# Errant gardeners: glial-cell-dependent synaptic pruning and neurodevelopmental disorders

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Abstract | The final stage of brain development is associated with the generation and maturation of neuronal synapses. However, the same period is also associated with a peak in synapse elimination — a process known as synaptic pruning — that has been proposed to be crucial for the maturation of remaining synaptic connections. Recent studies have pointed to a key role for glial cells in synaptic pruning in various parts of the nervous system and have identified a set of critical signalling pathways between glia and neurons. At the same time, brain imaging and post-mortem anatomical studies suggest that insufficient or excessive synaptic pruning may underlie several neurodevelopmental disorders, including autism, schizophrenia and epilepsy. Here, we review current data on the cellular, physiological and molecular mechanisms of glial-cell-dependent synaptic pruning and outline their potential contribution to neurodevelopmental disorders.

Neuromuscular junction
(NMJ). A peripheral synapse between one or more motor neurons and the motor endplate on a skeletal muscle fibre.

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Postnatal development of the nervous system is associated with the generation of excess neuronal synapses, their selective elimination and the maturation of surviving contacts[1–3]. The selective elimination of synapses that occurs during periods of synapse formation and refinement is called synaptic pruning and has an important functional role in the proper maturation of several synaptic inputs, including the neuromuscular junction (NMJ), cerebellum and visual system[4–6]. In all of these systems, an initial excess formation of synapses is followed by synaptic elimination before the adult synaptic architecture is established. Synaptic pruning is thought to be necessary for proper maturation and function of the mature synapses (BOX 1).

However, these studies have focused on relatively distinct sets of synaptic connections, and the contribution of pruning to synaptic maturation across the rest of the brain remains poorly described. Recently, a growing number of studies has highlighted an essential role for non-neuronal glial cells in pruning, and several neuron–glia signalling pathways have been identified that are crucial for synaptic maturation[7–12]. These studies are the first to offer molecular insight into synaptic pruning and open the door to a more widespread understanding of pruning in synapse development, plasticity and disease. Here, we review data on the cellular, physiological and molecular mechanisms underlying glial-cell-dependent synaptic pruning and consider how abnormal pruning may contribute to the risk of neurodevelopmental disorders. Most of the studies reviewed below were carried out in mice, although substantial work has been carried out in other systems, including *Drosophila melanogaster* (BOX 2).

## Synaptic pruning in various regions

Although synaptic pruning is described as a neuronal maturation process, there is mounting evidence that circuit refinement by the elimination of synapses requires the involvement of non-neuronal cells[1,13,14]. Supernumerary synapses can be removed both by glia of neuronal origin (including perisynaptic Schwann cells and astrocytes) and by glia of extra-neuronal origin (namely, myeloid-derived microglia)[8–10,15]. The first indication that glial cells are involved in synaptic pruning was a study that used light and electron microscopy to examine the developing corpus callosum in cats[16]. There, the large-scale axonal remodelling in embryonic and early postnatal development was accompanied by phagocytic activity of microglia and astrocytes, which were suggested to contribute to axon elimination[16]. Since then, synapse elimination by glial cells has been demonstrated in diverse model systems and circuits, ranging from peripheral synapses in the NMJ to central synapses in cortex, hippocampus, thalamus and cerebellum[4,8–10,17–19]. Glial-cell-dependent synaptic pruning has been suggested to contribute to both the primary phase of brain circuit refinement (in the first 3 weeks in mice) and to a second phase that is described during

Box 1 | **Contribution of synaptic pruning to synapse maturation and function**

Developmental synaptic pruning is required to refine emerging brain circuitry, and any perturbations in this process lead to both morphological and functional deficits[8–10,18,68,100]. However, the link between the failure to prune excessive synapses and its consequences for synaptic function has not yet been elucidated. Here, we speculate how synaptic pruning may support the proper maintenance and strengthening of remaining synapses.

**Input selectivity**

Synaptic pruning may contribute to the input selectivity of individual neurons by supporting synaptic competition. Selective elimination of a subset of synapses allows the strengthening of others, thus creating a specific input pattern and potentiating the efficiency of the remaining synapses (that is, leading to axon potential-induced simultaneous release at multiple synapses and thus increasing postsynaptic responses). As synaptic pruning has been repeatedly demonstrated to depend on synaptic activity[7,9,10,31] and to favour the maintenance of more-active synapses while removing the less-active ones, it may underlie the formation of receptive fields and topographic mappings. For example, the strengthening of the most responsive synapses and the subsequent pruning of irrelevant connections could explain the formation of receptive fields in the dorsal lateral geniculate nucleus and visual cortex, which are arranged topographically to encode the entire span of the visual field. However, whether developmental pruning of retinothalamic[9,10] and thalamocortical[17,45] synapses contributes to these processes is yet to be demonstrated.

