Mechanisms governing activity-dependent synaptic pruning in the developing mammalian CNS

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Abstract | Almost 60 years have passed since the initial discovery by Hubel and Wiesel that changes in neuronal activity can elicit developmental rewiring of the central nervous system (CNS). Over this period, we have gained a more comprehensive picture of how both spontaneous neural activity and sensory experience-induced changes in neuronal activity guide CNS circuit development. Here we review activity-dependent synaptic pruning in the mammalian CNS, which we define as the removal of a subset of synapses, while others are maintained, in response to changes in neural activity in the developing nervous system. We discuss the mounting evidence that immune and cell-death molecules are important mechanistic links by which changes in neural activity guide the pruning of specific synapses, emphasizing the role of glial cells in this process. Finally, we discuss how these developmental pruning programmes may go awry in neurodevelopmental disorders of the human CNS, focusing on autism spectrum disorder and schizophrenia. Together, our aim is to give an overview of how the field of activity-dependent pruning research has evolved, led to exciting new questions and guided the identification of new, therapeutically relevant mechanisms that result in aberrant circuit development in neurodevelopmental disorders.

Synaptic pruning
Developmental elimination of elements that comprise a bona fide structural synapse (presynaptic terminal and postsynaptic membranes), which might also include some pruning of small branches of axonal arbors and dendrites, while remaining synapses are maintained and strengthened.

Spontaneous neural activity
Neuronal activity that is not driven by an external stimulus.

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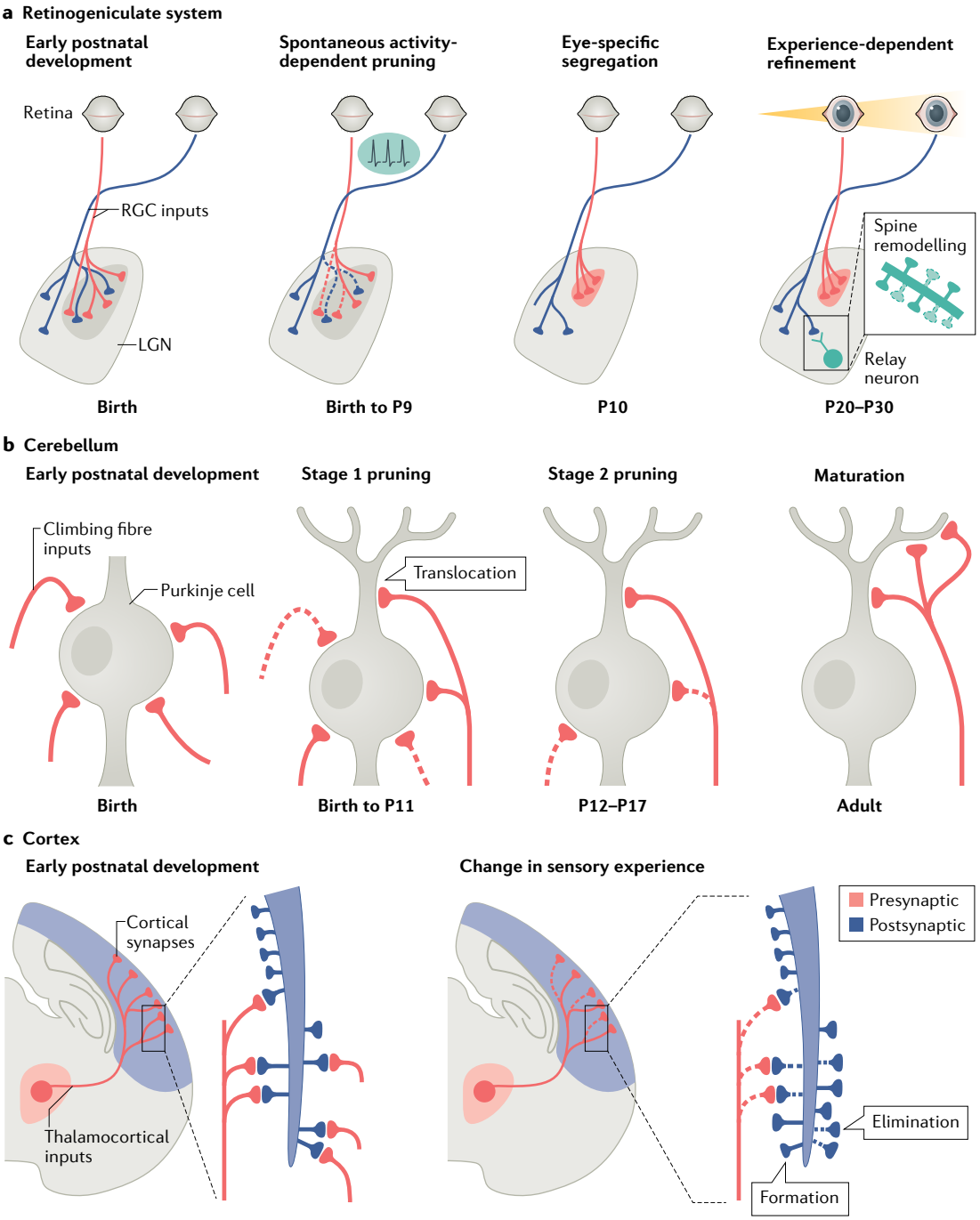
The developing nervous system undergoes remarkable remodelling to achieve the highly precise wiring diagram characteristic of mature neural circuits. This precision is achieved through pruning — an extensive process in which a large subset of axons, dendrites and synapses that initially form in abundance are eliminated over the course of development throughout the nervous system. While it is important to recognize the vast literature on large-scale pruning of axons and dendrites in the vertebrate and invertebrate peripheral nervous system and central nervous system (CNS)[1–4], in this Review we focus on developmental pruning of synapses in the mammalian CNS. We define synaptic pruning as a developmental process in which elements that comprise a bona fide structural synapse (presynaptic terminals and postsynaptic membranes) are eliminated, which may also include the removal of small segments of axonal and dendritic branches. Concomitant with elimination, remaining synapses are maintained, strengthened and elaborated. We review literature demonstrating roles for neural activity in developmental synaptic pruning and literature that has guided a new understanding of molecular mechanisms that may link changes in activity with the physical removal of some synapses and maintenance and strengthening of others. These mechanisms include emerging new roles for immune signalling and cell death molecules in synapse removal, which can be neuron intrinsic but can also include neuron-extrinsic, glial-driven mechanisms. Finally, we highlight how insights gained from the study of synaptic pruning in the healthy, developing nervous system have informed mechanisms of neurodevelopmental disorders, with a focus on autism spectrum disorder (ASD) and schizophrenia.

Background: activity-dependent pruning

Neuronal activity plays a central role in developmental synaptic pruning, which involves weakening and elimination of some synaptic connections and strengthening and maintenance of others[5–7]. In mammals, activity-dependent pruning occurs throughout the CNS at different periods in development, depending on the brain region and neuron subtype. In this section, we focus on model circuits that have been extensively studied in the context of activity-dependent synaptic pruning, given the stereotyped nature of the pruning and the experimental tractability of each circuit (FIG. 1). Through modulating either spontaneous neural activity or experience-driven neural activity, studies of these circuits have taught us guiding principles by which neuronal activity drives synaptic pruning during development. They have also led to the identification of molecules that



Experience-driven neural activity
Neuronal activity driven by external changes affecting sensory experience.

Eye-specific segregation
A process involving synaptic pruning by which spontaneous retinal activity drives presynaptic inputs from retinal ganglion cells to segregate and synapse in discrete, non-overlapping territories within the lateral geniculate nucleus during postnatal development.

link changes in activity with the physical elimination of synapses, which we outline in the next section as well as in following sections.

Spontaneous neural activity-driven synaptic pruning. Among the first studies to identify roles for neural activity in synaptic pruning in the CNS were those in the retinogeniculate system examining synapses from retinal ganglion cells (RGCs) onto relay neurons within the lateral geniculate nucleus (LGN) of the thalamus (FIG. 1a). Initially, right eye and left eye RGC inputs form exuberant synapses onto relay neurons within overlapping territories of the LGN. Before eye opening (before postnatal day 10 (P10) in mice), waves of spontaneous activity in the retina drive the segregation of RGC inputs into eye-specific territories in the LGN (that is, eye-specific segregation). This process involves elimination of a subset of presynaptic inputs and strengthening and maintenance of others[8–20]. Appropriate refinement of eye-specific territories requires that spontaneous activity among neurons of the same retina is synchronized, while activity among the two retinas must be asynchronous[17]. If spontaneous activity is blocked in both eyes during eye-specific segregation or synchronized through optogenetic stimulation, RGC inputs fail to prune into eye-specific territories[11,19,21]. If spontaneous activity is blocked or increased in only one eye, synaptic territory from the less active RGCs is reduced and

