependent specifiers of synapse properties by combining local concentrations of different neurexin and neuroligin isoforms in specific neural circuits (Chen et al., 2017; Futai et al., 2013; Kim et al., 2017; Südhof, 2017b). Neuroligin-1 and neuroligin-2 act as key synapse organizers for excitatory and inhibitory synapse development, respectively, through corresponding interactions with unique sets of extracellular and intracellular mechanisms [reviewed in (Bemben et al., 2015; Katzman



and Alberini, 2017)]. Neurexins are expressed in both excitatory and GABAergic interneurons (particularly, fast-spiking PV-positive interneurons) in the mouse hippocampus (Nguyen et al., 2016). Intriguingly, the presence of cell type-specific alternative splicing factors results in the expression of different neurexin isoforms in the mouse hippocampus: neurexin variants without an insert in splice site #4 are mainly expressed in excitatory neurons, whereas neurexin variants with an insert at this splice site #4 are primarily expressed in PV-positive interneurons (Nguyen et al., 2016). These results suggest the possibility that synaptic specificity conferred by neurexin-neuroigin synaptic adhesion pathways could be partially determined by expression levels of neurexin splice variants and the number of partner neuroigins (Fig. 2A). Although neurexin-1 $\beta$  is primarily expressed in excitatory presynaptic terminals of hippocampal neurons, forced expression of neurexin-1 $\beta$  in presynaptic interneurons has been shown to induce neuroigin-1 to act towards inhibitory synapses, rather than excitatory synapses (Futai et al., 2013). Neuroigin-2 and neuroigin-3 also function to specify specific synaptic properties depending on the identity of presynaptic partners (Foldy et al., 2013; Gibson et al., 2009). Collectively, these reports suggest the tantalizing hypothesis that neurexins and neuroigins differentially control the connectivity and activity of specific neural circuits to orchestrate distinct signaling pathways in a context-dependent manner.

## CEREBELLAR CIRCUITS

During postnatal development of the cerebellar cortex, Purkinje cells act as the principal output neurons and feature a regular and highly stereotyped pattern of glutamatergic and GABAergic synaptic connections, such that certain presynaptic neurons preferentially synapse onto the Purkinje cell body and axon initial segment, whereas others selectively target distinct dendritic domains (Sassoe-Pognetto and Patrizi, 2017) (Fig. 2B). The neural circuitry involving Purkinje cells has been used as an experimental model system for investigating molecular and cellular mechanisms underlying synaptic specificity (reviewed (Cerminara et al., 2015; Kano and Hashimoto, 2009; Mapelli et al., 2015; Uesaka et al., 2015)). In this minireview, we focus on two major glutamatergic circuits: parallel fiber (PF) circuits originating in deep granule neurons, and climbing fiber (CF) circuits originating from inferior olive neurons. The formation and maintenance of both PF and CF synapses depend on distinct sets of molecular players acting through distinct signaling pathways, particularly cell-surface proteins (see below). Purkinje cells also receive GABAergic inputs from molecular layer interneurons (stellate cells and basket cells), and form inhibitory circuits together with Golgi cells (Hull and Regehr, 2012). Neurofascin 186 and related immunoglobulin domain-containing cell surface proteins are involved in inhibitory synapse formation at distinct subcellular compartments of Purkinje cells. Because of space constraints, inhibitory circuits of the cerebellum are not discussed further in the current review.

### CF-Purkinje neuron synapses

CFs form synapses on thorny spines located on the proximal

dendritic domain. Purkinje cells are initially innervated by multiple CFs that make synapses on the cell body, but only a single CF translocates from the cell body to the proximal dendrites and, starting at postnatal day 9, the remaining perisomatic contacts are eliminated, through a defined sequence of molecular events (Watanabe and Kano, 2011) (Fig. 2B). Not surprisingly, structural defects during early stages of circuit development caused by abnormal Purkinje cell activity lead to multi-innervation or mis-patterning of CFs, resulting in movement disorders, such as ataxia (Miterko and Sillitoe, 2017). Thus, elimination of early-formed, redundant synapses is crucial for functional neural circuit development in the cerebellum. Activity-dependent cerebellar synapse elimination depends on functional NMDA-type glutamate receptors (Kakizawa et al., 2000), and on functional GABAergic inhibition (Nakayama et al., 2012) in specified developmental time window (postnatal days 15-16 for NMDA receptors and postnatal days 10-16 for GABA receptors). A recent study demonstrated that brain-derived neurotrophic factor (BDNF) secreted by Purkinje cells promotes CF synapse elimination from somata through retrograde BDNF-TrkB and mGluR1 signaling pathways (Choo et al., 2017). However, the molecular features governing how CF synapses are formed, specified, and eliminated have remained largely elusive.