**Signal-to-noise ratio**

Synaptic pruning may also improve neuronal signal-to-noise ratios. Developmental elimination of supernumerary synapses by microglia has been demonstrated to provide synaptic multiplicity in Schaffer collaterals of the hippocampus[18]. Formation of multi-synapse boutons during maturation ensures that action potentials passing down an axon induce simultaneous release of transmitter at multiple synapses on the same postsynaptic neuron. Membrane potential noise in the postsynaptic neuron due to spontaneous neurotransmitter release can be offset by averaging over multiple dendritic responses, thus strengthening the signal and preserving its fidelity[18]. Synaptic multiplicity ensures that even relatively weak signals are sufficiently enhanced to overcome the existing synaptic noise, and it upholds intact synaptic plasticity.

adolescence in mammals (3–8 weeks in mice)[13,20]. The primary phase of synaptic pruning ensures the proper formation of sensory circuits (for example, those processing tactile, visual and auditory information) and executive circuits (including those regulating memory and behaviours) of the brain[3]. By contrast, adolescent pruning is most pronounced in brain regions such as the prefrontal cortex (PFC) that require remodelling to achieve fully mature internally guided behaviour, goal planning and impulse control[21].

The distribution of glia such as astrocytes and microglia throughout the entire CNS indicates that glial-cell-dependent synaptic pruning is likely to be a widespread part of synapse development but leaves open the possibility that different types of pruning exist that may require distinct glial-cell-mediated pruning mechanisms. Here, we describe reports of glial-cell-mediated synaptic pruning in different parts of the nervous system (FIG. 1; TABLE 1).

***Neuromuscular junction.*** The NMJ undergoes synaptic pruning during development in order to transform the initial many-to-one connectivity — in which several terminals synapse onto one motor endplate on a muscle fibre — into the one-to-one connectivity found in the mature organism[4]. Synaptic refinement of the NMJ has been widely studied as a simple, accessible and easily manipulated synaptic structure, and the NMJ remains the best-understood example of synaptic pruning in terms of its structure and development.

During mouse embryonic development, motor endplates each become innervated by approximately ten synaptic inputs from different motor neurons. In the first and second postnatal weeks (in mice), multi-innervated endplates lose synaptic inputs to become singly or sparsely innervated. During synaptic elimination, some synapses are strengthened, and others are weakened, in a process that is thought to be driven by competition for limited postsynaptic resources and that can be biased by manipulations that increase or decrease the activity of individual competing inputs[15,22,23]. Ultimately, the mature NMJ is innervated by a single motor neuron that shows high synaptic efficacy. Interestingly, nearby Schwann cells — neuron-derived glial cells known to be crucial for muscle maintenance — surround synaptic inputs during NMJ refinement and engulf and phagocytose pruned synaptic material[22,24–26]. Engulfment by Schwann cells is associated with the activation of apoptotic signals in postsynaptic sites of associated NMJ synapses[27]. In mice, inhibition or genetic ablation of caspase 3, a protease associated with apoptosis, blocks the transition from multi- to single-innervated fibres[27].

Moreover, Schwann cells have been shown to exhibit intracellular $Ca^{2+}$ spikes in response to neurotransmitter acetylcholine released from motor neurons. These spikes decode the synaptic efficacy of each terminal based on levels of presynaptic transmitter release[15,28] and are thus hypothesized to play a part in determining which competing inputs shall subsequently be selectively phagocytosed. These findings suggest that Schwann cells actively respond to neural activity to select weak motor neuron inputs for engulfment and elimination. However, it should be pointed out that it has been difficult to perform high-resolution time-lapse imaging of the entire NMJ pruning process, and it remains possible that motor neuron boutons first degenerate via non-glial mechanisms and that their debris is subsequently cleared by Schwann cells. As we discuss further below, the question

Receptive fields
Particular regions of a sensory space, such as the visual field, in which a stimulus will modify the firing of an individual sensory neuron.

Topographic mappings
Ordered projections of a sensory surface, such as the retina, to one or more structures of the central nervous system (such as the lateral geniculate nucleus and the visual cortex).

Box 2 | **Glial-cell-mediated synaptic pruning in *Drosophila melanogaster***

In addition to rodents, synaptic pruning has also been investigated in *Drosophila melanogaster*. Although the neuronal circuitry and, in particular, glial cells in the fly differ considerably from those in mammals, the mechanisms of phagocytosis have been mostly conserved through evolution. Therefore, molecular mechanisms of phagocytic pruning in *Drosophila* may suggest homologous molecules involved this process in mammals.

In the fly, the refinement of neuronal connections has been investigated in the mushroom body and in the ventral nerve cord. The mushroom body is the insect brain structure that is responsible for olfactory learning and memory. The γ neurons are the first neurons to appear in the mushroom body during embryonic development, and their axons and their dendrites are pruned during metamorphosis to allow the formation of adult circuitry[114]. The γ neurons receive the input from olfactory projection neurons that relay olfactory experiences to the brain. Olfactory projection neurons also undergo stereotyped pruning of their dendrites and axon-terminal branches during metamorphosis[115]. In particular, the synapses of olfactory projection neurons and of the γ neurons are engulfed by glia during metamorphic circuit remodelling[115]. In the ventral nerve cord, the neurites of peptidergic neurons expressing Corazonin are also eliminated during metamorphosis, in a process that depends on astrocytic phagocytosis[116].

