Mechanisms governing activity-dependent synaptic pruning in the mammalian CNS

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Abstract

Nearly 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 time, 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 synaptic components, 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 programs may go awry in neurodevelopmental disorders of the human CNS with a focus on autism spectrum disorders (ASD) and schizophrenia (SZ). Together, we intend to give an overview of how the field of activity-dependent pruning has evolved, has led to exciting new questions, and has guided the identification of new, therapeutically-relevant mechanisms by which circuit development can go awry in neurodevelopmental disorders.

Introduction

The developing nervous system undergoes remarkable remodeling 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 vertebrate and invertebrate peripheral and central nervous systems (CNS)[1–4], this review will focus on developmental pruning of synapses in the mammalian CNS. We define synapse 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 will review literature

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demonstrating roles for neural activity in developmental synapse 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 signaling and cell death molecules in synapse removal, which can be neuron-intrinsic but can also include neuron-extrinsic, glia-driven mechanisms. Finally, we will highlight how lessons we have learned by studying synapse pruning in the healthy, developing nervous system have informed mechanisms of neurodevelopmental disorders with a focus on autism spectrum disorders (ASD) and schizophrenia (SZ).

Activity-dependent synaptic pruning: a historical perspective

Neuronal activity plays a central role in developmental synapse pruning, which involves weakening and elimination of synaptic connections and strengthening and maintenance of others[5–7]. In mammals, activity-dependent pruning occurs throughout the CNS at different developmental periods, depending on the brain region and neuron subtype. In this section, we will 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). Either through modulating spontaneous neural activity or sensory-evoked 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 link changes in activity with the physical elimination of synapses, which we outline in the section below as well as in following sections.

Spontaneous neural activity-driven synapse pruning

Among the first studies to identify roles for neural activity in synaptic pruning in the CNS were those in the retinogeniculate circuit, examining synapses from retinal ganglion cells (RGCs) onto relay neurons within the lateral geniculate nucleus (LGN) of the thalamus (Fig. 1a). Initially, right and left eye RGC inputs form exuberant synapses onto relay neurons within overlapping territories of the LGN. Before eye opening (<P10 in mice), waves of spontaneous activity in the retina drive the segregation of RGC inputs into eye-specific territories in the LGN (*i.e.* 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 between neurons of the same retina is synchronized, while activity between 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 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]. This circuit, thus, 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 fibers from the inferior olivary nucleus synapse on Purkinje cell somas. By ~P11 in mice, many somatic climbing fiber synapses are eliminated and a 'winning' climbing fiber translocates and synapses on the Purkinje cell dendrites[22,23]. In a second stage (~P12-P17), any remaining somatic climbing fiber synapses are pruned away. Similar to the retinogeniculate circuit, Hebbian-type plasticity is required for climbing fiber pruning. This includes strengthening of a single climbing fiber input, while other inputs weaken and are eliminated[24]. This selective strengthening requires postsynaptic activity as the climbing fiber input most synchronous to 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 fiber inputs during the first stage of pruning[30]. During the later stage of pruning, signaling downstream of metabotropic glutamatergic receptors (mGluR1) in Purkinje cell dendrites drives the pruning of any remaining somatic climbing fiber synapses[31–36].This mGluR1 signaling stimulates the expression of membrane tethered semaphorin Sema7a[37,38] and the release of brain-derived neurotropic factor (BDNF)[39] from Purkinje neurons onto remaining somatic climbing fiber synapses to facilitate their removal.

In addition to signals that lead to the elimination of 'losing' climbing fibers, other molecules stabilize the 'winning' climbing fibers. For example another semaphorin, Sema3A, is secreted from Purkinje neurons to promote the stabilization and maturation of 'winning' climbing fibers throughout all stages of pruning[37]. Acting in parallel to Sema3A is progranulin, which is derived from Purkinje cells and binds climbing fibers via Sort1 to promote climbing fibers stabilization and strengthening[40]. Finally, it is important to consider the influence of glia. Wrapping of climbing fiber synapses by specialized cerebellar astrocytes called Bergmann glia is required to strengthen the 'winning' synapses and prevent exuberant climbing fiber innervation along Purkinje cell dendrites during later pruning stages[41]. There are additional influences of microglia to promote weakening of a subset of climbing fiber synapses, which is discussed under immune-mediated synaptic pruning[42,30]. Together, the retinogeniculate and the cerebellar climbing fiber circuits demonstrate how differential activity in neurons drives a competitive pruning process. Cerebellar climbing fiber pruning has provided further insight into activity-dependent molecules that drive this competitive process.

Experience-driven changes in neural activity and synapse pruning

The effects of neural activity on synapse pruning have also been studied through the modulation of sensory experience. First demonstrated by Hubel and Weisel[43] in the cat visual cortex, sensory deprivation caused by suturing one eye closed during development (i.e. monocular deprivation) results in the weakening of synapses corresponding to the sutured eye and strengthening of synapses corresponding to the open eye. This process is 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 (Fig. 1c)[44–52]. Linking changes in activity with structural plasticity is evidence that long-term depression (LTD) also occurs during monocular deprivation[53,54], which has previously been linked to spine shrinkage and pruning in the hippocampus[55–57]. Also, GABAergic innervation of cortical pyramidal neurons within the visual cortex by parvalbumin (PV)[+] interneurons is required to detect spatiotemporal differences in thalamocortical inputs during monocular deprivation and is necessary for subsequent spine pruning[58,59]. Downstream of GABAergic transmission during monocular deprivation is the activation of the proteolytic enzyme tissue-type plasminogen activator (tPA), which is proposed to remodel the extracellular matrix to accommodate spine remodeling[59]. Similar to the visual cortex, visual experience also affects a later stage of synapse remodeling (P20-P30) in the retinogeniculate circuit when RGC synapses in the LGN consolidate and strengthen, a process that requires visual experience[60]. While some of this remodeling involves clustering of presynaptic terminals along a 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]. This also occurs in adult rodents, albeit less robustly[63–65]. However, there are also reports of increased spine density within the barrel cortex following whisker deprivation[66,67], possibly due to an increase in cortico-cortical synapses. It is notable that synapse pruning occurs with increased activity in the somatosensory cortex, too. For example, stimulating whisker activity through environmental enrichment accelerates thalamocortical synapse maturation and pruning[68]. Data suggests that spines compete for synapse-stabilizing cadherin/catenin complexes when activity is increased during whisker stimulation. Upstream of the barrel cortex in this circuit is the ventral posteromedial nucleus (VPM) of the thalamus, which also undergoes experience-dependent pruning. Sensory information originating from the whisker pad is conveyed to the VPM through synapses originating from the brainstem principal trigeminal nucleus (PrV). As active whisking begins (~P14 in mice), a subset of whisker-specific inputs in the VPM are strengthened while others are pruned away[69]. Evidence suggests that astrocytic calcium signaling induces purinergic signaling, which then impinges on P2Y1 receptors on VPM neurons to facilitate the pruning of a subset of synapses over 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 synapse pruning in mammalian circuits, there are still major gaps 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 this progress is that the developmental timing of this pruning is different across CNS regions. In addition, measuring the timing and neuron subtype undergoing pruning throughout the CNS is a major undertaking and work in one species may not necessarily align with another 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 where the pre- and post-synaptic partners are clearly defined. These model circuits have further paved the way to identifying exciting new molecular mechanisms that are activity-dependent and regulate synaptic pruning (discussed below).

Immune-mediated synaptic pruning

Besides the mechanisms described above, 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]. 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 below.

Neuron-intrinsic immune signaling regulating synaptic pruning

Excitement for immune-mediated synaptic pruning began with the discovery of activity-dependent regulation of class I major histocompatibility complex antigens (MHC class I) in the LGN[72]. Later, it was shown that that MHC class I molecules were neuronally expressed[73]. Further, mice lacking all cell surface MHC class I expression ($\beta_2 M^{-/-}$ or TAP $1^{-/-}$ mice), mice lacking the MHC receptor component CD3ζ, and mice deficient in 2 of the 50 MHC class I genes ($K^b D^{b-/-}$ mice) all had deficits in retinogeniculate eye-specific segregation[73,74]. Mice deficient in MHC class I genes or the MHC receptor PirB 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 they may also affect initial synapse formation, as suggested by a study showing elevated synaptogenesis in cultured $\beta_2 M^{-/-}$ neurons[77]. It also remains unclear how MHC class I-receptor signaling leads to pruning[78]. In the immune system, these molecules are key factors involved in antigen presentation, which activates neighboring adaptive immune cells to initiate destruction of foreign antigens, such as viruses. The T cell receptors that bind MHC I complexes are not expressed in the CNS, but the coreceptors (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 interacts with these receptors to regulate pruning is not known. MHC Class I-mediated pruning could represent a separable phenomenon from its function in the immune system, but MHC Class I presentation could also interact with other immune molecules, like neuronal pentraxins and complement proteins (discussed below).