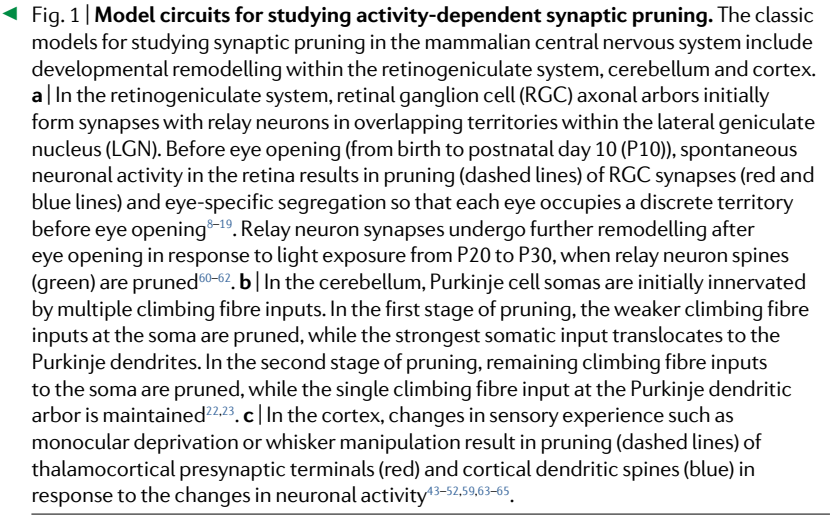


Fig. 1 | **Model circuits for studying activity-dependent synaptic pruning.** The classic models for studying synaptic pruning in the mammalian central nervous system include developmental remodelling within the retinogeniculate system, cerebellum and cortex. **a** | In the retinogeniculate system, retinal ganglion cell (RGC) axonal arbors initially form synapses with relay neurons in overlapping territories within the lateral geniculate nucleus (LGN). Before eye opening (from birth to postnatal day 10 (P10)), spontaneous neuronal activity in the retina results in pruning (dashed lines) of RGC synapses (red and blue lines) and eye-specific segregation so that each eye occupies a discrete territory before eye opening[8–19]. Relay neuron synapses undergo further remodelling after eye opening in response to light exposure from P20 to P30, when relay neuron spines (green) are pruned[60–62]. **b** | In the cerebellum, Purkinje cell somas are initially innervated by multiple climbing fibre inputs. In the first stage of pruning, the weaker climbing fibre inputs at the soma are pruned, while the strongest somatic input translocates to the Purkinje dendrites. In the second stage of pruning, remaining climbing fibre inputs to the soma are pruned, while the single climbing fibre input at the Purkinje dendritic arbor is maintained[22,23]. **c** | In the cortex, changes in sensory experience such as monocular deprivation or whisker manipulation result in pruning (dashed lines) of thalamocortical presynaptic terminals (red) and cortical dendritic spines (blue) in response to the changes in neuronal activity[43–52,59,63–65].

synaptic territory from the active RGCs is expanded[11,20]. If asynchronous activity between the two eyes is driven by optogenetics earlier in development, eye-specific segregation is achieved earlier[21]. Thus, this circuit supports a model in which spontaneous neuronal activity drives a Hebbian competition between synapses. The relative timing of activity between the two eyes and within a given eye is critical to dictate the elimination of some synapses and strengthening and maintenance of others.

Spontaneous neural activity also regulates pruning in the developing cerebellum (FIG. 1b). Initially, multiple climbing fibres from the inferior olivary nucleus synapse onto Purkinje cell somas. By approximately P11 in mice, many somatic climbing fibre synapses are eliminated and a 'winning' climbing fibre translocates and synapses onto the Purkinje cell dendrites[22,23]. In a second stage (approximately P12–P17), any remaining somatic climbing fibre synapses are pruned away. Similarly to the retinogeniculate system, Hebbian-type plasticity is required for climbing fibre pruning. This plasticity includes strengthening of a single climbing fibre input, while other inputs weaken and are eliminated[24]. This selective strengthening requires postsynaptic activity as the climbing fibre input that is most synchronous with the Purkinje neuron burst output becomes the 'winner'[25–29]. Increased GABAergic innervation onto the Purkinje cell soma from cerebellar basket cells further drives the relative weakening of the 'losing' climbing fibre inputs during the first stage of pruning[30]. During the later stage of pruning, signalling downstream of metabotropic glutamatergic receptors (mGluR1) in Purkinje cell dendrites drives the pruning of any remaining somatic climbing fibre synapses[31–36]. This mGluR1 signalling stimulates expression of the membrane-tethered semaphorin SEMA7A[37,38] and release of brain-derived neurotropic factor (BDNF)[39] from Purkinje neurons onto remaining somatic climbing fibre synapses by binding to plexin C1/integrin β1and TrkB, respectively, to facilitate their removal.

In addition to signals that lead to the elimination of 'losing' climbing fibres, other molecules stabilize the 'winning' climbing fibres. For example, another semaphorin, SEMA3A, is secreted from Purkinje neurons to promote the stabilization and maturation of 'winning' climbing fibres throughout all stages of pruning[37]. Acting in parallel to SEMA3A, progranulin derived from Purkinje cells is taken up by climbing fibres through binding to sortilin 1 (SORT1) to promote the stabilization and strengthening of these climbing fibres[40]. Finally, it is important to consider the influence of glia. Wrapping of climbing fibre synapses by specialized cerebellar astrocytes called 'Bergmann glia' is required to strengthen the 'winning' synapses and prevent exuberant climbing fibre innervation along Purkinje cell dendrites during later pruning stages[41]. Microglia promote weakening of a subset of climbing fibre synapses in other ways[30,42] (discussed later). Together, the retinogeniculate and cerebellar climbing fibre circuits demonstrate how differential activity in neurons drives a competitive pruning process. Cerebellar climbing fibre pruning has provided further insight into activity-dependent molecules that drive this competitive process.

Experience-driven changes in neural activity and synaptic pruning. The effects of neural activity on synaptic pruning have also been studied by the modulation of sensory experience. First demonstrated by Hubel and Weisel[43] in the cat visual cortex, monocular deprivation results in the weakening of synapses corresponding to the sutured eye and strengthening of synapses corresponding to the open eye, a process termed 'ocular dominance plasticity'. Studies in rodents have since revealed a decrease in thalamocortical arborization and a reduction in postsynaptic dendritic spines corresponding to the deprived eye, while thalamocortical projections from the open eye increase their arborization and synaptic territory[44–52] (FIG. 1c). Linking changes in activity with structural plasticity is evidence that long-term depression (LTD) also occurs during monocular deprivation[53,54], which has been linked to spine shrinkage and pruning in the hippocampus[55–57]. In addition, GABAergic innervation of cortical pyramidal neurons within the visual cortex by parvalbumin-positive interneurons is required to detect spatio-temporal differences in thalamocortical inputs during monocular deprivation and is necessary for subsequent spine pruning[58,59]. Downstream of GABAergic transmission during monocular deprivation, the proteolytic enzyme tissue-type plasminogen activator is activated and the active protease is proposed to remodel the extracellular matrix to accommodate spine remodelling[59]. Similarly to in the visual cortex, visual experience also affects a later stage of synapse remodelling (P20–P30) in the retinogeniculate system when RGC synapses in the LGN consolidate and strengthen, a process that requires visual experience[60]. While some of this remodelling involves clustering of presynaptic terminals along an RGC arbor[61], it also involves pruning of dendritic spines in the LGN[62].

Experience-dependent synaptic pruning has also been well documented in the somatosensory system. For example, whisker deprivation leads to dampened neural activity in the barrel cortex and the elimination of thalamocortical presynaptic inputs onto layer IV cortical neurons in developing rodents[63–65], which also occurs in adult rodents, albeit less robustly[63–65]. However, increased spine density within the barrel cortex following whisker

Monocular deprivation
The loss of sensory input to one eye, typically performed by suturing one eye closed for a defined period.

Ocular dominance plasticity
A process by which monocular deprivation results in strengthening of synaptic inputs from the open eye and weakening and elimination of synapses corresponding to the sutured, deprived eye.

Long-term depression
(LTD). A process by which changes in neuronal activity, such as sustained low-frequency stimulation, induce a reduction in synaptic strength.

deprivation, possibly owing to an increase in cortico-cortical synapses, has been reported[66,67]. Of note, synaptic pruning also occurs with increased activity in the somatosensory cortex. For example, stimulating whisker activity through environmental enrichment accelerates thalamocortical synapse maturation and pruning[68], which seems to involve competition between spines for synapse-stabilizing cadherin–catenin complexes. Experience-dependent pruning also occurs upstream of the barrel cortex in this circuit, in the ventral posteromedial nucleus (VPM) of the thalamus. Sensory information originating from the whisker pad is conveyed to the VPM through synapses originating from the brainstem principal trigeminal nucleus. As active whisker movement begins (around P14 in mice), a subset of whisker-specific inputs in the VPM are strengthened, while others are pruned[69]. Evidence suggests that astrocytic calcium signalling induces purinergic signalling, which then impinges on P2Y1 receptors on VPM neurons to facilitate the pruning of a subset of synapses during development[70].

Together, these studies emphasize the importance of activity for driving the selective elimination and stabilization of synaptic sites. While the model circuits described above established fundamental principles of activity-driven synaptic pruning in mammalian circuits, major gaps still remain in our knowledge. To what extent does pruning occur throughout the entire CNS? Are all neurons and circuits pruned or is pruning limited to a subset of circuits or neurons? If so, why? A major impediment to progress in answering these questions is that the developmental timing of this pruning is different across CNS regions. In addition, the timing and neuron subtype undergoing pruning throughout the CNS in one species may not necessarily align with those in other species. In many complex circuits, including the cortex, it is also often difficult to discern the cellular source of presynaptic inputs for each synapse, which is needed to selectively modulate activity during pruning. Instead, it has been more tractable to focus on these classic model circuits in which the presynaptic and postsynaptic partners are clearly defined. These model circuits have further paved the way to the identification of exciting new molecular mechanisms that are activity dependent and regulate synaptic pruning (discussed next).