Developmental synaptic pruning in fly axonal degeneration commences with glia infiltrating into axonal bundles and engulfing neuronal varicosities in an endocytosis-dependent manner[114]. Glial synaptic pruning during metamorphosis requires the apoptotic cell engulfment genes *draper* and *ced-6* (fly homologues of the genes encoding MEGF10 (multiple epidermal growth factor-like domains protein 10) and GULP (PTB domain-containing engulfment adaptor protein 1), respectively), as well as the Crk–myoblast city (mbc)–Ced-12 signalling pathway (which is homologous to the mammalian CRK–dedicator of cytokinesis protein 1 (DOCK1)–engulfment and cell motility protein 1 (ELMO1) pathway)[116,117]. Loss of function of any of these genes suppresses glial engulfment, resulting in the inhibition of pruning[116,117].

Although the importance of MEGF10 in synaptic pruning in mammalian brains has already been confirmed[10], other molecular components homologous to those mediating elimination of unnecessary synapses in *Drosophila* are yet to be investigated. As all of them are known to have an important role in phagocytosis, such studies would be highly promising.

of whether glia have a rate-limiting role in initiating the pruning process or merely clean up after synapses have degenerated remains an active area of debate in the field.

***Cerebellum.*** A large literature has been devoted to studying the activity-dependent synaptic pruning of supernumerary synapses between climbing fibres and Purkinje cells in the rodent cerebellum during the second and third postnatal weeks[5]. Similar to motor endplates in the NMJ, Purkinje cells initially receive approximately four or more climbing fibre synaptic inputs, most of which are then gradually removed to leave behind a single, strong input[5,29]. Elimination of climbing fibre inputs depends on synaptic activity, with weaker climbing fibres that exhibit a lower probability of neurotransmitter release being preferentially eliminated[30]. Disruption of the activity of the climbing fibre specifically between postnatal day 9 (P9) and P12 impairs the pruning of excessive input fibres and results in sustained double innervation of Purkinje cells[31]. Although morphological analysis suggests that the retraction of climbing fibres is at least partly mediated by cell-autonomous axonal degeneration[32], a substantial number of Purkinje cell spines become surrounded by the processes of Bergmann glia during the synaptic pruning period[19,33], suggesting they may play a part in this process. Furthermore, synapse elimination is associated with increased lysosomal activity around Purkinje cells and localized within ring-shaped structures that resemble Bergmann glia[25]. Together, these findings indicate that weak neural activity attracts and may elicit increased phagocytic activity in nearby Bergmann glia, which thus contribute to pruning of climbing fibres by phagocytosing supernumerary synapses.

Molecular evidence for a non-cell-autonomous mechanism in the pruning of climbing fibre synapses comes from data showing that the complement component C1q-like protein 1 (C1QL1) is required for synaptic pruning of Purkinje cell inputs[34]. However, as in the NMJ, the active engulfment of synapses by Bergmann glia has not yet been shown, and it is also not clear whether apoptotic signalling is necessary for synaptic pruning of climbing fibres and, if so, at what stage of elimination glial cells are involved. Cell-type-specific manipulations of molecular components and time-lapse imaging will be necessary to shed light on the signalling between neurons and glia that underlies synaptic pruning in the developing cerebellum.

***Retinothalamic system.*** Synaptic pruning in the visual system is essential for the segregation of inputs from each eye to the thalamus and for the synaptic maturation of these sensory inputs. During the final week of embryonic development in mice, axonal inputs from retinal ganglion cells (RGCs) terminate in the dorsal lateral geniculate nucleus (dLGN) of the thalamus. At this stage, the ratio of ipsilateral to contralateral retinal inputs in each dLGN is approximately 1 to 2, and they are partially overlapping[35]. During the first postnatal week, before the onset of vision in mice, the RGC inputs undergo major eye-specific segregation through elimination of overlapping inputs by microglia and astrocytes in a process that is dependent on spontaneous activity[7], resulting in a separate map of the visual fields of each eye in each dLGN[7,9,10]. In this map, ipsilateral and contralateral eyes are represented in distinct, non-overlapping dLGN areas, with the contralateral area almost tenfold larger[35]. Glial-cell-dependent pruning ensures that, at the end of segregation period, each dLGN neuron is innervated by only one or two stable RGC axons from either the ipsilateral or the contralateral eye[7]. Circuit remodelling then continues over the next 2 weeks to further refine monocular regions and achieve adult synaptic input strength[36].