Other neuronal immune-related molecules called neuronal pentraxins also regulate synapse pruning. Pentraxins are cyclic multimeric proteins involved in calcium-dependent ligand binding and innate immune response[79]. Neurons express the neuronal pentraxin receptor (NPR) and also neuronal pentraxin 1 (NP1) and neuronal pentraxin 2 (NP2/Narp). These neuronal pentraxins share 20–30% homology with short pentraxins in the peripheral immune system, but they have different tertiary structure and little evidence supports roles for them in innate immunity[80–82]. NP1/NP2 complexes regulate AMPA receptor clustering and synapse plasticity[83] and knockdown of NP1 in cultured rat neurons results in increased

numbers of synapses[84]. Mice lacking NP1 and NP2 also have deficits in eye-specific segregation in the retinogeniculate circuit, implicating these molecules in pruning of presynaptic terminals[85]. In peripheral immunity, other pentraxin molecules can activate the classical complement cascade through C1q and play a role in phagocytosis[86,87]. One possibility is that neuronal pentraxins and MHC class I molecules could be working together with complement to mediate synapse pruning via microglia (discussed in the following section). Indeed, very recent work in adult mice has shown that NPs co-immunoprecipitate with C1q in cortex-derived synaptosomes and that these molecules are co-localized *in vivo* at cortical synapses[88], but it remains unknown if these interactions occur during developmental synapse pruning.

Microglial-dependent immune signaling and synapse pruning

Microglia are resident macrophages of the CNS. Similar to other tissue resident macrophages, microglia are efficient phagocytes and key regulators of innate immune signaling in the CNS. The first evidence that microglia were involved in synaptic pruning was provided by work in the developing hippocampus, visual cortex, and retinogeniculate circuit[51,89,90]. These studies revealed that microglia engulfed synaptic components. In the visual cortex and retinogeniculate circuit, developmental synapse pruning by microglia is regulated by activity as microglia engulf synapses from less active neurons[51,90,91]. Microglial engulfment of synapses has also now been observed in other developing brain regions 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, it remains to be fully deciphered whether microglia more passively "clean up" synaptic debris or play a more active role in initiating synapse pruning. Arguing for the latter, live and static imaging in rodents, and now Xenopus, have demonstrated that microglia "trogocytose" (*trogo-*: nibble) intact presynaptic membranes but not postsynaptic structures[96,97], which is consistent with other work showing selective phagocytosis of presynaptic membranes[65,90]. Further arguing for a more active role, multiple groups have shown that disrupting microglial engulfment results in sustained increases in numbers of structural and functional synapses[65,89,90,94,98–100]. For example, mice lacking the fractalkine receptor CX3CR1 (*Cx3cr1*^{−/−}), a G protein-coupled chemokine receptor (GPCR) highly enriched in microglia in the healthy CNS[101], have delayed microglial infiltration into the hippocampus concomitant with delays in synapse maturation and a transient elevation in spine density[89]. It was also shown that adolescent to early adult *Cx3cr1*^{−/−} mice had increased excitatory synaptic connectivity in the hippocampus and defects in sociability[99]. More recently, CX3CR1 signaling was shown to directly impact 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 and this process was blocked in *Cx3cr1*^{−/−} mice and in mice lacking the canonical ligand fractalkine (CX3CL1). It was further shown that whisker removal elicited an increase in a metalloprotease ADAM10 that cleaves CX3CL1 into a secreted form. Pharmacological inhibition of ADAM10 phenocopied

the synapse engulfment and pruning defects in *Cx3cr1*^{−/−} and *Cx3cl1*^{−/−} mice. Together, these results suggest a mechanism by which CX3CL1 is cleaved by ADAM10 in an activity-dependent manner to bind microglial CX3CR1 and induce pruning of presynaptic terminals (Fig. 2b). In the immune system, the canonical function for CX3CR1 is to regulate recruitment of myeloid cells[102]. However, in the barrel cortex following whisker ablation, microglial recruitment is unaffected and only engulfment is blocked in *Cx3cr1*^{−/−} and *Cx3cl1*^{−/−} mice. Interestingly, CX3CR1 is required for phagocytosis of necrotic fibers after acute skeletal muscle injury by macrophages *in vivo* and phagocytosis of apoptotic cells by bone marrow-derived macrophages *in vitro*[103,104]. Still, the downstream signaling of this GPCR, which leads to regulation of engulfment, is an open question.

Besides CX3CR1, the classical complement cascade also regulates microglial synapse engulfment and pruning (Fig. 2c). In the innate immune system, the initiating molecule C1q induces the formation of the C3 convertase through complement factors C2 and C4. This C3 convertase then cleaves and activates C3, which then binds pathogens and cellular debris to induce cell lysis or clearance by phagocytic cells (reviewed in[105,106]). Similarly, C1q and C3 proteins localize to retinogeniculate synapses during developmental pruning and microglia then engulf and remove presynaptic terminals via complement receptor 3 (CR3), a C3 receptor[90,98]. Importantly, C1q^{−/−}, C3^{−/−}, and CR3^{−/−} mice have defects in eye-specific segregation, impaired removal of structural presynaptic terminals, and failure to eliminate functional synapses in the retinogeniculate circuit[98]. In other brain regions, C1q^{−/−} mice have increased numbers 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 adolescent male mice[94]. It remains to be determined if data in the nucleus accumbens is a reflection of removal of postsynaptic receptors from the postsynaptic membrane or reflective of a physical 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 elimination of some synapses, but not others. A few recent breakthroughs suggest that molecular inhibitors of microglial synaptic engulfment are involved. First, it was shown that a canonical "don't eat me" immune system molecule, CD47, is expressed by RGCs. CD47 and its cognate receptor SIRPα on microglia were required for activity-dependent retinogeniculate synaptic pruning (Fig. 2b)[108]. Another study identified an endogenous C1q inhibitor, SRPX2, is expressed by neurons[109]. SRPX2^{−/−} mice have increased C3 deposition, elevated engulfment of presynaptic terminals by microglia, and abnormal eye-specific segregation in the retinogeniculate circuit (Fig. 2c)[109]. In addition to complement inhibition, there is evidence that changes in sialyation and/or externalization of phosphatidyl-serine residues on synapses in the retinogeniculate circuit and hippocampus target complement binding to a specific subset of synapses (discussed later)[110–112].

An emerging concept from these microglia-mediated pruning studies is that mechanisms are region-specific and context-dependent. For example, early pruning in the developing retinogeniculate circuit is dependent on complement, but not CX3CR1[113]. CX3CR1 is also dispensable for climbing fiber pruning in the cerebellum[114]. Conversely, developmental pruning of hippocampal synapses and pruning of barrel cortex synapses are CX3CR1-dependent, but not CR3-dependent[65,96,115]. In the visual cortex, microglia engulf synaptic material during visual deprivation[51,116] but CX3CR1 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 recently been shown in other brain regions to modulate neural excitability[118,119]. This P2RY12-dependent modulation of neuronal activity by microglia, in turn, stimulates engulfment of synapses by a yet-to-be identified mechanism. Recent work has further suggested other non-phagocytic mechanisms by which microglia regulate pruning. In the cerebellum, microglia impact climbing fiber pruning by promoting GABAergic innervation of cerebellar Purkinje cell somas, which influences weakening of a subset of climbing fiber synapses[42,30]. Also, during a later stage of experience-dependent pruning in the retinogeniculate circuit, microglia-derived TNF family cytokine TWEAK acts via neuronal Fn14 to facilitate strengthening of synapses along thalamic relay neuron dendritic spines[62]. Precisely how different mechanisms drive pruning in specific circuits at specific times remains a key open question, which we discuss extensively later in this review.