Immune-mediated synaptic pruning

Besides the mechanisms described so far herein, molecules canonically involved in immune system function have also been identified as key regulators of developmental, activity-dependent synaptic pruning. In some cases, these immune molecules and cells regulate pruning in a fashion very similar to how they function in the immune system[71]. In this section, we give a historical perspective of these findings and highlight the most recent work outlining key roles for these molecules in shaping developing neural circuits.

Neuron-intrinsic immune signalling that regulates synaptic pruning. Excitement about the involvement of immune signalling in synaptic pruning began with the discovery of activity-dependent regulation of major histocompatibility complex (MHC) class I antigen expression in the LGN[72]. Later, MHC class I molecules were shown to be expressed by neurons[73]. Furthermore, mice lacking all cell surface expression of MHC class I (including *B2m*$^{-/-}$ mice lacking the MHC class I component β_2-microglobulin (β_2M) and *Tap1*$^{-/-}$ mice lacking the antigen peptide transporter TAP1), mice lacking the MHC receptor component CD3ζ and mice deficient in two of the 50 MHC class I genes ($K^bD^{b-/-}$ mice) all have deficits in retinogeniculate eye-specific segregation[73,74]. Mice deficient in MHC class I genes or the MHC receptor PirB (also known as LIR3) also had enhanced ocular dominance plasticity, diminished LTD[73,75,76] and elevated spine density in the visual cortex[76]. While these results demonstrate a role for MHC molecules and receptors in pruning, it is important to consider that MHC molecules and receptors may also affect initial synapse formation, as suggested by a study showing elevated synaptogenesis in cultured *B2m*$^{-/-}$ neurons[77]. It is also unclear how MHC class I–receptor signalling leads to pruning[78]. In the immune system, these molecules are key factors involved in antigen presentation, which activates neighbouring adaptive immune cells to initiate destruction of foreign antigens, such as viruses. The T cell receptors that bind to MHC class I complexes are not expressed in the CNS, but the co-receptors CD3ζ and CD3ε are present. The natural killer cell receptors for MHC class I molecules, PirB and Ly49, are expressed in the CNS, but exactly how MHC class I molecules interact with these receptors to regulate pruning is not known. Synaptic pruning could represent a separate function from the antigen presentation function of MHC class I in the immune system, including interactions with other immune-related molecules involved in pruning, such as neuronal pentraxins and complement proteins.

Pentraxins form two subfamilies on the basis of their length, namely the short pentraxins and the long pentraxins, which include pentraxin 3 (PTX3), PTX4 and the neuronal pentraxins. Pentraxins are cyclic multimeric proteins that are characterized by calcium-dependent ligand binding, and some are involved in the innate immune response[79]. Neurons express neuronal pentraxin 1 (NP1; encoded by *NPTX1*) and NP2 (also known as NARP; encoded by *NPTX2*), as well as neuronal pentraxin receptor (NPR). These neuronal pentraxins share 20–30% sequence homology with the short pentraxins in the peripheral immune system. In contrast to short pentraxins, neuronal pentraxins have a different tertiary structure, and there is little evidence to support roles for them in innate immunity[80–82]. NP1 and NP2 form heteromeric complexes that regulate AMPA receptor clustering and synapse plasticity[83], and knockdown of *Nptx1* in cultured rat neurons results in increased numbers of synapses[84]. Mice deficient in NP1 and NP2 also have deficits in eye-specific segregation in the retinogeniculate system, implicating these molecules in pruning of presynaptic terminals[85]. In peripheral immunity, non-neuronal pentraxin molecules can activate the classical complement cascade through complement component 1q (C1q) and play a role in phagocytosis[86,87]. Therefore, one possibility is that, in the CNS, neuronal pentraxins and MHC class I molecules could be working

Engulfment
The internalization or phagocytosis of material by a cell for degradation.

Trogocytosis
Partial phagocytosis of membrane material (*trogo* means 'nibble') while leaving the remaining membrane intact.

together with complement to mediate synaptic pruning by modulating microglial phagocytosis (discussed in the next section). Indeed, in adult mice, neuronal pentraxins co-immunoprecipitate with C1q in cortex-derived synaptosomes, and these molecules are colocalized in vivo at cortical synapses[88], but it remains unknown whether these interactions occur during developmental synaptic pruning.

Microglia-dependent immune signalling and synaptic pruning. Microglia are resident macrophages of the CNS. Similarly to other tissue-resident macrophages, microglia are efficient phagocytes and key regulators of innate immune signalling in the CNS. The first evidence that microglia are involved in synaptic pruning was obtained from studies of the developing hippocampus, visual cortex and retinogeniculate system[51,89,90], which revealed that microglia engulf synaptic components. In the visual cortex and retinogeniculate system, developmental synaptic pruning by microglia is regulated by activity, as microglia engulf synapses from less active neurons[51,90,91]. Microglial engulfment of synapses has also been observed in other regions of the developing brain, such as the auditory brainstem, auditory cortex, primary and secondary somatosensory cortices, and nucleus accumbens[65,92–95].

Despite mounting evidence that microglial engulfment mechanisms underlie activity-dependent synaptic pruning, whether microglia more passively 'clean up' synaptic debris or play a more active role in initiating synaptic pruning remains to be fully deciphered. Arguing for a more active role, live and static imaging in rodents and *Xenopus laevis* demonstrated microglial trogocytosis of intact presynaptic membranes but not postsynaptic structures[96,97], which is consistent with other studies showing selective phagocytosis of presynaptic membranes[65,90]. Furthermore, studies from multiple groups showed that disrupting microglial engulfment results in sustained increases in the number of structural and functional synapses[65,89,90,94,98–100]. For example, mice lacking the fractalkine (also known as CX_3CL1) receptor CX_3CR1 (*Cx3cr1*$^{-/-}$ mice), a G-protein-coupled chemokine receptor that is highly enriched in microglia in the healthy CNS[101], show delayed microglial infiltration into the hippocampus concomitant with delays in synapse maturation and a transient elevation in spine density[89]. Adolescent to early adult *Cx3cr1*$^{-/-}$ mice also had increased excitatory synaptic connectivity in the hippocampus and defects in sociability[99]. More recently, CX_3CR1 signalling was shown to directly affect microglial synapse engulfment and activity-dependent pruning of presynaptic terminals, but not postsynaptic structures, in the somatosensory cortex following neonatal whisker lesioning[65]. Following whisker removal, microglia engulf and remove thalamocortical presynaptic inputs in the neonate somatosensory cortex. This process was blocked in *Cx3cr1*$^{-/-}$ mice and in *Cx3cl1*$^{-/-}$ mice, which lack the canonical CX_3CR1 ligand CX_3CL1. Furthermore, transcriptional profiling revealed that whisker removal elicited an increase in the expression of the metalloproteinase ADAM10, which cleaves CX_3CL1 into a secreted form, in layer IV *Rorb*$^{+}$ neurons and microglia. Pharmacological inhibition of ADAM10 phenocopied the defects in engulfment and pruning of synapses in *Cx3cr1*$^{-/-}$ and *Cx3cl1*$^{-/-}$ mice. Together, these results suggest a mechanism by which CX_3CL1 is cleaved by ADAM10 in an activity-dependent manner; CX_3CL1 then binds to microglial CX_3CR1 and induces pruning of presynaptic terminals (FIG. 2a). In the immune system, the canonical function of CX_3CR1 is to regulate recruitment of myeloid cells[102]. However, in the barrel cortex following whisker ablation, microglial recruitment is unaffected and only synapse engulfment is blocked in *Cx3cr1*$^{-/-}$ and *Cx3cl1*$^{-/-}$ mice. Interestingly, CX_3CR1 is required for phagocytosis of necrotic fibres after acute skeletal muscle injury by macrophages in vivo and phagocytosis of apoptotic cells by bone marrow-derived macrophages in vitro[103,104]. However, the signalling downstream of this G-protein-coupled chemokine receptor that leads to regulation of engulfment remains to be ascertained.