The selective elimination of retinogeniculate inputs and the maintenance of a subset of synaptic connections have been demonstrated to depend on the phagocytic activity of glial cells — specifically, microglia and astrocytes. Astrocytes in the dLGN at the end of the first postnatal week have been observed to contain more engulfed synaptic material than do the local microglia[10], but whether this reflects a greater contribution to synapse elimination is not clear, as the capacities of these cell types to degrade ingested material may not be equivalent. Synaptic pruning in the retinogeniculate system is diminished by using minocycline to interfere with microglial activity[7], further providing support for the involvement of microglia in this process.

Climbing fibres
Axons of inferior olivary neurons that form excitatory synapses with Purkinje cells in the cerebellum.

Bergmann glia
Radial astrocytes in the cerebellar cortex that are involved in early cerebellar development, glutamate diffusion control, synaptogenesis and synaptic pruning.

Minocycline
A tetracycline antibiotic that has been shown to inhibit inflammatory activation of microglia by blocking the nuclear translocation of the pro-inflammatory transcription factor nuclear factor-κB.

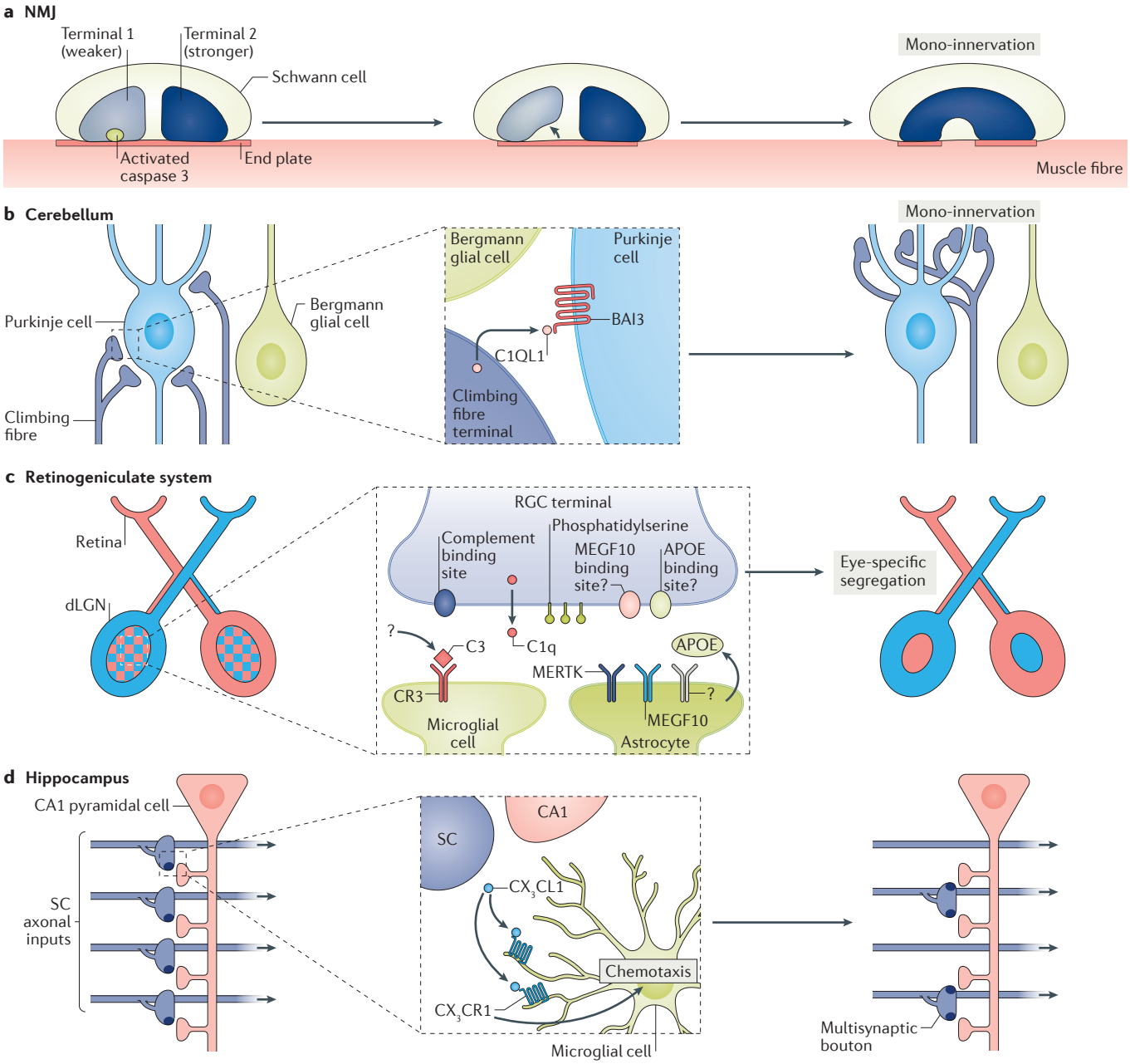


Figure 1 | **Molecular mechanisms of synaptic pruning by glial cells. a** | At the developing neuromuscular junction (NMJ), Schwann cells envelop terminals synapsing onto the motor endplate of a muscle fibre[22]. In order to achieve mono-innervation of the endplate, all but one of the terminals will be removed. Synaptic pruning is associated with activation of the apoptotic protease caspase 3 in the postsynapse of the terminal to be eliminated[22], before the terminal is thought to be phagocytosed by the Schwann cell. **b** | In the developing cerebellar cortex, many climbing fibres synapse onto each Purkinje cell but are eliminated to achieve mono-innervation. Bergmann glia surround Purkinje cell spines during synaptic pruning[19,33], and the process of climbing fibre elimination depends on interactions between C1q-like protein 1 (C1QL1) and brain-specific angiogenesis inhibitor 3 (BAI3; also known as ADGRB3)[34]. **c** | During development, retinal ganglion cells (RGCs) in the eye send projections mostly to the contralateral dorsal lateral geniculate nucleus (dLGN), with a smaller proportion projecting to the ipsilateral dLGN. In order to achieve the segregation of eye-specific inputs seen in the mature dLGN (on the right), immature projections from the contralateral eye that terminate in the region receiving ipsilateral inputs (chequered region) are eliminated. Synapses are eliminated by microglia in a manner dependent on complement proteins (specifically, complement component C1q, which is produced by neurons; complement component C3, which is of undefined origin; and complement receptor 3 (CR3), which is expressed by microglia)[9] and by astrocytes through MER receptor tyrosine kinase (MERTK), multiple epidermal growth factor-like domains protein 10 (MEGF10) and apolipoprotein E (APOE)[10,11]. MERTK is a known receptor for phosphatidylserine; however, the role of phosphatidylserine in synaptic pruning has not yet been demonstrated[10]. MEGF10 is a receptor that may bind C1q[124], whereas APOE is an opsonin that may act through glial low-density-lipoprotein receptor-related protein, although its target on synapses has not been identified[125]. **d** | In the developing hippocampus, Schaffer collateral (SC) inputs in the CA1 region form excessive numbers of synapses with CA1 pyramidal cells. Neuronal CX3C-chemokine ligand 1 ($CX_3CL1$) binds to microglial CX3C-chemokine receptor 1 ($CX_3CR1$), and this interaction is thought to promote chemotaxis and synaptic pruning, which in turn is required for establishing synaptic multiplicity[18].