Microglia-astrocyte immune crosstalk in synapse pruning

In addition to microglia, studies have also implicated astrocytes in developmental synapse pruning. Like microglia, astrocytes engulf retinogeniculate synapses in an activity-dependent manner[120]. Mice lacking the astrocyte-enriched engulfment receptor MEGF10 or the TAM receptor MerTK, typically used by phagocytes to engulf and clear apoptotic cells, have reduced astrocytic synapse engulfment and fail to properly prune retinogeniculate synapses (Fig. 2d)[120]. While MEGF10 is highly enriched in astrocytes in the mammalian CNS, both astrocytes and microglia express MerTK. In contrast to astrocytes, microglia in MerTK$^{-/-}$ mice engulfed more retinogeniculate inputs, but not at sufficient levels to rescue synaptic pruning deficits[120]. These data raise the question as to which cell type is most important for pruning. Immunofluorescence microscopy shows more engulfed material per cell in microglia, but more total engulfed material in the larger population of astrocytes[120]. It could be that astrocytes carry out the bulk of the pruning of retinogeniculate axons and synapses and microglia are involved in more local pruning and refinement. Interestingly, in the adult hippocampus, astrocytes were recently shown to more readily engulf synaptic material[121]. However, it is important to note that the kinetics of phagocytosis and degradation of phagocytosed material *in vitro* are faster in microglia compared to astrocytes[122]. Therefore, *in vivo* engulfment of synaptic material may be more easily detected at static imaging points in astrocytes compared to microglia, provided the kinetics of phagocytosis is similar between *in vitro* and *in vivo* contexts.

Besides engulfment, astrocyte secreted factors have also been shown to regulate pruning of synapses. For example, the astrocyte-secreted molecule Hevin is necessary for the elimination of exuberant synapses in the developing visual cortex[123]. It is intriguing to consider that this molecule is signaling to microglia. Indeed, astrocyte-microglia crosstalk has been shown to regulate microglial engulfment and pruning in other contexts (Fig. 2e). For example, astrocyte-derived transforming growth factor beta (TGF-β) induces C1q expression, which is necessary for complement-mediated retinogeniculate synapse pruning by microglia[100]. In sensorimotor circuits, astrocyte-derived IL-33 binds its receptor IL1RL1/ST2 on microglia to stimulate synapse engulfment and pruning[124]. Conversely,

microglia may also direct astrocytic synapse engulfment. This has been suggested by data in the hippocampus where microglia were recently shown to regulate synapse pruning via triggering receptor expressed on myeloid (TREM2)[112,125,126], in addition to CX3CR1. One of these studies suggested that TREM2 deficiency in microglia results in disrupted signaling of microglia to astrocytes, which subsequently results in failure of astrocytes to engulf synaptic material during hippocampal development[126]. However, this TREM2-deficient signal derived from microglia to stimulate astrocyte engulfment is unknown.

These studies have identified a diverse array of immune signaling mechanisms required for developmental synaptic pruning. Emphasizing the importance, many of these mechanisms are evolutionarily conserved (Box 1). However, there are still fundamental questions that remain. First, many studies only look at one timepoint once pruning is over. This raises the possibility that some of these molecules could regulate initial synaptogenesis, which would result in a phenotype that is identical to a pruning defect at older ages. Assessing synapse density pre and post pruning in mutant animals is key. Second, given the diversity of molecular players identified across different circuits, immune signals during synapse 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 signaling cascades in neurons, which could then elicit diversity in glial cell responses. Third, while it is clear that immune molecules are changing expression and/or localization in response to activity, it is unclear how. One possibility is through the newly identified mechanism by which JAK2-STAT1 signaling modulates activity-dependent axonal and synaptic pruning. Recent work has provided evidence in the developing retinogeniculate and callosal projections in mice that the protein tyrosine kinase JAK2 is activated in inactive neurons and synapses, but only when neighboring neurons and synapses remain activated[127]. This leads to the pruning of inactive synapses via the downstream transcriptional regulator STAT1. JAK2-STAT1 is a canonical signaling cascade in the immune system and driver of cytokine signaling. Precisely which molecules are transcriptionally regulated by JAK2-STAT1 signaling to induce the selective elimination of inactive synapses is unclear, but it is intriguing to consider that many immune molecules mentioned above are impacted.

Cell death molecules at the synapse

Besides immune signaling, evolutionarily conserved molecules (Box 1) canonically involved in cell death signaling have been implicated in activity-dependent synaptic pruning. The mechanisms described below are recognized as primary drivers of programmed cell death/apoptosis and clearance of apoptotic debris by phagocytes. However, it is becoming increasingly appreciated that these mechanisms can be subcellularly localized and temporally restricted to avoid cell death and, instead, drive pruning. In some cases, they may also be operating upstream of immune molecules to localize them to synapses prior to pruning.

Activation of caspases and phosphatases

Caspase-3 is a protease activated during mitochondria-mediated apoptosis. It cleaves hundreds of different protein substrates that mediate DNA fragmentation, phosphatidyl-serine (PtdSer) exposure, and membrane blebbing[128]. *In vitro* studies have revealed that mitochondrial capase-3 activation can be compartmentalized in dendritic spines, leading to LTD via AKT1 cleavage and AMPAR internalization[129,130], and subsequent spine pruning[131]. Additionally, caspase-3 knockout mice have defects in hippocampal spine pruning *in vivo*[131]. Compared to apoptosis, LTD and pruning involve lower levels and durations of caspase-3 activation, possibly due to proteasomal degradation of caspase-3 in the dendrites to prevent precocious cell death[130,131]. Besides caspase-3, caspase-2 has also been implicated in dendritic spine pruning[132]. Similar to caspase-3 knockout mice[129,130], deficiency in caspase-2 results in defects in LTD and increased numbers of dendritic spines in hippocampal neurons both *in vitro* and *in vivo*. Finally, another molecule involved in cell death[133], which has been implicated in pruning, is the calcium-dependent protein phosphatase calcineurin. In mammalian systems, calcineurin has been shown to be regulated by myocyte enhancer factor 2 (MEF2) to initiate degradation of PSD-95 in an activity-dependent manner, leading to dendritic spine elimination[134–136].

Phosphatidyl-serine exposure

Downstream of caspases, phosphatidylserine (PtdSer) residues normally restricted to the inner leaflet of the plasma membrane become externalized on the cell surface by scramblases, which are activated by caspases. During apoptosis, TAM receptor complexes on phagocytes bind externalized PtdSer, leading to engulfment and clearance of apoptotic cell bodies[137]. Similarly, recent studies have indicated that PtdSer is exposed on a subset of largely presynaptic terminals during developmental synaptic pruning in the retinogeniculate circuit and hippocampus, which are subsequently cleared by phagocytes[111,112,138]. It is unclear whether TAM receptors, such as MerTK, bind PtdSer during synapse pruning. However, other immune molecules required for synaptic pruning have been shown to bind PtdSer, including C1q[139], GPR56[138], and TREM2[140]. Annexin-V treatment, which binds externalized PtdSer, or deletion of TREM2 *in vitro* leads to reduced microglial engulfment and inhibition of hippocampal synapse pruning[112]. In addition, deletion of C1q *in vivo* leads to accumulation of exposed PtdSer, primarily at presynaptic structures, and reduced microglia-mediated pruning of presynaptic terminals in the retinogeniculate circuit[112,138]. Although an earlier study provided *ex vivo* data to support that C1q-tagged synapses were enriched for activated caspase-3 and exposed PtdSer compared to untagged synapses[111], this later work in the retinogeniculate circuit showed that stimulation of microglia-mediated synaptic pruning by PtdSer exposure was not downstream of caspase-3 activation[112]. Published in parallel, another group showed that the microglial adhesion G-protein coupled receptor, GPR56 binds directly to PtdSer[138]. Similar to C1q-deficient mice, PtdSer exposure and pruning of presynaptic terminals is blocked when GPR56 is conditionally ablated in microglia. Thus, data points towards GPR56, TREM2, and complement proteins working in concert to regulate microglial pruning of synapses via binding to PtdSer. It remains to be determined whether these pathways regulate each other or whether they are working in parallel to perform a similar function.