Besides CX_3CR1, microglial engulfment and pruning of synapses are also regulated by the classical complement cascade (FIG. 2b). In the innate immune system, the classical complement cascade-initiating molecule C1q forms part of the C1 complex that cleaves the complement factors C2 and C4. The C2b and C4b fragments then form C3 convertase, which cleaves and activates C3. C3, in turn, binds to pathogens and cellular debris to induce cell lysis and/or clearance by phagocytes (reviewed elsewhere[105,106]). Similarly, C1q and C3 proteins localize to retinogeniculate synapses during developmental pruning, and microglia then engulf and remove presynaptic terminals via microglia-expressed C3 receptor complement receptor 3 (CR3)[90,98]. Importantly, *C1q*$^{-/-}$ (also known as *C1qa*$^{-/-}$), *C3*$^{-/-}$ and *Cr3*$^{-/-}$ (also known as *Itgam*$^{-/-}$) mice have defects in eye-specific segregation, impaired removal of structural presynaptic terminals and failure to eliminate functional synapses in the retinogeniculate system[98]. In other brain regions, *C1q*$^{-/-}$ mice have an increased number of axonal boutons and elevated cortical seizure activity[107], and CR3-dependent phagocytosis has been implicated in the removal of neuronal dopamine D1 receptors in the nucleus accumbens in adolescent male mice[94]. However, it remains to be determined whether pruning in the nucleus accumbens involves removal of postsynaptic receptors from the postsynaptic membrane or elimination of structurally intact synapses. One of the biggest questions emerging from these complement-dependent pruning studies is how does complement, a secreted protein, lead to the elimination of some synapses but not others? A few recent breakthroughs suggest that molecular inhibitors of microglial synapse engulfment are involved. First, a canonical 'do not eat me' immune system molecule, CD47, is expressed by RGCs, and CD47 and its cognate receptor SIRPα on microglia are required for activity-dependent synaptic pruning in the retinogeniculate system[108] (FIG. 2a). Second, an endogenous C1q inhibitor, SRPX2, is expressed by neurons[109], and *Srpx2*$^{-/-}$ mice have increased C3 deposition, elevated engulfment of presynaptic terminals by microglia and abnormal eye-specific segregation in the retinogeniculate system[109] (FIG. 2b). Last, in addition to complement inhibition, changes in sialyation and/or externalization

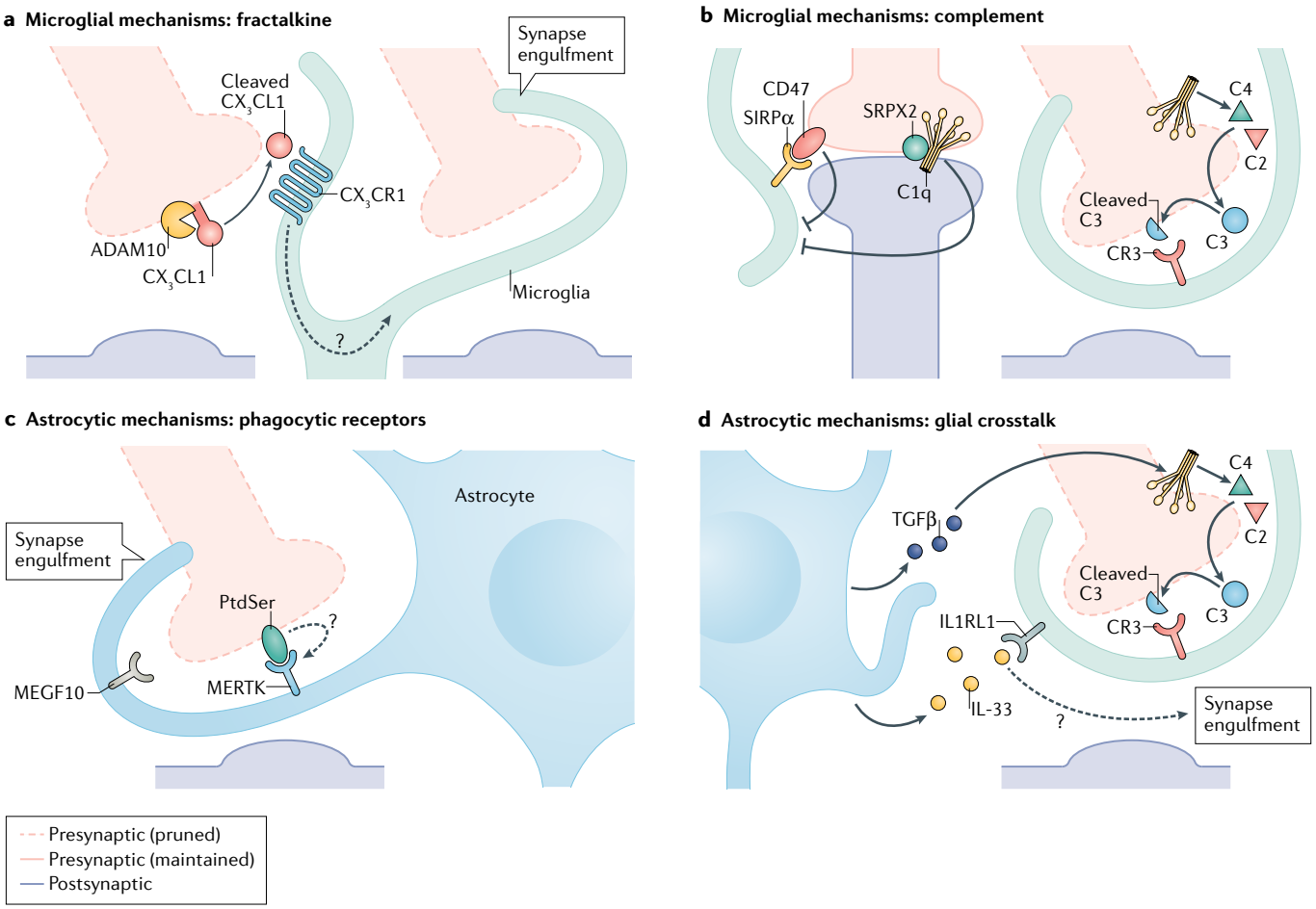


Fig. 2 | **Glial cell engulfment mechanisms that regulate synaptic pruning. a** | Microglial synaptic pruning through signalling involving C-X_3-C motif chemokine ligand 1 (CX_3CL1; also known as fractaline), C-X_3-C motif chemokine receptor 1 (CX_3CR1) and a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10). The protease ADAM10 cleaves neuron-expressed CX_3CL1 from the membrane, which binds to its receptor CX_3CR1 on microglia and initiates signalling. CX_3CR1 signalling induces synapse engulfment by microglia by a yet-to-be-identified mechanism[65]. **b** | Microglial synaptic pruning through the complement signalling cascade. Complement component 1q (C1q) induces the formation of C3 convertase through cleavage of the complement factors C2 and C4. C3 convertase then cleaves and activates C3, which induces engulfment of synapses by microglia expressing complement receptor 3 (CR3)[90,98]. By contrast, CD47 binding to its receptor signal regulatory protein-α (SIRPα) on microglia inhibits engulfment of another subset of synapses[108]. Similarly, the complement inhibitor sushi repeat-containing protein X-linked 2 (SRPX2) binds to C1q at the synaptic membrane, thereby preventing C1q-mediated engulfment and pruning of synapses[109]. **c** | Astrocytes also prune synapses through the phagocytic receptors multiple epidermal growth factor-like domains protein 10 (MEGF10) and MER proto-oncogene tyrosine kinase (MERTK). MERTK may bind directly to externalized phosphatidylserine (PtdSer) at the synaptic membrane[121]. **d** | Astrocyte–microglia crosstalk has been implicated in synaptic pruning. For example, astrocyte production of transforming growth factor-β (TGFβ) induces C1q production by retinal ganglion cells, which influences microglial pruning[100]. In addition, interleukin-33 (IL-33) produced by astrocytes binds to the IL-33 receptor IL-1 receptor-like 1 (IL1RL1) on microglia and induces microglia to engulf and prune synapses by a yet-to-be-identified downstream mechanism[125].

of phosphatidylserine (PtdSer) residues on synapses in the retinogeniculate system and hippocampus target the binding of complement to a specific subset of synapses[110–112] (discussed later).

An emerging concept from these studies is that the mechanisms regulating synaptic pruning by microglia are region specific and context dependent. For example, early pruning in the developing retinogeniculate system is dependent on complement but not on CX_3CR1 (REF.[113]). CX_3CR1 is also dispensable for climbing fibre pruning in the cerebellum[114]. Conversely, developmental pruning of hippocampal or barrel cortex synapses is dependent on CX_3CR1 but not on CR3 (REFS[65,96,115]). In the visual cortex, microglia engulf synaptic material during visual deprivation[51,116] but CX_3CR1 and complement do not regulate ocular dominance plasticity[113,117]. Instead, deficiency in the microglial purinergic receptor P2RY12 results in reduced microglial synapse engulfment and defects in ocular dominance plasticity[91]. One possibility is that microglia influence neuronal activity via P2RY12, which has been shown to modulate neural excitability in other brain regions[118,119]. This P2RY12-dependent modulation of neuronal activity by microglia, in turn, stimulates engulfment of synapses by a yet-to-be-identified mechanism. Furthermore, recent studies suggest that microglia regulate pruning by other non-phagocytic mechanisms. In the cerebellum, microglia affect climbing fibre pruning by promoting GABAergic innervation of cerebellar Purkinje cell somas, which influences weakening of a subset of climbing fibre synapses[30,42]. In addition,

during a later stage of experience-dependent pruning in the retinogeniculate system, microglia-derived TWEAK (a TNF family cytokine also known as TNFSF12) acts by binding to neuronal FN14 (also known as TNFRSF12A) to facilitate strengthening of synapses along thalamic relay neuron dendritic spines[62]. Another contrast may be between glutamatergic vs. GABAergic synapses which were recently shown to be pruned by microglia in the developing somatosensory cortex via a mechanism dependent on microglial "$GABA_B$" receptors[120]. Precisely how these different mechanisms drive pruning in specific circuits at specific times remains a key open question, which we discuss extensively below.