Selective elimination of retinogeniculate synapses by glial cells is driven by input activity. In mice, pharmacological blockade of binocular spontaneous retinal waves on P4 markedly reduced engulfment of both contralateral and ipsilateral projections, implying that the selection of terminals for phagocytosis depends on retinal activity[10]. By contrast, blockade of RGC activity in only one eye selectively promotes phagocytic uptake of less-active, weaker inputs, even more so than in control animals[9,10]. These studies revealed that those terminals that are weaker than their neighbours are targeted for phagocytosis by glial cells; however, glial phagocytosis is impaired when there is no difference in activity between two inputs, demonstrating the central role of retinal activity in synaptic pruning.

The process of synapse elimination also depends on molecular signals, including components of the classical complement system such as C1q and C3, which are secreted by glial cells and to some extent by neurons, the microglial complement receptor CR3[7,9] (reviewed in REF. 37), and elements of other phagocytic pathways, including astrocytic multiple epidermal growth factor-like domains protein 10 (MEGF10) and glial MER receptor tyrosine kinase (MERTK) and astrocytic apolipoprotein E (APOE)[10,11] (which is a putative opsonin). Investigation of the molecular components required for retinothalamic remodelling revealed that a subset of RGC synapses are decorated with the complement opsonins C1q and C3 at P5 (REFS 7,9). The colocalization of C1q with presynaptic and postsynaptic structures demonstrated preferential enrichment at smaller synaptic structures that lacked a synaptic partner, suggesting that they may be immature and more likely to be targeted for elimination[7]. The expression of complement proteins by RGCs is developmentally regulated, with the highest immunoreactivity observed during the period of eye-specific segregation, followed by a rapid decline[7,9].

In the developing brain, the major complement receptor CR3 is exclusively expressed by microglia, arguing for a convergence of complement signalling on this glial cell type. CR3 binds iC3b (the proteolytically inactive cleavage product of C3) and stimulates phagocytic activity of the cell through myeloid differentiation primary response protein 88 (MYD88)-independent signalling pathways[38]. Experiments examining CR3-expressing macrophages in the periphery show that CR3 recognizes and binds C3 on target cells, debris or desialylated neuronal material. Moreover, gene-knockout studies have shown that CR3 is required for microglial engulfment of RGC inputs in the dLGN[9,39,40]. Genetic disruption of C1q, C3, or CR3 markedly reduces eye-specific segregation in the retinothalamic pathway (by diminishing the elimination of inputs from both ipsilateral and contralateral sides and leaving them partially overlapping in dLGN), but does not completely prevent it[9], suggesting that complement signalling is an important, but probably not the only, pathway for synapse elimination in this system.