Potential role for mitochondria

While data are suggestive that molecules that normally regulate cell death are compartmentalized at the synapse to regulate synapse pruning, it remains less clear how they are initially activated at some synapses and not others. One potential mechanism is through JAK-STAT signaling (described above), which is activated in inactive synapses leading to their removal[127]. In addition to regulating immune molecules, JAK-STAT signaling is also known to regulate 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 signaling, and the physical removal of the synapse. Indeed, elevated mitochondrial calcium is required for LTD-dependent elimination of spines in cultured hippocampal neurons[129]. Also, 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]. Further, live imaging in adult mouse cortex and hippocampal slices has revealed that presynaptic terminals with more mitochondria are more stable and decreased presynaptic mitochondria motility is associated with increased synaptic strength[143,144]. It remains critical to understand how these mitochondrial changes may be relevant to synapse pruning during development *in vivo* as this 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 are used to facilitate synapse pruning. Externalized PtdSer, which may be downstream of local caspase activation, provides a substrate to initiate immune-mediated synapse pruning by glial cells (Fig. 3). Upstream JAK-STAT signaling and/or mitochondria may be key.

Disease-associated genes, environment, and synaptic pruning: implications for neurodevelopmental disorders

In humans, synaptic pruning primarily occurs during two developmental periods: the first two years after birth and adolescence (Fig. 4)[1]. Studies suggest defects in synaptic pruning during both developmental periods underlie several neurodevelopmental disorders[145–153]. Here, we focus on autism spectrum disorders (ASD) and schizophrenia (SZ) to highlight how, in addition to the molecules described above, 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 autism spectrum disorders (ASD)

Individuals with ASD are typically diagnosed within the first five years of life coincident with the first wave of synaptic pruning[1,154]. Analyses of postmortem ASD tissue have shown increased synapse density compared to neurotypical controls in the frontal, temporal, and parietal lobes, suggesting an under-pruning phenotype[155,156]. Transcriptomic analyses of postmortem ASD brains further show significant changes in genes associated with pruning[157] and hypomethylation of immune genes involved in synaptic pruning including

C1Q, C3, and C3R[158]. Genetic analyses also point to the involvement of molecules that regulate synapse formation and synaptic plasticity[159,160].

In addition to these genetic associations, recent studies in mice harboring mutations or deletions in syndromic ASD-linked genes further support activity-dependent synaptic pruning deficits. One example is Fragile X Mental Retardation Protein (FMRP). Mutations in FMRP cause Fragile-X Syndrome (FXS), a disorder with high incidence of ASD. FMRP is a known regulator of mRNA translation through eIF4E[161], is regulated by neuronal activity[162,163], and regulates activity-dependent synaptic plasticity[164,165]. FMRP also works in concert with another ASD-linked gene, MEF2[166–168], which can no longer carry out its normal role in activity-dependent spine elimination in *Fmr1*^{−/−} mice[136,169–171]. In addition, increased spines have been reported in the neocortex in FXS[172,173] and there are increased spines in the hippocampus and cortex and impaired ocular dominance plasticity in mice deficient in FMRP (*Fmr1*^{−/−} mice)[174–176]. Linking immune mechanisms discussed previously with FMRP regulation of pruning, data suggests 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]. Also, other studies find the opposite or no change in spines in *Fmr1*^{−/−} mice, likely due to differences in age, region, and methodologies[174]. Still, supportive of aberrant synaptic pruning, a time-course of paired electrophysiological recordings of L5 pyramidal neurons demonstrated that excess 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 has been noted in mice harboring genetic mutations linked to syndromic ASD: 15q11–13 duplication and neuroligin-3 R451C point mutation[180].

Other syndromic ASD-linked genes that regulate synaptic pruning are molecules belonging to the mTOR signaling pathway, another set of mRNAs that are regulated by FMRP[181]. For example, deletion of phosphatase and tensin homolog (PTEN) in neurons, a gene that accounts for ~1–4% of ASD cases[182] and an upstream inhibitor of mTOR, leads to increased spine density and altered social behavior in mice[183]. Other negative regulators of mTOR, tuberous sclerosis 1 (TSC1) and 2 (TSC2), cause tuberous sclerosis (TSC), which is another disorder with a high incidence of ASD. In mice deficient in *Tsc1* (*Tsc1*^{−/−}) or heterozygous for *Tsc2* (*Tsc*^{+/−}), spine density is increased in the cerebellum and cortex[155,184]. These synaptic changes occur concomitant with overactive mTOR signaling in both ASD tissue and in *Tsc*^{+/−} neurons[154]. Tang *et al.* further showed that overactive mTOR signaling in ASD and in *Tsc2*^{+/−} mice likely exerts its effects on spines and behavior by modulating neuronal autophagy[155], a cellular degradative process for proteins and organelles. The exact mechanism by which autophagy leads to synaptic pruning remains unknown. However, there is evidence that mitochondria, well-known targets of autophagy, are depleted at presynaptic sites in Tsc1/2 deficient neurons[185], which could impact synaptic pruning. Autophagy can also be induced presynaptically by neuronal activity[186,187] and autophagy can regulate synaptic transmission and plasticity by degrading synaptic vesicles and postsynaptic proteins[188–191]. Thus, autophagy via mTOR signaling may play a similar

role in activity-dependent pruning and disruptions may contribute to aberrant pruning in ASD.

These studies demonstrate clear links between alterations in mRNA translation and autophagy in neurons to alterations in synapse numbers in ASD. Yet, all of these molecules are ubiquitously expressed by numerous cell types throughout the CNS and the contribution of non-neuronal cell types to synaptic alterations in ASD must be considered. In fact, a recent study showed that increasing protein synthesis specifically in microglia by overexpressing eIF4E, which is regulated by FMRP, results in increased spine density and ASD-like behaviors[192]. This phenotype largely recapitulates phenotypes observed in mice with global overexpression of eIF4E[193,194]. A separate study found that selectively disrupting a 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 (SZ)

SZ is diagnosed later than ASD, typically between late adolescence and mid to late 20's[195]. However, even before the full onset of SZ, 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 SZ[197]. In contrast to ASD, data from SZ patients and animal models suggest an over-pruning defect. For instance, studies in SZ patients indicate progressive loss of gray matter volume[198], especially during the initial phase of the disease[199]. Examination of postmortem 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, new PET imaging with a ligand for the synaptic marker SV2A has revealed reduced density of presynaptic terminals in SZ brains[205].

Synaptic pruning defects in SZ are also supported by genetics. Genetic variations overrepresented in SZ predominantly affect synaptic genes[160,206,207]. In several cases, reduced spine density has been observed in transgenic mouse models lacking or harboring mutations in SZ risk genes[208–210], including recent studies of *Setd1a*$^{+/-}$ mice. However, it is unknown if these changes are due to over-pruning or reduced spine formation. The most significant genetic associations for SZ are within the extended MHC locus, which spans ~8Mb 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 due to genetic variations in MHC molecules can result in SZ[78,212]. This MHC locus gained even more attention recently with work showing that allelic variations in another gene within this locus, complement C4, confer genetic risk for SZ[213]. C4 is downstream of C1q in the classical complement cascade, which is necessary to form the C3 convertase to cleave and activate C3[90,98] (Fig 2c). C4 exists as two isoforms: C4A and C4B. Sekar *et al.* showed allelic variations that increase C4A, but not C4B, are correlated with heightened SZ risk[213]. In addition, synaptic pruning in the retinogeniculate circuit was defective in *C4* knockout mice. Supporting this initial work, AAV-mediated overexpression of C4 leads to microglial synapse engulfment and hypoconnectivity in the mouse prefrontal cortex,

which is accompanied by defects in social interactions[214]. Most recently, human C4A, the isoform that confers higher SZ risk, was shown to more efficiently bind synapses compared to C4B[215]. Also, C3 deposition and microglial synapse engulfment in microglia-neuron co-cultures was higher in cells derived from SZ patients harboring C4A allelic variants[216]. Finally, overexpressing human C4A *in vivo* in mice resulted in increased synapse engulfment by microglia, enhanced synaptic pruning in the cortex, and social and cognitive behaviors related to SZ[215]. Intriguingly, co-expression analysis of SZ brains show that C4A has a selective, inverse correlation with synaptic SZ risk genes[217]. Together, these studies provide evidence linking SZ-risk genes, particularly C4, with over-pruning in SZ.

ASD and SZ: Environmental influences leading to pruning and behavioral defects

In addition to genetics, there are clear indications that environmental factors play roles in the etiology and progression of ASD, SZ, and related disorders. For example, epidemiological evidence suggests that environmental factors, which 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 impact susceptibility, but also require a secondary environmental influence to initiate abnormal developmental programs, including synaptic pruning[219].