Microglia–astrocyte immune crosstalk in synaptic pruning. In addition to microglia, astrocytes have also been implicated in developmental synaptic pruning. Like microglia, astrocytes engulf retinogeniculate synapses in an activity-dependent manner[121]. Mice lacking the astrocyte-enriched engulfment receptor MEGF10 or the TAM receptor MERTK, which are typically used by phagocytes to engulf and clear apoptotic cells, have reduced astrocytic synapse engulfment and fail to properly prune retinogeniculate synapses[121] (FIG. 2c). While MEGF10 is highly enriched in astrocytes in the mammalian CNS, MERTK is expressed by both astrocytes and microglia. In contrast to astrocytes, microglial engulfment of retinogeniculate inputs is increased in *Mertk*$^{-/-}$ mice, but not at sufficient levels to rescue synaptic pruning deficits[121]. These data raise the question as to which cell type is most important for developmental synaptic pruning. Immunofluorescence microscopy showed more engulfed material per cell in microglia than in astrocytes, but owing to their larger population, astrocytes engulfed more material in total[121]. It is conceivable that astrocytes perform the bulk of the pruning of retinogeniculate axons and synapses, whereas microglia are involved in more local pruning and refinement. Interestingly, in the adult mouse hippocampus, astrocytes more readily engulf synaptic material[122], although it is important to note that in vitro experiments have revealed that the kinetics of phagocytosis and degradation of phagocytosed material are faster in microglia than in astrocytes[123]. Therefore, if the kinetics of phagocytosis are similar in vitro and in vivo, engulfment of synaptic material in vivo may be more easily detected with static imaging for astrocytes than for microglia.

Besides engulfment, astrocyte-secreted factors have also been shown to regulate pruning of synapses. For example, the astrocyte-secreted molecule hevin (also known as SPARCL1) is necessary for the elimination of exuberant synapses in the developing visual cortex[124]. It is intriguing to consider that this molecule might be signalling to microglia, as astrocyte–microglia crosstalk has been shown to regulate microglial engulfment and pruning of synapses in other contexts (FIG. 2d). For example, astrocyte-derived transforming growth factor-β (TGFβ) induces expression of C1q, which is necessary for complement-mediated retinogeniculate synapse pruning by microglia[100]. In sensorimotor circuits, astrocyte-derived interleukin-33 (IL-33) binds to its receptor IL1RL1 (also known as ST2) on microglia to stimulate engulfment and pruning of synapses[125]. Conversely, microglia may also direct synapse engulfment by astrocytes, as suggested by studies showing that microglia in the hippocampus regulate synaptic pruning by triggering receptor expressed on myeloid cells 2 (TREM2)-mediated signalling[112,126,127], in addition to CX_3CR1. One of these studies suggested that TREM2 in microglia normally limits synaptic pruning by astrocytes in the developing hippocampus[127]. TREM2 deficiency removes this limit and results in exuberant pruning by astrocytes during hippocampal development. However, the identity of the TREM2-dependent signal from microglia that restrains astrocyte engulfment remains unknown.

These studies have identified a diverse array of immune signalling mechanisms that are required for developmental synaptic pruning. Emphasizing their importance, many of these mechanisms are evolutionarily conserved (BOX 1). However, fundamental questions remain about the timing and extent of immune signalling involvement in developmental synaptic pruning. First, many studies examine synaptic changes at only one time-point, usually once pruning is finished. Therefore, it is possible that, in addition to or instead of pruning, some of these molecules might regulate initial synaptogenesis, which would result in a phenotype that is identical to a pruning defect at older ages. Assessing synapse density before and after pruning in mutant animals is key.

Box 1 | **Conserved pruning mechanisms in invertebrates**

Two of the major immune signalling pathways that are implicated in synaptic pruning — major histocompatibility complex (MHC) class I and complement — seem to be specific to vertebrates. However, many of the other molecules involved in activity-dependent synaptic pruning were first shown to function in axonal or dendrite pruning in invertebrates. For example, glial cell-mediated engulfment of neuronal compartments during pruning is highly conserved. Indeed, glial cells in the fruit fly *Drosophila melanogaster* engulf axons and sensory endings during developmental pruning[234]. This process is regulated by Draper[235–237], the fly homologue of mammalian MEGF10, which regulates astrocyte-mediated pruning[121]. The *Caenorhabditis elegans* Draper homologue CED-1 performs a similar function in cholinergic synapse pruning[238]. Furthermore, secreted immune molecules involved in mammalian synaptic pruning also seem to regulate pruning in invertebrates. For example, the *Drosophila* chemokine-like protein Orion, which has some functional similarity to mammalian fractalkine (also known as CX_3CL1), regulates glial cell recruitment and engulfment of axons during mushroom body pruning[239]. In addition, just as astrocyte-derived transforming growth factor-β (TGFβ) signalling has been implicated in driving complement-mediated pruning in mammals[100], so too does the *D. melanogaster* TGFβ family member Myoglianin drive axonal pruning in developing fly embryos[240]. However, unlike TGFβ in mammals, Myoglianin seems to be acting neuron intrinsically to promote pruning in *D. melanogaster*.

Cell death signalling during pruning is also evolutionarily conserved. The role of caspases in programmed cell death was first demonstrated in *C. elegans*, in which the mammalian caspase 9 (CASP9) homologue CED-3 regulates cell survival[241]. It is now appreciated that this signalling can be compartmentalized to regulate cellular remodelling without inducing cell death[242]. In the *C. elegans* nervous system, CED-3 cleaves and activates the actin-severing protein gesolin (GLSN-1) to promote disassembly of presynaptic machinery during synapse remodelling[243]. In addition, in GABAergic neurons, local calcium concentration increases activates calcineurin, which in turn activates CED-3 to promote dismantling of the presynaptic machinery[244]. Downstream of caspase activation and similarly to in mammals, local exposure of phosphatidylserine in the outer membrane layer regulates subsequent amphid sensory organ sheath glial cell engulfment of sensory endings in *C. elegans*[245].

Together, these data point to evolutionarily conserved mechanisms driving synaptic pruning by glia and immune and cell death molecules. A better understanding of how changes in activity regulate invertebrate pruning could open up powerful new systems to provide insights into activity-dependent synaptic pruning in mammals.

Second, given the diversity of molecular players identified across different circuits, immune signals during synaptic pruning are circuit specific rather than a 'one-size-fits-all' mechanism. How this circuit specificity is achieved is unclear and could be a result of molecular and functional diversity of neurons and/or glia. Differing gene expression and/or levels of activity could elicit different signalling cascades in neurons, which could then elicit diversity in glial cell responses. Third, while it is clear that the expression and/or localization of immune molecules changes in response to activity, it is unclear how. One possibility is through a newly identified mechanism — modulation of activity-dependent axonal and synaptic pruning by JAK2–STAT1 signalling. In the developing retinogeniculate and callosal projections in mice, the protein tyrosine kinase JAK2 is activated in inactive neurons and synapses, but only when neighbouring neurons and synapses remain activated[128]. This JAK2 activation leads to the pruning of inactive synapses through activation of the downstream transcription regulator STAT1. JAK2–STAT1 is a canonical signalling cascade in the immune system and mediates cytokine receptor signalling. Which molecules are transcriptionally regulated by JAK2–STAT1 signalling to induce the selective elimination of inactive synapses is unclear, but it is intriguing that the expression of many of the immune molecules discussed above is altered by JAK2–STAT1 signalling.

Cell death molecules at the synapse

Besides immune molecules and signalling pathways, evolutionarily conserved molecules involved in cell death signalling (BOX 1) have been implicated in activity-dependent synaptic pruning. The mechanisms described in the following sections are recognized as primary drivers of apoptosis and clearance of apoptotic debris by phagocytes. However, it is becoming increasingly appreciated that these mechanisms can be spatially and temporally restricted to drive pruning in the absence of cell death. In some cases, they may also be operating upstream of immune molecules to regulate their localization to synapses during pruning.

Activation of caspases and phosphatases. The protease caspase 3 (CASP3) is activated during mitochondrion-mediated apoptosis and cleaves hundreds of different protein substrates that mediate DNA fragmentation, PtdSer exposure and membrane blebbing[129]. In vitro studies have revealed that mitochondrial CASP3 activation can be compartmentalized in dendritic spines, leading to LTD via AKT1 cleavage and AMPA receptor internalization[130,131], and subsequent spine pruning[132]. In addition, hippocampal spine pruning is defective in *Casp3*$^{-/-}$ mice[132]. Of note, the level and duration of CASP3 activation during LTD pruning are lower than in apoptosis, possibly due to proteasomal degradation of CASP3 in the dendrites to prevent precocious cell death[131,132]. CASP2 has also been implicated in dendritic spine pruning[133]. Similarly to *Casp3* knockout[130,131], *Casp2* knockout results in defects in LTD and increased numbers of dendritic spines in hippocampal neurons, both in vitro and in vivo. Finally, another cell death molecule, the calcium-dependent protein phosphatase calcineurin[134], has been implicated in pruning. In mammals, calcineurin is regulated by myocyte-specific enhancer factor 2 (MEF2) to initiate degradation of PSD95 in an activity-dependent manner, which leads to dendritic spine elimination[135–137].