Phagocytic pruning of retinothalamic inputs by astrocytes, in contrast to microglial-cell-mediated pruning, has been demonstrated to depend on the receptors MEGF10 and MERTK (REF. 10). At P5, these receptors function in parallel, contributing independently and additively to eye-specific pruning of RGC projections by promoting the phagocytic activity of astrocytes, and potentially supplementing or complementing synaptic pruning by microglia. Mice lacking MEGF10 and MERTK fail to complete eye-specific segregation, and thalamic neurons in the dLGN of these animals retain multiple retinal inputs[10]. A recent study demonstrated that astrocytic phagocytosis of RGC terminals is modulated by different alleles of the gene encoding APOE; whereas mice carrying the human hypermorphic allele *APOE*2* showed increased synaptic pruning, mice carrying the hypomorphic allele *APOE*4* showed less-efficient elimination of synapses[11]. However, the mechanisms and cell types involved in APOE-dependent modulation of neuron–glial-cell signalling and synaptic pruning remain to be identified.

Microglia have also been shown to have a role in late retinothalamic circuit refinement between P30 and P60 — a period marked by dynamic spatial reorganization and clustering of RGC boutons, as well as a considerable decrease in RGC arbors and a reduction in the density of presynaptic boutons[36,41]. Through an engulfment assay followed by immunolabelling, microglia were observed to engulf RGC presynaptic terminals, with peak phagocytosis observed at P40 (REF. 41). As components of the complement system show reduced expression at P30 (REF. 7), other neuron–glial-cell signalling pathways may govern this late form of synaptic remodelling — for example, those involving the phagocytosis-promoting receptors MEGF10 or MERTK, which are expressed throughout the lifetime of the animal[10,42].

***Cerebral cortex.*** Synaptic pruning in the cerebral cortex has been studied less than that in other areas, primarily because the complex synaptic connectivity of cortical regions makes it more difficult to trace pathway-specific synaptic inputs. Several other neural circuits have been shown to undergo substantial synaptic pruning in rodents during development, including corticospinal projections[2,43] and hippocampal mossy fibre projections[44], but a role for microglia in the maturation of these systems has not yet been examined. Nonetheless, in the developing mouse hippocampus (which can be described as archicortex), the maturation of excitatory Schaffer collateral inputs synapsing onto pyramidal neurons in the CA1 region has been shown to depend on neuron–microglial-cell signalling via the neuronal chemokine CX3C-chemokine ligand 1 ($CX_3CL1$; also known as fractalkine) and its microglial receptor, CX3C-chemokine receptor 1 ($CX_3CR1$)[8]. In mice, microglia achieve a ramified, mature morphology and infiltrate the developing cortex and hippocampus during the first 2 postnatal weeks. The infiltration of microglia into the cortex and hippocampus is accelerated by the release of the chemoattractant $CX_3CL1$ by resident neurons and is delayed by genetic loss of $CX_3CR1$ (REF. 8). Notably, the period of peak microglial infiltration to the hippocampus occurs at ~P8, just before the period of maximal synaptogenesis.

Retinal waves
Spontaneous bursts of action potentials that propagate in a wave-like fashion across the developing retina.

Apolipoprotein E
(APOE). A major cholesterol carrier that is also hypothesized to serve as an opsonin. The *APOE*4* allele is a major genetic risk factor for Alzheimer disease.

Opsonin
A protein, such as an antibody or complement protein, that binds to a phagocytic target (such as a pathogen), thus rendering it more susceptible to phagocytosis, in a process known as opsonization.

Archicortex
A phylogenetically old part of the cerebral cortex that constitutes the hippocampal formation.

Table 1 | **The evidence for glial-cell involvement in synaptic pruning in different systems**

| Region | Synapse | Age* | Processes and mediators involved | Refs |
|---|---|---|---|---|
| NMJ | Motor neuron to motor endplate on muscle fibre | P3–P16 | Caspase 3 in the presynapse of the motor neuron that is subsequently pruned. | 27 |
| Cerebellum | Climbing fibre to Purkinje cell | P7–P17 | C1QL1 from climbing fibres binds to BAI3. | 34 |
| dLGN | Retinal ganglion cell to dLGN | P5–P8 | C1q and C3 (expressed by glia and neurons) opsonize synapses targeted for elimination, which are then recognized by microglial CR3. | 7,9 |
| | | | Astrocytic MERTK and MEGF10 function in parallel to eliminate unnecessary synapses. On such synapses, they may bind phosphatidylserine and C1q, respectively. | 10 |
| | | | Astrocytic APOE may act as an opsonin for synapse elimination. It may act through glial low-density lipoprotein receptor-related protein. | 11 |
| Hippocampus | Schaffer collateral to CA1 pyramidal neuron | P8–P28 | Microglial $CX_3CR1$ recognizes neuronal $CX_3CL1$, and this interaction is required for timely migration of microglia into the hippocampus and for subsequent synaptic pruning. | 8,18 |
| Primary visual cortex | LGN to layer 4 pyramidal neuron | P21–P35 | Microglial P2Y12 receptor recognizes ADP that may be released by neurons to stimulate microglial migration and synaptic pruning. | 12 |