Mechanistic evidence for environmental influences impacting developmental synaptic pruning and subsequent ASD or SZ-like behaviors largely stems from animal models. For example, an immune challenge in pregnant rodents (i.e. maternal immune activation/MIA), which is known to induce ASD and SZ-like behaviors in offspring[223], increases neuronal spine density and reduces expression of the microglial pruning gene *Cx3cr1* in the hippocampi of offspring[224]. Two other studies found decreased microglial synapse engulfment in a similar model[225] and synaptic and behavioral deficits of MIA were prevented by postnatal depletion of microglia[226]. Thus, microglia may be the conduit by which early immune activation results in synaptic pruning defects and behavioral changes (reviewed in[227]). Other prenatal environmental risk factors for ASD and SZ such as insufficient maternal dietary omega-3 fatty acids (n-3 PUFAs)[228] also result in increased microglial engulfment of synaptic material, decreased hippocampal synapses, and altered spatial working memory in the offspring[229]. Connecting with immune-mediated pruning, in n-3 PUFA-deficient offspring, C1q, C3, and CR3 are elevated in the hippocampus and spine and memory defects are dependent on C3. With dietary changes, it was shown that n-3PUFAs deficiency affects brain lipids, which then stimulates 12/15-LOX/12-HETE signaling in microglia to induce changes in CR3 expression[229]. Another possibility is that these environmental stressors first impact the peripheral immune system, including the microbiome, which then impacts immune signaling in the brain. This has been shown in rodent MIA in which the autism-like behaviors and cortical circuit abnormalities in offspring manifest as a result of gut microbiome-dependent development of T helper 17 cells (TH17) and their release of IL17a[230–232]. Thus, it could be that, for those with genetic susceptibility, this environmental influence becomes the "second hit" to induce changes in developmental pruning. This may affect pruning in early life in the case of ASD. It could also "prime" the system for later disruptions in pruning during SZ.

In summary, multiple lines of evidence point towards impaired synaptic pruning as an important mechanism in ASD, SZ, and related disorders (summarized in Table 1). As discussed previously, many studies look at only one endpoint and compare synapse density. It is important to consider that besides the physical elimination of synapses, these changes in synapse densities could be a consequence of exuberant synaptogenesis or increased turnover of synaptic structures. In ASD, most evidence points towards elevated synaptic connectivity and an under-pruning phenotype. However, depending on the mutation, neuron type, and brain region affected, over or under-pruning could have similar functional outcomes. In SZ, over-pruning prevails across multiple studies. Animal models demonstrate that many genetic risk factors that underlie syndromic forms of these disorders impact pruning. In SZ, there is the clearest link to disruptions in MHC and complement-mediated pruning. Further linking to immune-mediated pruning is work showing that environmental stressors may work cooperatively to impact the immune system, pruning, and risk of developing ASD, SZ, and related disorders (Fig. 4).

Perspectives and outstanding questions

Synaptic pruning in the CNS is a developmental program 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 signaling mechanisms and cell death pathways spatially restricted to synapses are activated downstream of changes in neural activity and lead to synaptic pruning. Yet, whether and how activity directly regulates these molecules and cells is unknown. One possibility is JAK2-STAT1 signaling. Canonically, JAK2-STAT1 is involved in innate immune and cell death transcriptional programs. During pruning, it was very recently shown to be activated specifically in less active neurons and localized to less active synapses, which were subsequently eliminated[138]. It remains unknown how this transcriptional program 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 due to, for example, LTD results in perturbations of local mitochondria. These early mitochondrial changes can then elicit recruitment and/or activation of cell death signaling molecules, which then stimulates immune signaling and glial cells for synapse removal. It also remains unknown whether pruning occurs in all neurons and circuits. Also, for those circuits that do prune, it remains a mystery why different mechanisms are used for pruning depending on the brain region and circuit. The extent of pruning could prove challenging to measure given the differences across species, and the timing of pruning will be different depending on the circuit. Understanding why different pruning mechanisms are elicited for different types of neurons should be more tractable. This could result from regional molecular and functional heterogeneity of neurons and glia throughout the mammalian CNS, which is becoming increasingly more appreciated with single-cell RNAseq and spatial transcriptomic studies. Finally, the lessons learned regarding the mechanistic underpinnings of synaptic pruning are now informing disease. This is reflected by a number of studies, which point to synaptic pruning as a common feature disrupted across multiple neurodevelopmental

disorders, including ASD and SZ. However, in many of these studies, as well as studies of the fundamental biology underlying activity-dependent pruning, only one timepoint is assessed. It is possible that synaptic 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 has made tremendous progress since the initial studies by Hubel and Wiesel. A picture is emerging by which activity is eliciting immune and cell death signaling that, ultimately, leads to the clearance of some synapses and maintenance and strengthening of others. We are now at a point to address these critical questions in the field. This will result in fundamental new insight into how CNS circuits develop and how defects in pruning can drive changes in connectivity and behavior in a myriad of neurodevelopmental and neuropsychiatric disorders.

Acknowledgments:

Glossary

Synapse pruning

A developmental process in which elements that comprise a *bona fide* structural synapse (presynaptic terminal and postsynaptic membranes) are eliminated, which may also include some pruning back 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

Experience-driven neural activity

Neuronal activity driven by external changes affecting sensory experience

Long-term depression

A process by which changes in neuronal activity, such as sustained low-frequency stimulation, induces a reduction in synaptic strength.

Eye-specific segregation

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

Monocular deprivation

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

Ocular dominance plasticity

A process by which disruption of visual input to one eye (see Monocular deprivation) results in strengthening of synaptic inputs from the open eye and weakening and elimination of synapses corresponding to the sutured, deprived eye.

Critical window

A limited period during development in which a cellular developmental process, such as synaptic pruning, is responsive to intrinsic or extrinsic (environmental) factors, including changes in neuronal activity.

Engulfment

The internalization or phagocytosis of material into a cell for degradation.

Trogocytosis

Partial phagocytosis of material ("trogo" – "nibble") while leaving the remaining membrane intact.

Opsonization

The process by which molecules, such as complement, adhere to material destined for removal by phagocytes or cell lysis.

Apoptosis

A well-controlled process of programmed cell death which may naturally occur in development and involves membrane blebbing, cell shrinkage, and DNA fragmentation.

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Box 1.

Evolutionarily conserved pruning mechanisms: Immune and cell death signaling in invertebrate systems.

Two of the major immune signaling pruning pathways, MHC class I and complement, appear to be vertebrate-specific synaptic pruning pathways. However, many of the other molecular players identified in activity-dependent synaptic pruning were first identified during axonal or dendrite pruning in invertebrates. For example, glial cell-mediated engulfment of neuronal compartments during pruning is highly conserved. Indeed, in *Drosophila* glial cells engulf axons and sensory endings during developmental pruning[238]. This process is regulated by Draper[239–241], the homologue of the mammalian MEGF10 which regulates astrocyte-mediated pruning[120]. The homologue in *C. Elegans* CED-1 performs similar functions in cholinergic synapse pruning[242]. There is also evidence that secreted immune molecules involved in mammalian synaptic pruning regulate pruning in invertebrates. For example, in *Drosophila* the chemokine-like protein Orion, which has some similarity to mammalian fractalkine (CX3CL1), was recently shown to regulate glial recruitment and engulfment of axons during mushroom body pruning[243]. In addition, as astrocyte-derived TGF-β signaling has been implicated in driving complement-mediated pruning in mammals[100], TGF-β/Myoglianin drives axonal pruning in developing *Drosophila*[244]. Unlike mammals, TGF-β appears to be acting neuron-intrinsically to promote pruning in *Drosophila*.

Cell death signaling during pruning is also evolutionarily conserved. Caspase was first identified in *C. elegans* whereby the homologue *ced-3* regulated cell survival[245]. It is now appreciated that this signaling can be compartmentalized to regulate cellular remodeling without inducing cell death[246]. In the *C. elegans* nervous system, caspase CED-3 cleaves and activates the actin severing protein gesolin/GLSN-1 to promote disassembly of presynaptic machinery during synapse remodeling[247]. In addition, in GABAergic neurons, local calcium increases activates calcineurin, which, in turn, activates the caspase CED-3 to promote dismantling of presynaptic machinery[248]. Downstream of caspase activation and similar to the mammalian system, it has recently been shown in *C. elegans* that local PtdSer exposure regulates subsequent AMsh glial cell engulfment of sensory endings[249].