C1ql1, a member of the C1q protein superfamily, is expressed in inferior olivary neurons, secreted by CFs (Iijima et al., 2010), and directly interacts with cell-adhesion G protein-coupled BAI3 (receptor brain-specific angiogenesis inhibitor 3), which is strongly expressed on postsynaptic Purkinje cells (Bolliger et al., 2011; Kakegawa et al., 2015). Strikingly, the C1ql1/BAI3 complex is crucial for regulating the formation and maintenance of a 'single-winner' CF during mouse cerebellar development and adulthood (Kakegawa et al., 2015; Sigoillot et al., 2015). Moreover, C1ql1/BAI3 signaling is required for motor learning, but not for gross motor performance or coordination (Kakegawa et al., 2015). However, it remains to be determined how the C1ql1/BAI3 signaling pathway specifically confers the specificity of CF synapses. Given the distinct expression patterns of C1ql members and other C1q superfamily proteins (Iijima et al., 2010; Miura et al., 2006), it is likely that these molecules act as distinct synapse specifiers across various brain regions. Indeed, C1ql3 is expressed in the basolateral amygdala and is specifically required for the formation and maintenance of basolateral-medial prefrontal cortical synapses that contribute to fear memories (Martinelli et al., 2016).

Neuroigins and neurexins are also important for Purkinje cell synapses in a context-dependent and isoform-dependent manner (Chen et al., 2017; Zhang et al., 2015). Conditional deletion of all three neuroigins in cerebellar Purkinje cells leads to a specific decrease in CF excitatory synapse size and excitatory postsynaptic currents without affecting PF synapse properties (Zhang et al., 2015). These phenotypes are largely replicated in neuroigin-1/neuroigin-2 double-knockout mice and neuroigin-1 or neuroigin-3 single-knockout mice (Zhang et al., 2015). Moreover, deletion of all three neuroigins increases the size of GABAergic basket/stellate cell synapses, but paradoxically decreases inhibitory postsynaptic

currents in Purkinje cells; notably, most of these observations are recapitulated in neuroligin-2 single-knockout mice (Zhang et al., 2015). In addition, neuroligins are essential for PV-positive stellate synapses through extrasynaptic NMDA receptor signaling (Zhang and Südhof, 2016). Neurexins are also specifically required for the formation and function of CF synapses (Chen et al., 2017). These studies suggest that neuroligins and neurexins function as synapse organizers to determine the properties of specific cerebellar synapse types.

### PF-Purkinje neuron synapses

PFs form a large number of synapses in the distal dendritic domain of Purkinje cells (Napper and Harvey, 1988) (Fig. 2B). The  $K_v3$  type of voltage-dependent potassium channel regulates synaptic transmission dynamics at PF synapses and motor performance (Matsukawa et al., 2003). Cerebellin 1 precursor protein (Cbln1), another member of the C1q protein superfamily, is specifically required for formation and maintenance of PF synapses, together with the  $\delta 2$  glutamate receptor (GluD2) (reviewed in (Emi et al., 2013; Mishina et al., 2012)). Although GluD1 is also expressed in molecular layer interneurons of the cerebellum and is concentrated at PF synapses on interneuron somata (Konno et al., 2014), the functional significance of the link between Cblns and GluD1 at the cerebellum has been largely uninvestigated. Cbln1 is released from cerebellar granule cells and forms complexes with GluD2 and specific splice variants of neurexins (Matsuda and Yuzaki, 2011; Uemura et al., 2010). These complexes play an instrumental role in mediating specific synaptic adhesion and dynamic axonal anatomical changes at PF synapses (Ito-Ishida et al., 2012; 2014). These complexes are also important for both motor and non-motor functions in multiple regions of the mouse brain (Emi et al., 2013; Otsuka et al., 2016). Collectively, these studies have started to paint a picture of the synaptic pathways that encompass the specificity of PF synapses in the cerebellum, but more studies are warranted to understand how Purkinje cells and their stereotyped connectivity are spatially and temporally regulated by distinct sets of molecular components.

### PERSPECTIVES

The functions of various synaptic molecules have begun to be investigated in a variety of neural circuits in recent years, a development that has been accelerated by the use of conditional transgenic animals in which specific genes are manipulated in specific cell types. Hippocampal and cerebellar circuits are better established than those in other brain regions; thus, many molecular mechanisms that relate to how specific synapses are formed, maintained, refined, or eliminated have been revealed in these neural circuits. Intriguingly, neurexin-3 has been shown to act through distinct mechanisms in different brain regions (Aoto et al., 2013; 2015), whereas the roles of PSD-95 and gephyrin are largely similar across various brain regions (Choi and Ko, 2015; Kim and Sheng, 2004). Thus, it will be crucial to validate the functions of synaptic molecules in the context of various neural circuits. Future advances in molecular, cellular, and systems-level neuroscientific techniques will bring us closer to an

understanding of how the properties of various synapses and neural circuits are specified, and lead to the design of more precise therapeutic strategies against synaptopathy-related brain disorders.

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