*All ages described for mouse. APOE, apolipoprotein E; BAI3, brain-specific angiogenesis inhibitor 3; C1q, complement component C1q; C3, complement component C3; C1QL1, C1q-like protein 1; CR3, complement receptor 3; $CX_3CL1$, CX3C-chemokine ligand 1; $CX_3CR1$, CX3C-chemokine receptor 1; dLGN, dorsal lateral geniculate nucleus; P, postnatal day; P2Y12, purinergic receptor P2Y12; MEGF10, multiple epidermal growth factor-like domains protein 10; MERTK, MER receptor tyrosine kinase; NMJ, neuromuscular junction.

Mice lacking $CX_3CR1$ show an excess of Schaffer collateral synaptic inputs and features of immature synapses, such as increased long-term depression (LTD) and low synaptic multiplicity[8]. Although the excess of inputs and LTD defects are transient in these knockout animals, the deficiency in synaptic multiplicity remains into adulthood, when it is associated with decreased functional connectivity across brain regions (as measured by resting-state functional MRI (fMRI) and local field potential coherence), social deficits and increased novelty-induced grooming[18]. These findings suggest that microglia contribute to specific features of synapse maturation during an important developmental time window and that the development of synaptic multiplicity might require microglial-cell-dependent synaptic elimination (although the relationship between synaptic elimination and synaptic multiplicity remains only correlative).

Synapse maturation in the neocortex also depends on neuron–microglial-cell signalling. Mice lacking $CX_3CR1$ showed a delay in microglial infiltration in the barrel cortex during the first postnatal week and a delay in synaptic maturation (which usually involves a reduction in the AMPA receptor-to-NMDA receptor ratio and a switching of NMDA receptor subunits from NMDA2B to NMDA2A)[17]. These observed deficits in synaptic maturation were transient and corrected by P30, although physiological changes that have previously been found to be persistent in the hippocampus of *Cx3cr1*-knockout mice, such as the reduction in synaptic multiplicity, were not examined in this study.

Synaptic development in the visual cortex is also dependent on microglia. *In vivo* time-lapse imaging using window-on-the-brain technology in rodents showed that microglia in the primary visual cortex of mice continually extend and retract their processes[45], which make contacts with postsynaptic dendritic spines, showing a preference for smaller, immature spines[45]. Binocular visual deprivation from the beginning to the peak of the critical period (P20–P28) reduces microglial motility in V1 but enhances microglial contacts with spines and increases the number of phagocytic inclusions found in microglia at P28, suggesting that an absence of sensory input may increase cortical synaptic pruning[45]. With monocular deprivation from P28 to P35, inputs carrying information from the deprived eye are preferentially eliminated first, after which inputs corresponding to the open eye are strengthened (see REF. 46 for a review of this classic form of cortical plasticity), and microglia seem to play an important part in the initial pruning phase[12]. They show rapid changes in morphology and increase their motility as soon as 12 hours after monocular deprivation, even before synaptic pruning takes place, and subsequently show more interaction with synaptic clefts, increased phagocytic inclusions and engulfment of GluR1-subunit-containing receptors[12]. All of these changes seem to depend on purinergic neuron–microglial-cell signalling, as they are absent in mice lacking the purinergic P2Y12 receptor, which is exclusively expressed by microglia[12]. Interestingly, purinergic signalling for microglial homing and engulfment of apoptotic neurons has been reported to be necessary in

Synaptic multiplicity
A feature of mature circuits in which afferent inputs make more than one synapse onto a single target neuron.

Window-on-the-brain technology
A technique in which the skull is thinned or opened and capped with a transparent implant to allow two-photon or near-infrared *in vivo* imaging of cortical function.

other systems — for example, for microglial chemotaxis to brain lesion sites in zebrafish and mice[47,48] — suggesting that apoptosis-related signalling pathways may also be recruited for neuron–microglial-cell signalling in the uninjured brain.

Some evidence from studies in non-human primates (NHPs) also points to a role for microglia in synaptic pruning in the cerebral cortex[49,50]. In the macaque, over 70% of initially formed callosal connections are lost in the first 6 months of life, and this has been argued to reflect the coordinated elimination of some synapses and the elaboration of others[51], although the elimination of axons that have not yet formed synapses may also contribute to this reduction[2]. In both the macaque and the marmoset, synapse density in the cortex reaches a peak at 2–3 months of age and then shows a modest and gradual decrease[49,52]. Intriguingly, this trajectory is matched by those of microglial activation[49,53] and of the expression of neuron–microglial-cell signalling factors (including neuronal $CX_3CL1$ and microglial $CX_3CR1$ in mouse hippocampus between P15 and P30 (REF. 54)). Furthermore, in marmosets, proteins reported to protect cells against reactive oxygen species (which are known to activate microglial cells[55]), caspases and excessive activation of the complement system show peak expression at 6 months of age, suggesting that microglial-cell-dependent synaptic remodelling may be suppressed at this stage[50]. Nevertheless, several components of the complement system are still upregulated at 6 months[49], raising the possibility that a later phase of synaptic pruning, similar to that thought to accompany remodelling of the adolescent human brain, may be complement-dependent. Although causal experiments investigating these processes in NHPs are currently lacking, these findings argue that microglia might also have a functional role in circuit refinement in NHP cortex, in which phases of synaptic maturation and pruning are considerably longer than in rodents.