Together, these data point to evolutionary conserved mechanisms driving synaptic pruning by glia and immune and cell death molecules. Better understanding how changes in activity could regulate invertebrate pruning could open up powerful new systems to better understand activity-dependent synapse pruning across species.
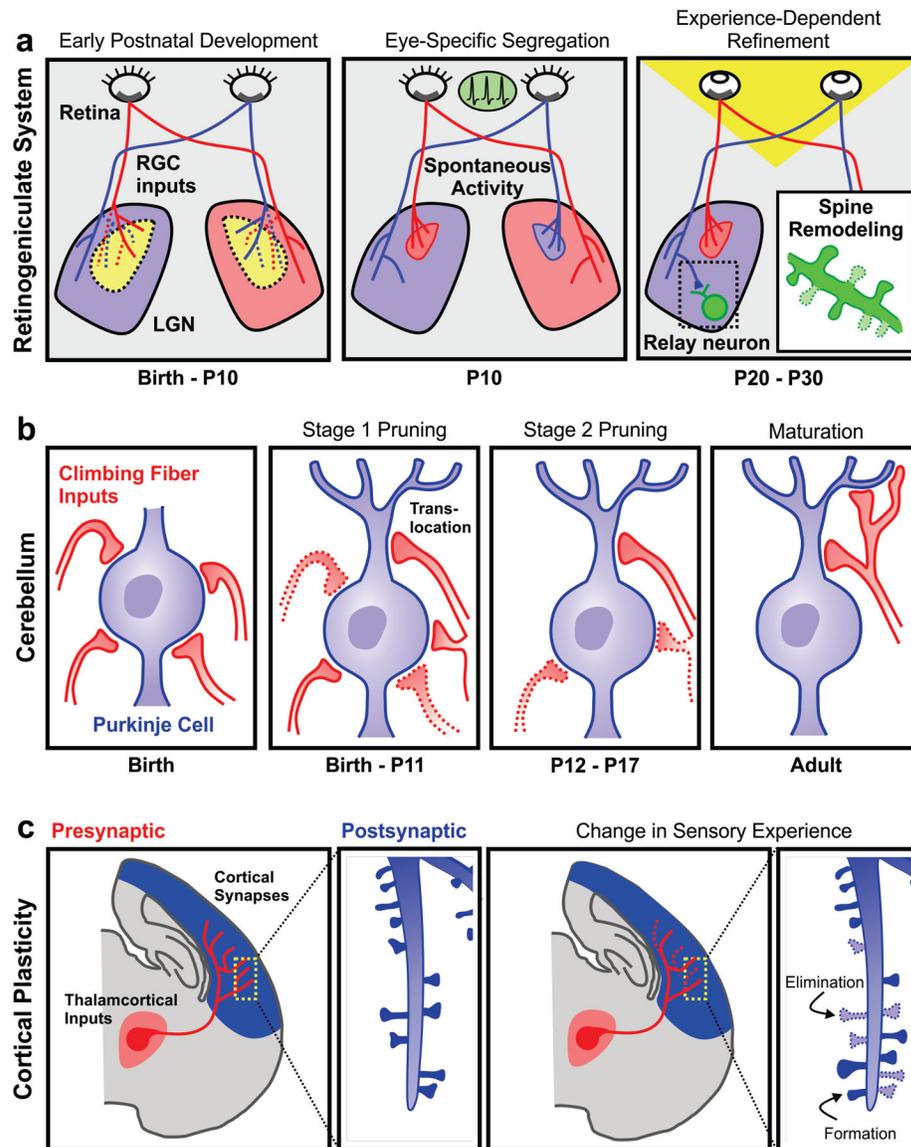
Figure 1. Model circuits for studying activity-dependent synaptic pruning.
The classic models for studying synaptic pruning in the mammalian CNS include developmental remodeling at the retinogeniculate circuit (a), cerebellum (b), and cortical synapses (c). Within the retinogeniculate circuit (a), retinal ganglion cell (RGC) axonal arbors initially form synapses with relay neurons in overlapping territories within the lateral geniculate nucleus (LGN) (far left panel in a). Prior to eye opening (Birth-P10), spontaneous neuronal activity in the retina results in pruning of RGC synapses (dotted red and blue lines) and eye-specific segregation so that each eye occupies a discrete territory prior to eye opening (middle panel in a)[8–19,233]. Relay neuron synapses undergo further remodeling after eye opening in response to light exposure from P20-P30 when relay neuron spines are pruned (far right panel, green)[60–62]. In the cerebellum (b), Purkinje cell somas are initially innervated by multiple climbing fiber inputs (far left panel in b). In the first stage of pruning (2nd panel in b), the weaker climbing fiber inputs at the soma are pruned, while the strongest

somatic input translocates to the Purkinje dendrites. In the second stage of pruning (3rd panel in b), remaining climbing fiber inputs to the soma are pruned, while the single climbing fiber input at the Purkinje dendritic arbor is maintained[22,23]. In the cortex (c), changes in sensory experience such as monocular deprivation or whisker manipulation (right panel in c) results in pruning of thalamocortical presynaptic terminals (red, dotted lines) and cortical dendritic spines (blue) in response to the changes in neuronal activity[43–52,59,63–65].
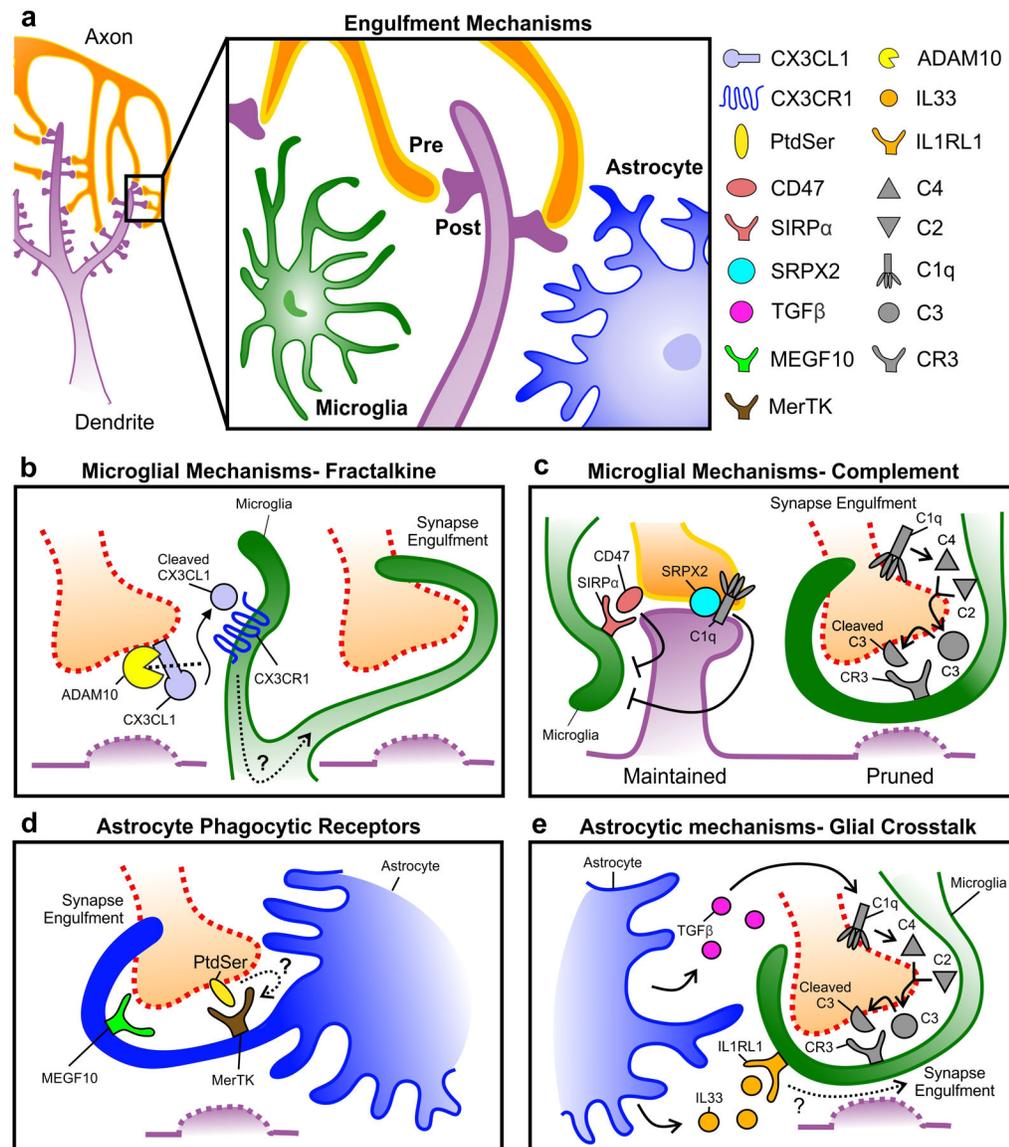
Figure 2. Summary of glial cell engulfment mechanisms regulating synaptic pruning.
Multiple glial engulfment pathways have been identified to regulate synaptic pruning (a).
A mechanism by which microglia prune synapses is through CX3CR1-CX3CL1-ADAM10
signaling (b). Neuron-expressed CX3CL1 is cleaved from the membrane by the protease
ADAM10 which initiates signaling to its microglial receptor CX3CR1. CX3CR1 signaling
instructs microglial synapse engulfment by a, yet-to-be identified mechanism[65]. Another
mechanism involved in microglial synapse pruning is the complement signaling cascade.
C1q induces the formation of the C3 convertase through complement factors C2 and
C4. C3 convertase then cleaves and activates C3, which then directs microglia to engulf
synapses via CR3 (c)[90,98]. In contrast, CD47 binding to its receptor SIRPα on microglia
inhibits engulfment of another subset of synapses[108]. Similarly, the complement inhibitor
SRPX2 binds C1q at the membrane in order to prevent C1q-mediated synapse engulfment
and pruning[109]. Astrocytes also play roles in synapse pruning through the phagocytic