PtdSer exposure. Downstream of caspases is PtdSer. PtdSer is normally restricted to the inner leaflet of the plasma membrane but it becomes externalized on the surface of apoptotic cells by scramblases, which are activated by caspases. During apoptosis, TAM receptor complexes on phagocytes bind to externalized PtdSer, leading to engulfment and clearance of apoptotic cell bodies[138]. Similarly, during developmental synaptic pruning in the retinogeniculate system and hippocampus, PtdSer is exposed on a subset of mostly presynaptic terminals, which are subsequently cleared by microglia[111,112,139]. It is unclear whether TAM receptors, such as MERTK, bind to PtdSer during synaptic pruning. However, other immune molecules required for synaptic pruning have been shown to bind to PtdSer, including C1q[140], GPR56 (REF.[139]) and TREM2. Treatment with annexin V, which binds to externalized PtdSer, or deletion of *Trem2* in vitro leads to reduced microglial engulfment and inhibition of hippocampal synaptic pruning[112]. In addition, C1q deficiency in mice leads to accumulation of exposed PtdSer, primarily at presynaptic structures, and reduced microglia-mediated pruning of presynaptic terminals in the retinogeniculate system[112,139]. Although an earlier study provided ex vivo data to support that C1q-tagged synapses are enriched in activated CASP3 and exposed PtdSer compared with untagged synapses[111], this later work in the retinogeniculate system showed that stimulation of microglia-mediated synaptic pruning by PtdSer exposure was not downstream of CASP3 activation[112]. Published in parallel, another group showed that the microglial adhesion G-protein-coupled receptor GPR56 binds directly to PtdSer[139]. Similarly to in C1q-deficient mice, conditional ablation of GPR56 in microglia leads to reduced PtdSer exposure and pruning of presynaptic terminals. These data point to GPR56, TREM2 and complement proteins working in concert to regulate microglial pruning of synapses by binding to PtdSer. It remains to be determined whether these pathways regulate each other or whether they work in parallel to perform a similar function.

A potential role for mitochondria. Although the available evidence suggests that molecules that normally regulate cell death are compartmentalized at the synapse to regulate synaptic pruning, it remains less clear how they are initially activated at some synapses and not others. One potential mechanism is through the aforementioned JAK–STAT signalling, which is activated in inactive synapses, leading to their removal[128]. In addition to regulating immune molecules, JAK–STAT signalling also regulates the expression of caspases during cell death in other contexts (reviewed in[141]). Another possibility is that mitochondria may be an important link between changes in activity at a given synapse, compartmentalized cell death signalling and the physical removal of the synapse. Indeed, elevated mitochondrial calcium concentration is

Apoptosis
A canonical highly regulated process of programmed cell death that occurs in multiple contexts, including during development, and involves membrane blebbing, cell shrinkage and DNA fragmentation.

required for LTD-dependent elimination of spines in cultured hippocampal neurons[130]. In addition, reducing the amount of mitochondria in dendrites leads to a reduction in dendritic spine density in cultured neurons, while an increase in mitochondrial content supports synaptogenesis and plasticity[142]. Furthermore, live imaging in adult mouse cortex and hippocampal slices has revealed that presynaptic terminals with more mitochondria are stabler and decreased presynaptic mitochondria motility is associated with increased synaptic strength[143,144]. It is crucial to understand how these mitochondrial changes may be relevant to synaptic pruning during development in vivo, as mitochondrial changes may be an important link by which activity drives the selective elimination of some synapses and maintenance of others.

Together, these data demonstrate that calcium-dependent cell death pathways involving caspases and calcineurin facilitate synaptic pruning. Externalized PtdSer, which may be downstream of local caspase activation, provides a substrate to initiate immune-mediated synaptic pruning by glial cells (FIG. 3). Upstream JAK–STAT signalling and/or mitochondria may be key in selective elimination of synapses based on activity.

Disease mechanisms in synaptic pruning

In humans, synaptic pruning occurs primarily during two developmental periods: the first 2 years after birth and during adolescence[1] (FIG. 4). Studies suggest that defects in synaptic pruning during both developmental periods underlie several neurodevelopmental disorders[145–153]. Here we focus on ASD and schizophrenia to highlight how, in addition to the molecules described so far herein, many disease-associated genes contribute to changes in neural circuit structure and function through disruptions in synaptic pruning (summarized in TABLE 1).

Genes associated with ASD. ASD is typically diagnosed within the first 5 years of life, coincident with the first wave of synaptic pruning[1,154]. Analyses of post-mortem ASD tissue samples have shown increased synapse density compared with neurotypical controls in the frontal, temporal and parietal lobes, suggesting an underpruning phenotype[155,156]. Transcriptomic analyses of post-mortem ASD brain tissue further show significant changes in the expression of genes associated with pruning[157] and hypomethylation of immune genes involved in synaptic pruning, including *C1QA, C3* and *C3R* (REF.[158]). Whole-exome sequencing and genome-wide association studies also point to the involvement of molecules that regulate synapse formation and synaptic plasticity in ASD[159,160].

In addition to these genetic associations, recent studies in mice harbouring mutations or deletions in syndromic ASD-linked genes provide further support for the involvement of activity-dependent synaptic pruning deficits in ASD pathogenesis. For example, *Fmr1*$^{-/-}$ mice have been used to study fragile X syndrome, a disorder with high incidence of ASD that is caused by mutations in *FMR1*, the gene encoding fragile X mental retardation protein (FMRP). FMRP is a known repressor of mRNA translation through binding to eIF4E[161], is regulated by neuronal activity[162,163] and regulates activity-dependent synaptic plasticity[164,165]. FMRP also works in concert with the ASD-linked MEF2 transcription factor family (REFS[166–168]) which no longer performs its normal role in activity-dependent spine elimination in *Fmr1*$^{-/-}$ mice[137,169–171]. In addition, an increased number of spines has been reported in the neocortex in individuals with fragile X syndrome[172,173] and in the hippocampus and cortex in *Fmr1*$^{-/-}$ mice, which also show impaired ocular dominance plasticity[174–176]. A link between FMRP and the immune mechanisms discussed previously is provided by a study showing an impairment in microglia-mediated engulfment of postsynaptic structures in the hippocampus of *Fmr1*$^{-/-}$ mice[175]. However, it is important to note that assessment of synaptic pruning is complicated by evidence that there are defects in synapse stabilization and synaptogenesis in *Fmr1*$^{-/-}$ mice[177,178]. Furthermore, other studies find the opposite or no change in spine number in *Fmr1*$^{-/-}$ mice, which are likely due to differences in the age of the mice used in the study, the brain region assessed and the methods used for counting spines[174]. However, in support of aberrant synaptic pruning in fragile X syndrome, a time course of paired electrophysiological recordings of layer V pyramidal neurons demonstrated that excess

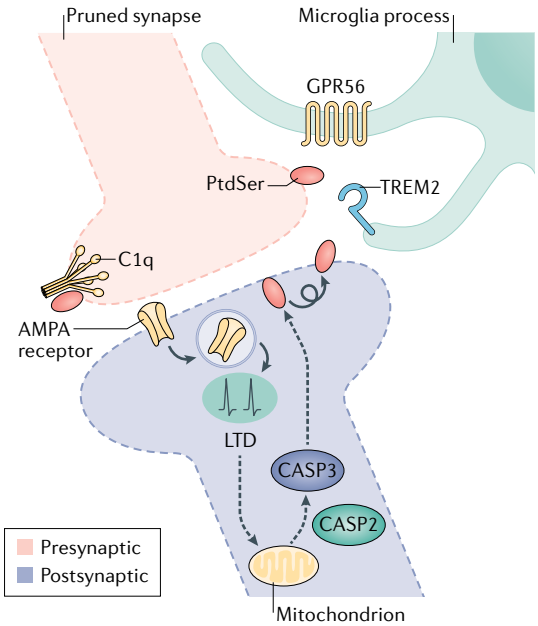


Fig. 3 | **Cell death molecules involved in synaptic pruning.** During synaptic pruning, mitochondrial molecules in the dendrite activate caspase 3 (CASP3), which drives α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid (AMPA) receptor internalization from the postsynaptic membrane and results in long-term depression (LTD)[130,131]. Caspase 2 (CASP2) also induces AMPA receptor internalization, although this occurs by a separate pathway[133]. Activity-dependent changes at the synapse, for example via LTD, may also influence mitochondrial processes and compartmentalized cell death molecules to regulate pruning (dashed arrows). In some but not all cases, caspase signalling leads to externalization of phosphatidylserine (PtdSer) to the outer membrane at synapses. Molecules such as complement component 1q (C1q), G-protein-coupled receptor 56 (GPR56) and triggering receptor expressed on myeloid cells 2 (TREM2) can bind to this exposed PtdSer to regulate pruning by microglia[112,126,139].

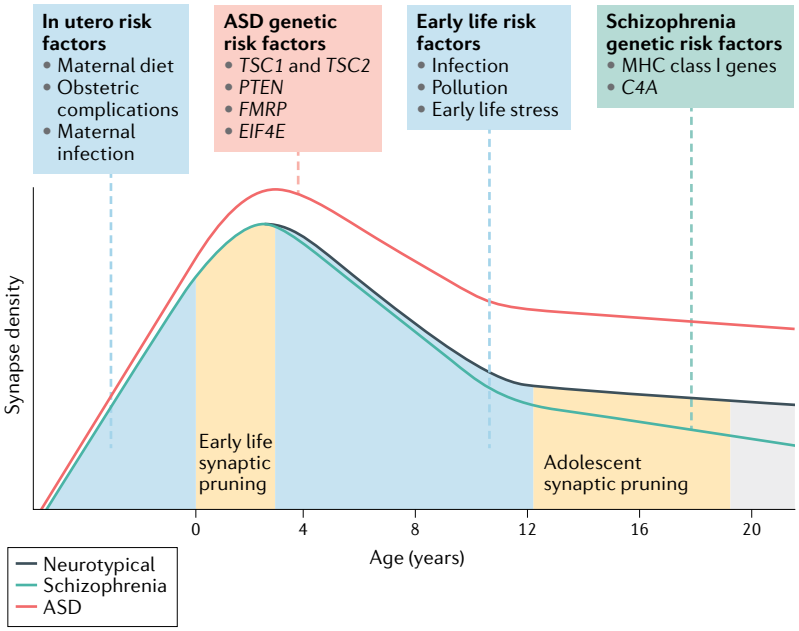


Fig. 4 | **Synaptic pruning and neurodevelopmental disorders.** Schematic depicting putative development changes in synapse density in the brains of neurotypical individuals compared with patients with autism spectrum disorder (ASD)[155,156] or schizophrenia[198–205]. The developmental periods in early life and adolescence when synaptic pruning primarily occurs are highlighted in yellow[1]. Genetic risk factors that are implicated in aberrant pruning during these two periods include the genes encoding tuberous sclerosis 1 protein (TSC1) and TSC2[155,184], phosphatase and tensin homologue (PTEN), fragile X mental retardation protein (FMRP)[137,161,169–175,177–179] and eukaryotic translation initiation factor 4E (eIF4E)[192–194] in ASD, and complement C4A (REFS[213–216]) and major histocompatibility complex class I (MHC class I) genes[78,212] in schizophrenia. Environmental risk factors that may, in concert, affect pruning[218–223] include maternal infection[223,225–228], obstetric complications and maternal diet[229,230] in utero and pollution, infection and early life stress later during early life development. Adapted from REF.[153], Springer Nature Limited.