receptors MEGF10 and MerTK. MerTK may directly bind externalized PtdSer at the synaptic membrane (d)[120]. Further, astrocyte-microglia crosstalk has been implicated in the pruning process (e). For example, astrocyte production of TGFβ directs C1q production by RGCs to influence microglial pruning[100]. Also, astrocytic IL33 expression signals to its microglial receptor IL1RL1 and instructs microglial synapse engulfment and pruning via a, yet-to-be-identified, downstream mechanism[124]. Cluster of differentiation 47 (CD47), signal regulatory protein α (SIPRα), sushi repeat containing protein X-linked 2 (SRPX2), transforming growth factor β (TGFβ), multiple epidermal growth factor-like domains protein 10 (MEGF10), Mer proto-oncognene tyrosine kinase (MerTK), phosphatidylserine (PtdSer), a disintegrin and metalloproteinase domain-containing protein 10 (ADAM10), C-X3-C motif chemokine ligand 1 (CX3CL1), C-X3-C motif chemokine receptor 1 (CX3CR1), interleukin 33 (IL33), interleukin 1 receptor like 1 (IL1RL1), complement receptor 3 (CR3), complement component 3 (C3), complement component 1q (C1q), complement component 4 (C4), complement component 2 (C2).

Cell Death Molecules
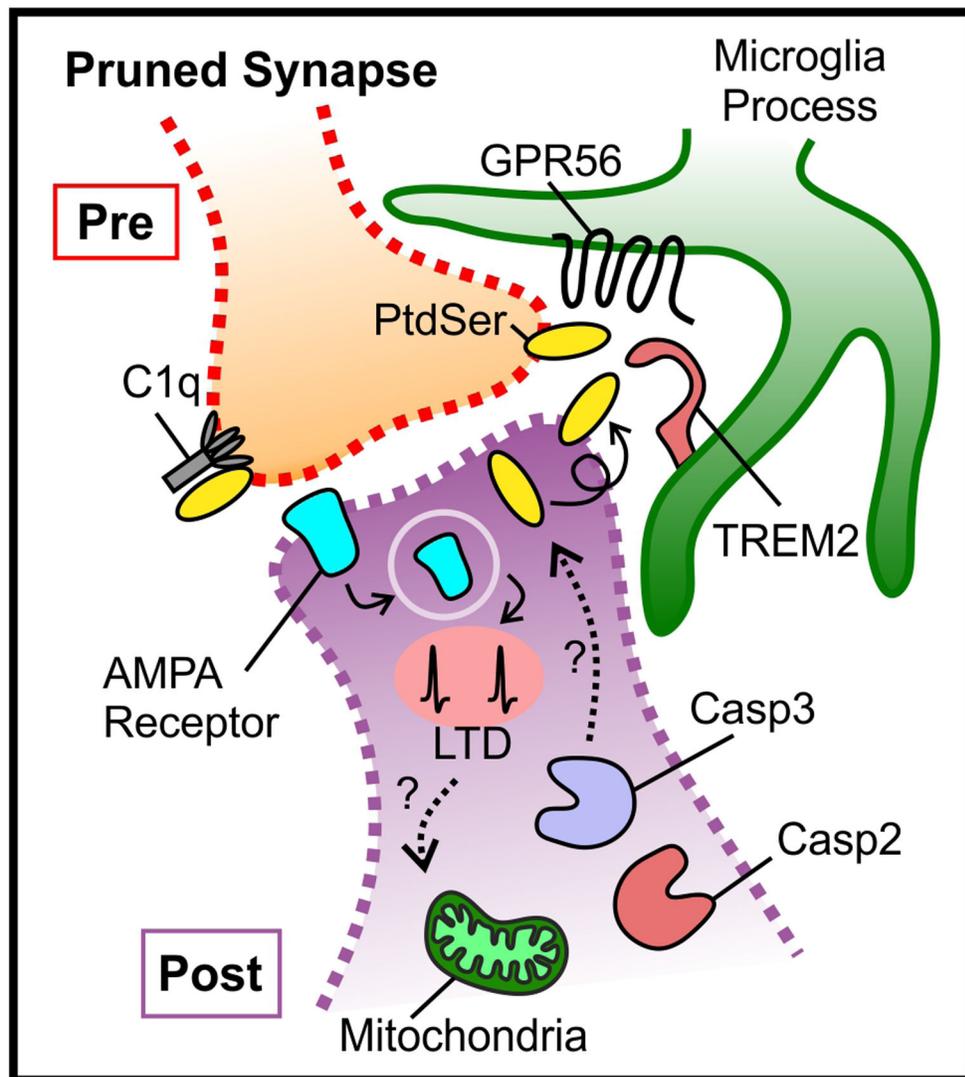
Figure 3. Local "death of a synapse."

During synaptic pruning, mitochondrial molecules in the dendrite activate Caspase 3 which drives AMPA receptor internalization from the postsynaptic membrane and LTD[129,130]. Caspase 2 also impacts AMPAR internalization although though a separate pathway[132]. Activity-dependent changes at the synapse, for example via LTD, may also influence mitochondrial processes and compartmentalized cell death molecules in order to regulate pruning (dotted lines). In some, but not all cases, caspase signaling leads to externalization of to the outer membrane at synapses. This exposed PtdSer can subsequently bind molecules such as C1q, GPR56, and TREM2 to induce pruning[112,125,138]. α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic-acid (AMPA), phosphatidylserine (PtdSer), G protein coupled receptor 56 (GPR56), triggering receptor expressed on myeloid cells (TREM2), complement component 1q (C1q).
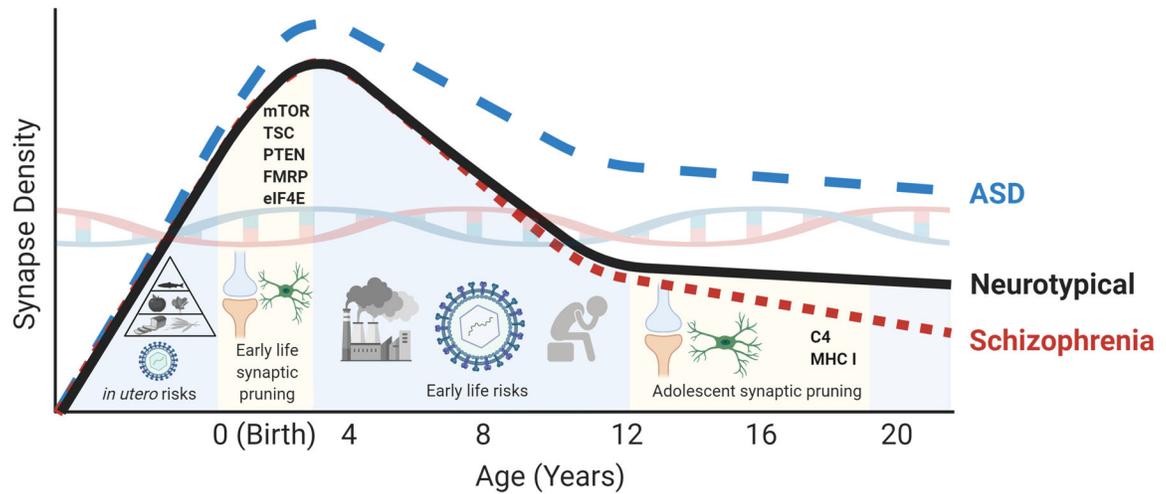
Figure 4. Synaptic pruning and neurodevelopmental disorders.
A summary of published findings depicting development changes in synapse density
in the brains of neurotypical individuals compared to patients with autism spectrum
disorders (ASD)[155,156] or schizophrenia (SZ)[198199200,201202–204205] (based on figure
from[153]). The developmental periods in early life and adolescence when synaptic pruning
primarily occurs are highlighted in yellow[1]. Key molecules implicated in aberrant
pruning during these two periods in ASD (mTOR[18218315595], TSC[155,184], PTEN[234235236],
FMRP[161172,173174,175179136,169–171177,178], eIF4E[192193,194]) and SZ (C4[213214215216], MHC
class I[21178,212]) are also indicated. Environmental risk factors which may, in concert, affect
pruning are also indicated[218–223219], such as maternal infection[223225226227] and diet[228229] *in
utero* and pollution, infection, and stress later in development. Genetic factors, which likely
interact with environment factors[237], are present throughout life (signified by the double
helix). Mammalian target of rapamycin (mTOR), tuberous sclerosis (TSC), phosphatase and
tensin homolog (PTEN), fragile X mental retardation protein (FMRP), eukaryotic translation
initiation factor 4E (eIF4E), major histocompatibility complex class I (MHC I), complement
4 (C4).

Table 1.

Summary of synaptic pruning studies in neurodevelopmental disorders discussed in this review

Species	Evidence for changes in pruning	Brain Region	Reference
Human			
ASD	Increased spine density Failure to eliminate spines over development	Temporal cortex	Tang, *Neuron*, 2014, Ref #155
	Increased spine density	Temporal cortex, frontal cortex, parietal cortex	Hutsler, *Brain Research*, 2010, Ref #156
	Transcriptional profiling reveals changes in immune/glial mRNA related to pruning	Combined frontal/ temporal cortex	Voineagu, *Nature*, 2011, Ref #157
	Hypomethylation and overexpression of complement genes involved in pruning	Prefrontal cortex, cingulate cortex	Nardone, *Transl Psychiatry*, 2014, Ref #158
	Genetic association to synaptic plasticity genes	N/A	De Rubeis, *Nature*, 2014, Ref #160
Fragile-X	Increased immature spine morphology	Cingulate cortex, temporal cortex	Hinton, *Am J Med Genet*, 1991, Ref #172
	Increased immature spine morphology	Cortex	Rudelli, *Acta Neuropathol*, 1985, Ref #173
Schiophrenia	Reduced gray matter volume	Cortex	Zipursky, *Arch Gen Psychiatry*, 1992, Ref #198
	Progressive loss of grey matter voume	Cerebral cortex, frontal cortex, thalamus	Andreasen, *Biologial Psychiatry*, 2011, Ref #199
	Reduced spine density Normal spine density	Dorsolateral prefrontal cortex Visual cortex	Glantz, *Arch Gen Psychiatry*, 2000, Ref #200
	Normal spine density	Dorsolateral prefrontal cortex	Kolluri, *Am J Psychiatry*, 2005, Ref #201
	Reduced synaptophysin immunoreactivity	Prefrontal cortex	Glantz, *Arch Gen Psychiatry*, 1997, Ref #202
	Reduced synaptic vesicle proteins	Frontal cortex, parietal cortex, cingulate cortex, hippocampus, thalamus	Davidsson, *Schizophr Research*, 1999, Ref #203
	Reduced VGluT1 boutons	Prefrontal cortex	Bitanihirwe, *BMC Psychiatry*, 2009, Ref #204
	Reduced SV2A (PET)	Frontal cortex, cingulate cortex	Onwordi, *Nature*, 2020, Ref #205
	Genetic association to synaptic gene loci	N/A	Fromer, *Nature*, 2014, Ref #206; SWG of PGC, *Nature*, 2014, Ref #207
	Genetic association to MHC gene locus	N/A	Stefansson, *Nature*, 2009, Ref #211
	Genetic association to C4 allelic variation	N/A	Sekar, *Nature*, 2016, Ref #213
Human Cell Models			
C4 variant microglia-neuron co-cultures	Increased microglial engulfment	N/A	Sellgren, *Nature Neuroscience*, 2019, Ref #216
Mouse Models			
Tsc2+/−	Failure to eliminate spines over development Increased levels of PSD95 and Synaptophysin	Temporal cortex	Tang, *Neuron*, 2014, Ref #155

Species	Evidence for changes in pruning	Brain Region	Reference
Atg7 flox/flox; CamKIIa-Cre	Failure to eliminate spines over development	Temporal cortex	Tang, *Neuron*, 2014, Ref #155
Atg7 flox/flox; CamKIIa-Cre; Tsc2+/−	Failure to eliminate spines over development	Temporal cortex	Tang, *Neuron*, 2014, Ref #155
Nse-Cre; Pten flox/flox	Macrocephaly Increased spine density	Hippocampus, cortex	Kwon, *Neuron*, 2006, Ref #183
L7-Cre; Tsc1 flox/flox	Increased spine density	Cerebellum	Tsai, *Nature*, 2012, Ref #184
Fmr1-KO [*]	Increased spine density Reduced microglial engulfment of PSD95	Hippocampus	Jawaid, *Glia*, 2018, Ref #175
	Impaired ocular dominance plasticity	Visual Cortex	Dolen, *Neuron*, 2007, Ref #176
	Failure to eliminate spines over development	Somatosensory Cortex	Patel, *J. Neuroscience*, 2014, Ref #179
	Impaired synapse elimination	Hippocampus	Pfeiffer, Neuron, 2010, Ref #169
	Impaired activity-dependent spine elimination	Somatosensory Cortex	Pan, *PNAS*, 2010, Ref #178
Lyz2-Cre; Atg7 flox/flox	Increased spine density Higher PSD-95, Shank3 protein levels Reduced degradation of synptophysin, PSD-95 by microglia	Somatosensory Cortex	Kim, *Molecular Psychiatry*, 2016, Ref #95
Cx3cr1-CreER/+; R26(Eif4e/Eif4e)	Increased spine density Altered microglial gene expression Impaired microglial phagocytosis	Prelimbic cortex, hippocampus	Xu, *Nature Communications*, 2020, Ref #192
eIF4E transgenic mice	Increased spine density	Prelimbic cortex	Santini, *Nature*, 2013, Ref #194
Setd1a+/−	Reduced spine density	Prelimbic cortex	Mukai, *Neuron*, 2019, Ref #208 Nagahama, *Cell Reports*, 2020, Ref #209
DISC1			
C4 KO	Impaired eye-specific segregation Reduction in synaptic C3 labeling	Thalamus	Sekar, *Nature*, 2016, Ref #213
Human *C4* overexpression (*in utero* electroporation)	Increased microglial engulfment of PSD-95 Increased spine elimination	Prefrontal cortex	Comer, *PLoS Biol*, 2020, Ref #214
Human *C4* overexpression (transgenic mice)	Reduced synapse density Increased microglial synapse engulfment	Thalamus, prefrontal cortex	Yilmaz, *Nature Neuroscience*, 2020, Ref #215
Maternal Immune Activation [**]	Increased synapse density Reduced *Cx3cr1* expression	Hippocampus	Fernandez de Cossio, *Brain, behavior, and Immunity*, 2017, Ref #224
	Increased synapse density Reduced microglia synaptic engulfment	Hippocampus	Andoh, *Cell Reports*, 2019, Ref #225
	Altered microglial gene expression Increased spine density Increased microglia-spine interations Hyperramified microglia	Prefrontal cortex	Ikezu, *Molecular Psychiatry*, 2020, Ref #226
Maternal n-3 PUFA deficiency	Decreased synapse density Increased microglial engulfement	Hippocampus	Madore, *Nature Communications*, 2020, Ref #229

[*] Note: Some studies also report normal or decreased spine densities depending on age, brain region, and method of analysis. See Review by He, *Neuroscience*, 2013, Ref #174

[**] Note: Some studies also report normal or decreased spine densities depending on age, brain region, and infection paradigm. See Review by Pekala, *Dev Neurobiol*, 2020, Ref #227