cell-to-cell connections in *Fmr1*$^{-/-}$ mice were indeed due to impaired pruning[179]. Given that activity-dependent pruning is dependent on the strengthening and stabilization of 'winning' synapses, it is possible that defects in spine stabilization or spine dynamics contribute to these changes in pruning. Indeed, enhanced spine dynamics have been noted in mice with genetic mutations linked to syndromic ASD, including 15q11–13 duplication and *Nlgn3*^{R451C} (REF.[180]).

Other syndromic ASD-linked genes that regulate synaptic pruning include mTOR signalling pathway components, which represent another set of mRNAs that are regulated by FMRP[181]. For example, neuronal deletion of the phosphatase and tensin homologue gene (*PTEN*), which is mutated in ~1–4% of ASD cases[182] and is an upstream inhibitor of mTOR, leads to increased spine density and altered social behaviour in mice[183]. Mutations in the genes encoding the negative regulators of mTOR tuberous sclerosis 1 protein (TSC1) and TSC2 cause tuberous sclerosis, which is another disorder with a high incidence of ASD. In *Tsc1*$^{-/-}$ mice or *Tsc2*$^{+/-}$ mice, spine density is increased in the cerebellum and cortex[155,184]. These synaptic changes occur concomitant with overactive mTOR signalling in both ASD brain tissue and *Tsc2*$^{+/-}$ neurons[154], which likely exerts its effects on spines and behaviour by modulating neuronal autophagy[155], a cellular process for the degradation of proteins and organelles. The exact mechanism by which modulation of autophagy leads to synaptic pruning remains unknown. However, there is evidence that mitochondria, well-known targets of autophagy, are depleted at presynaptic sites in TSC1-deficient and TSC2-deficient neurons[185], which could affect synaptic pruning. Autophagy can also be induced presynaptically by neuronal activity[186,187] and can regulate synaptic transmission and plasticity by degrading synaptic vesicles and postsynaptic proteins[188–191]. Thus, autophagy induced by changes in mTOR signalling may play a similar role in activity-dependent pruning, and disruptions in autophagy may contribute to aberrant pruning in ASD.

These studies demonstrate clear links between alterations in mRNA translation and autophagy in neurons and alterations in synapse numbers in ASD. However, all of these molecules are expressed by numerous cell types throughout the CNS, so the contribution of non-neuronal cell types to synaptic alterations in ASD must be considered. Selective overexpression of the FMRP target eIF4E in microglia results in increased spine density and ASD-like behaviours in mice[192], a phenotype that largely recapitulates that observed in mice with global overexpression of eIF4E[193,194]. Furthermore, selective disruption of the core autophagy gene *Atg7* in LYZ2^{+} myeloid cells, which may include microglia, also impairs synaptic pruning[95]. Thus, disruptions in mRNA translation and autophagy in multiple cell types may contribute to synaptic alterations in ASD.

Genes associated with schizophrenia. Schizophrenia is typically diagnosed at a later age than ASD, typically between late adolescence and the mid-to-late 20s[195]. However, even before the full onset of schizophrenia, cognitive deficits and structural changes can be detected in 'high-risk' individuals[196]. The emergence of symptoms towards the end of the second wave of pruning led to the hypothesis that aberrant synaptic pruning during adolescence contributes to schizophrenia[197]. In contrast to ASD, data from patients with schizophrenia and animal models of schizophrenia suggest the presence of an overpruning defect. For example, studies in patients with schizophrenia reveal progressive loss of grey matter volume[198], especially during the initial phase of the disease[199]. Examination of post-mortem tissue has also revealed a layer-specific loss of spines[200,201] and presynaptic proteins[202–204], particularly in layer III of the prefrontal cortex. Similarly, positron emission tomography with a ligand for the synaptic marker SV2A has revealed reduced density of presynaptic terminals in the brain of patients with schizophrenia[205].

Synaptic pruning defects in schizophrenia are also supported by genetic analyses. Genetic variations that are over-represented in schizophrenia predominantly affect synaptic genes[160,206,207]. In several cases, reduced spine density has been observed in transgenic mouse models lacking or harbouring mutations in schizophrenia risk genes[208–210], including mice haploinsufficient for the histone-lysine *N*-methyltransferase gene *Setd1a*. However, it is unknown whether these changes are due

Table 1 | **Summary of synaptic pruning studies in neurodevelopmental disorders discussed in this Review**

Disorder	Evidence for changes in pruning	Brain region	Refs
Humans			
Autism spectrum disorder	Increased spine density	Temporal cortex	155
	Failure to eliminate spines over development		
	Increased spine density	Temporal cortex, frontal cortex and parietal cortex	156
	Transcriptional profiling reveals changes in immune/glial mRNA related to pruning	Combined frontal and temporal cortex	157
	Hypomethylation and overexpression of complement genes involved in pruning	Prefrontal cortex and cingulate cortex	158
	Genetic association to synaptic plasticity genes	NA	160
Fragile X syndrome	Increased immature spine morphology	Cingulate cortex and temporal cortex	172
	Increased immature spine morphology	Cortex	173
Schizophrenia	Reduced grey matter volume	Cortex	198
	Progressive loss of grey matter volume	Cerebral cortex, frontal cortex and thalamus	199
	Reduced spine density	Dorsolateral prefrontal cortex	200
	Normal spine density	Visual cortex	
	Normal spine density	Dorsolateral prefrontal cortex	201
	Reduced synaptophysin immunoreactivity	Prefrontal cortex	202
	Reduced synaptic vesicle proteins	Frontal cortex, parietal cortex, cingulate cortex, hippocampus and thalamus	203
	Reduced VGLUT1 boutons	Prefrontal cortex	204
	Reduced SV2A (PET)	Frontal cortex and cingulate cortex	205
	Genetic association to synaptic gene loci	NA	206,207
	Genetic association to MHC gene locus	NA	211
	Genetic association to C4 allelic variation	NA	213
Human cell models			
C4 variant microglia–neuron co-cultures	Increased microglial engulfment	NA	216
Mouse models			
Tsc2$^{+/-}$	Failure to eliminate spines over development	Temporal cortex	155
	Increased levels of PSD95 and synaptophysin		
Atg7$^{flox/flox}$; *Camk2a*^{Cre}	Failure to eliminate spines over development	Temporal cortex	155
Atg7$^{flox/flox}$; *Camk2a*^{Cre}; *Tsc2*$^{+/-}$	Failure to eliminate spines over development	Temporal cortex	155
Nse^{Cre}; *Pten*$^{flox/flox}$	Macrocephaly	Hippocampus and cortex	183
	Increased spine density		
L7^{Cre}; *Tsc1*$^{flox/flox}$	Increased spine density	Cerebellum	184
Fmr1$^{-/-a}$	Increased spine density	Hippocampus	175
	Reduced microglial engulfment of PSD95		
	Impaired ocular dominance plasticity	Visual cortex	176
	Failure to eliminate spines over development	Somatosensory cortex	179
	Impaired synapse elimination	Hippocampus	169
	Impaired activity-dependent spine elimination	Somatosensory cortex	178

Table 1 (cont.) | **Summary of synaptic pruning studies in neurodevelopmental disorders discussed in this Review**

Disorder	Evidence for changes in pruning	Brain region	Refs
Mouse models (cont.)			
Lyz2^{Cre}; *Atg7*$^{flox/flox}$	Increased spine density Higher levels of PSD95 and SHANK3 Reduced degradation of synaptophysin and PSD95 by microglia	Somatosensory cortex	95
Cx3cr1$^{CreER/+}$; *R26*$^{eIF4E/eIF4E}$	Increased spine density Altered microglial gene expression Impaired microglial phagocytosis	Prelimbic cortex and hippocampus	192
Tg(ACTB^{eIF4E})	Increased spine density	Prelimbic cortex	194
Setd1a$^{+/-}$	Reduced spine density	Prelimbic cortex	208,209
C4b$^{-/-}$	Impaired eye-specific segregation Reduction in synaptic C3 labelling	Thalamus	213
Mouse *C4b* overexpression (in utero electroporation)	Increased microglial engulfment of PSD95 Increased spine elimination	Prefrontal cortex	214
Human *C4A* overexpression (transgenic mice)	Reduced synapse density Increased microglial synapse engulfment	Thalamus and prefrontal cortex	215
Maternal immune activation[b]	Increased synapse density Reduced *Cx3cr1* expression	Hippocampus	224
	Increased synapse density Reduced microglia synaptic engulfment	Hippocampus	225
	Altered microglial gene expression Increased spine density Increased microglia–spine interactions Hyper-ramified microglia	Prefrontal cortex	226
Maternal *n*-3 PUFA deficiency	Decreased synapse density Increased microglial synapse engulfment	Hippocampus	230

MHC, major histocompatibility complex; NA, not applicable; PUFA, polyunsaturated fatty acid; PET, positron emission tomography. [a]Some studies also report normal or decreased spine densities depending on age, brain region and method of analysis (reviewed elsewhere[174]). [b]Some studies also report normal or decreased spine densities depending on age, brain region and infection paradigm (reviewed elsewhere[228]).

to overpruning or reduced spine formation. The most significant genetic associations with schizophrenia are within the extended MHC locus, which spans ~8 Mb and contains numerous immune-related genes[211]. Given the role of MHC molecules in synaptic pruning, it is intriguing to speculate that defects in synaptic pruning owing to genetic variation in MHC molecules can result in schizophrenia[78,212]. This MHC locus gained even more attention recently with work showing that allelic variants of another gene within this locus, *C4A*, confers an increased risk of schizophrenia[213]. In humans, C4 exists as two isoforms, C4A and C4B, which are encoded by separate genes; allelic variants that increase expression of *C4A* but not of *C4B* are correlated with heightened schizophrenia risk[213]. C4 is particularly interesting as it is downstream of C1q in the classical complement cascade, which is known to regulate synaptic pruning (FIG. 2b). Mouse C4, encoded by *C4b*, has sequence homology to both human isoforms of C4. Supporting a role of C4 in pruning, *C4b*-knockout in mice leads to defective synaptic pruning in the retinogeniculate system. Supporting this initial work, in utero electroporation-mediated overexpression of mouse C4 leads to increased microglial synapse engulfment and hypoconnectivity in the mouse prefrontal cortex, which is accompanied by defects in social interactions[214]. Furthermore, binding of human C4A to synapses is more efficient than that of C4B, and overexpression of human *C4A* in mice results in increased synapse engulfment by microglia, elevated synaptic pruning in the cortex, and social and cognitive behaviours related to schizophrenia[215]. Moreover, C3 deposition and microglial synapse engulfment in co-cultures of microglia and neurons were higher for cells derived from patients with schizophrenia harbouring *C4A* allelic variants than those harbouring *C4B* allelic variants[216]. Intriguingly, co-expression network analysis of schizophrenia brains showed an inverse correlation between the expression of *C4A* and synaptic genes that do confer schizophrenia risk, a transcriptional signature linking *C4A* expression with increased synaptic pruning[217]. Together, these studies provide evidence linking schizophrenia risk genes, particularly *C4A*, with overpruning in schizophrenia.

Environmental influences leading to pruning and behavioural defects in ASD and schizophrenia. In addition to genetic factors, there are clear indications that environmental factors play roles in the origin and progression of

ASD, schizophrenia and related disorders. For example, epidemiological evidence suggests that environmental factors that affect the immune system, such as maternal infections, maternal obstetric complications, maternal nutrition and pollution, increase disease risk[218–223]. Thus, a 'two-hit' hypothesis has been proposed in which risk genes affect susceptibility, but a secondary environmental influence is also required to initiate abnormal developmental programmes, including aberrant synaptic pruning[219].

Mechanistic evidence for environmental influences affecting developmental synaptic pruning and subsequent ASD-like or schizophrenia-like behaviours largely stems from animal models. For example, an immune challenge in pregnant rodents (that is, maternal immune activation), which is known to induce ASD-like and schizophrenia-like behaviours in offspring[223], increases neuronal spine density and reduces expression of the microglial pruning gene *Cx3cr1* in the hippocampi of offspring[224]. Other studies found decreased microglial synapse engulfment in a similar model[225], and synaptic and behavioural deficits induced by maternal immune activation were prevented by postnatal depletion of microglia[226]. Early-life inflammation also increased microglial engulfment of dendritic spines in response to stressors later in life during adolescence, and was also prevented by depletion of microglia[227]. Thus, microglia might be the mechanism by which early immune activation results in synaptic pruning defects and behavioural changes (reviewed elsewhere[228]). Another prenatal environmental risk factor for ASD and schizophrenia is the diet, including insufficient maternal dietary *n*-3 polyunsaturated fatty acids (PUFAs)[229]. Recently, it was shown that offspring from mothers with reduced levels of dietary *n*-3 PUFAs had increased levels of C1q, C3 and CR3 and elevated microglial synapse engulfment in the hippocampus. This was accompanied by decreased numbers of hippocampal synapses, and altered spatial working memory in offspring[230]. *n*-3 PUFA-deficiency offspring have elevated ALOX15–12-hydroxyeicosatetraenoic acid signalling in microglia, which was suggested to elevate complement receptor expression and microglia-mediated phagocytosis[230]. One possibility is that these environmental stressors first affect the maternal peripheral immune system, including the gut microbiota, which then affects immune signalling and synapse development in the fetal brain. This potential link has been demonstrated in rodent maternal immune activation, in which the autism-like behaviours and cortical circuit abnormalities in offspring manifest themselves as a result of maternal gut microbiota-dependent development of T helper 17 cells. These T helper 17 cells release IL-17A, which enters the fetus and affects neurodevelopment[231–233]. Thus, it could be that, for those with genetic susceptibility, this environmental influence becomes the 'second hit' that induces changes in developmental pruning in early life in the case of ASD or that 'primes' the system for later disruptions in pruning in schizophrenia.

In summary, multiple lines of evidence point to impaired synaptic pruning as an important mechanism in ASD, schizophrenia and related disorders (summarized in TABLE 1). In ASD, most evidence indicates the presence of elevated synaptic connectivity and an underpruning phenotype. However, depending on the mutation, neuron type and brain region affected, overpruning or underpruning could have similar functional outcomes. In schizophrenia, overpruning is the prevalent finding across multiple studies. Animal models demonstrate that many genetic risk factors that underlie syndromic forms of these disorders affect pruning. In schizophrenia, there is a clear link with complement-mediated pruning. Further linking these disorders to immune-mediated pruning are studies showing that environmental stressors, which largely affect immune signalling, may work cooperatively to affect synaptic pruning and the risk of developing ASD, schizophrenia and related disorders (FIG. 4).

Conclusions

Synaptic pruning in the CNS is a developmental programme that is necessary for establishing appropriate brain wiring and function. In many mammalian circuits, spontaneous and experience-driven changes in neuronal activity drive an activity-dependent Hebbian competition between synaptic inputs for synaptic territory, which is further shaped by local LTD and GABAergic innervation at individual synapses. Neuronal and glial immune signalling mechanisms and cell death pathways are spatially restricted to synapses and are activated downstream of changes in neural activity, leading to synaptic pruning. However, whether and how activity directly regulates these molecules, pathways and cells is unknown. One possibility is JAK2–STAT1 signalling, which canonically is involved in innate immune and cell death transcriptional programmes. During pruning, JAK2–STAT1 signalling is activated specifically in less active neurons and localized to less active synapses, which are subsequently eliminated[139]. It remains unknown how this transcriptional programme is elicited by changes in activity or is compartmentalized, leading to pruning of some synapses but not others. Another possibility is that local changes in calcium levels owing to, for example, LTD result in perturbations of local mitochondria. These early mitochondrial changes can then elicit recruitment and/or activation of cell death signalling molecules, which stimulate immune signalling and glial cells for synapse removal. Furthermore, it is also unclear whether pruning occurs in all neurons and circuits, and for those circuits that are pruned, why different mechanisms are used for pruning depending on the brain region and circuit. The extent of pruning could prove challenging to measure given that there could be large variations in the circuits that are pruned and the timing of pruning across species. Understanding why different pruning mechanisms are elicited for different types of neurons within a given species should be more tractable. The different mechanisms used could result from regional molecular and functional heterogeneity of neurons and glia throughout the mammalian CNS, which is becoming increasingly appreciated from single-cell RNA sequencing and spatial transcriptomic studies. Finally, the lessons learned regarding the mechanistic underpinnings of synaptic pruning are now

informing our understanding of disease pathogenesis. For example, a number of studies point to immune and glia-mediated synaptic pruning as a common disruption across multiple neurodevelopmental disorders, including ASD and schizophrenia. However, in many of these studies, as well as in studies of the fundamental biology underlying activity-dependent pruning, only one time point is typically assessed. It is possible that synapse density changes could also result from defects in synaptogenesis. Also, while pruning defects could be reflective of increases or decreases in elimination of the 'losing' synapses, these phenotypes could also be a consequence of impaired stability or maintenance of the 'winning' synapses.

The field of activity-dependent synaptic pruning research has made tremendous progress since the initial studies by Hubel and Wiesel. A picture is emerging by which activity elicits immune and cell death signalling that, ultimately, leads to the clearance of some synapses and maintenance and strengthening of others. We are now at a point where these critical questions in the field can be addressed, which will result in fundamental new insights into how CNS circuits develop and how defects in pruning can drive changes in connectivity and behaviour in a myriad of neurodevelopmental and neuropsychiatric disorders.

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