## Synaptic pruning and brain disorders

Given the evidence presented above that glial-cell-dependent loss of synapses accompanies the successful formation of new synaptic contacts in different parts of the nervous system, it may be unsurprising that researchers have repeatedly proposed that deficits in synaptic pruning might have pathological consequences. In particular, aberrant synaptic pruning has been invoked as a possible mediating physiological substrate for autism spectrum disorder (ASD), schizophrenia and epilepsy — neurodevelopmental disorders that share genetic and environmental risk factors[56] (FIG. 2).

***Autism spectrum disorder.*** ASD is usually diagnosed in the first 3 years of life, a period that overlaps with the initial phase of cortical synaptogenesis in humans[57]. Genetic and environmental factors have been implicated in the pathophysiology of ASD, but the underlying physiological substrates remain obscure. Clues to a potential role for deficient cortical maturation in autism come from post-mortem and neuroimaging studies. Children with ASD show normal brain size at birth but significantly increased brain size in the first year of life, and this increase in volume is associated with an overabundance of cortical neurons and connections and excessive activation of nearby microglia (as measured by positron emission tomography imaging of peripheral benzodiazepine receptor levels)[58–60]. Although some reports challenge the findings of early head-circumference overgrowth[61,62], structural brain imaging studies reveal increased prevalence of macrocephaly in individuals with ASD during early childhood[58]. This abnormal brain growth rapidly plateaus and normalizes in most cases by adulthood[59], possibly as a result of homeostatic mechanisms that limit brain size.

Functionally, ASD is associated with reduced long-range functional connectivity over the entire cortex and corpus callosum[63,64] in favour of excess short-range connections (such as striatal or frontal lobe connectivity), as demonstrated by fMRI[65,66] and electroencephalogram studies[67]. Together with the reports of brain overgrowth in ASD, these findings suggest a link between a failure to eliminate neurons or synapses and a failure to appropriately strengthen other synaptic connections, as seen in *Cx3cr1*-deficient mice[18,68]. On the microstructural scale, synaptic densities of pyramidal neurons in the temporal lobe are observed in post-mortem studies to be increased in the brains of children and adults with ASD[68,69]. Moreover, the reduction in cortical spine density that is observed in brains of typically developing adolescents is diminished in individuals with ASD[68], suggesting a deficit in pruning, at least at this later age. Likewise, mice carrying rare, penetrant mutations that are found in individuals with ASD show elevated spine densities in the temporal cortex and cerebellum[68,70,71] and deficient adolescent pruning[68].

The importance of glia for the pruning deficits proposed in ASD is mainly supported by transcriptomic data. Gene co-expression network analysis of post-mortem ASD brain tissue identified an upregulation of a module enriched for immune genes and glial markers in the cortex, along with a downregulation of a module containing synaptic genes, compared with typically developing individuals[72]. Interestingly, genes in the immune–glial module showed considerably less enrichment for ASD-related mutations than did those in the synaptic module, a finding interpreted as indicating that the upregulation of the immune–glial module might be secondary to genetically driven synaptic changes[72]. An alternative interpretation is that environmental pathogens could act via immune–glial genes to trigger ASD in genetically susceptible individuals. Environmental pathogens may reprogramme gene expression through epigenetic mechanisms, and one study in individuals with ASD revealed decreased DNA methylation in parts of the genome associated with immune function, including on genes encoding the complement components C1q, C3, and CR3 (REF. 73), all of which have been implicated in synaptic pruning by microglia in rodent studies[7,9]. Other genes found to be epigenetically dysregulated in ASD include those encoding modulators of microglial activation, such as tumour necrosis factor (TNF), interferon regulatory factor 8 (IRF8) and transcription

Autism spectrum disorder
(ASD). A group of neurodevelopmental conditions characterized by social deficits, impaired language development, intellectual disability, increased repetitive or restrictive behaviours and motor abnormalities.

Peripheral benzodiazepine receptor
Translocator protein (TSPO) of the outer mitochondrial membrane that modulates bursts of reactive oxygen species in macrophages, including microglia, and is therefore used as a marker of inflammation.

Penetrant
Of a mutation, producing expression of associated phenotypic traits in a large proportion of individuals carrying the mutation.

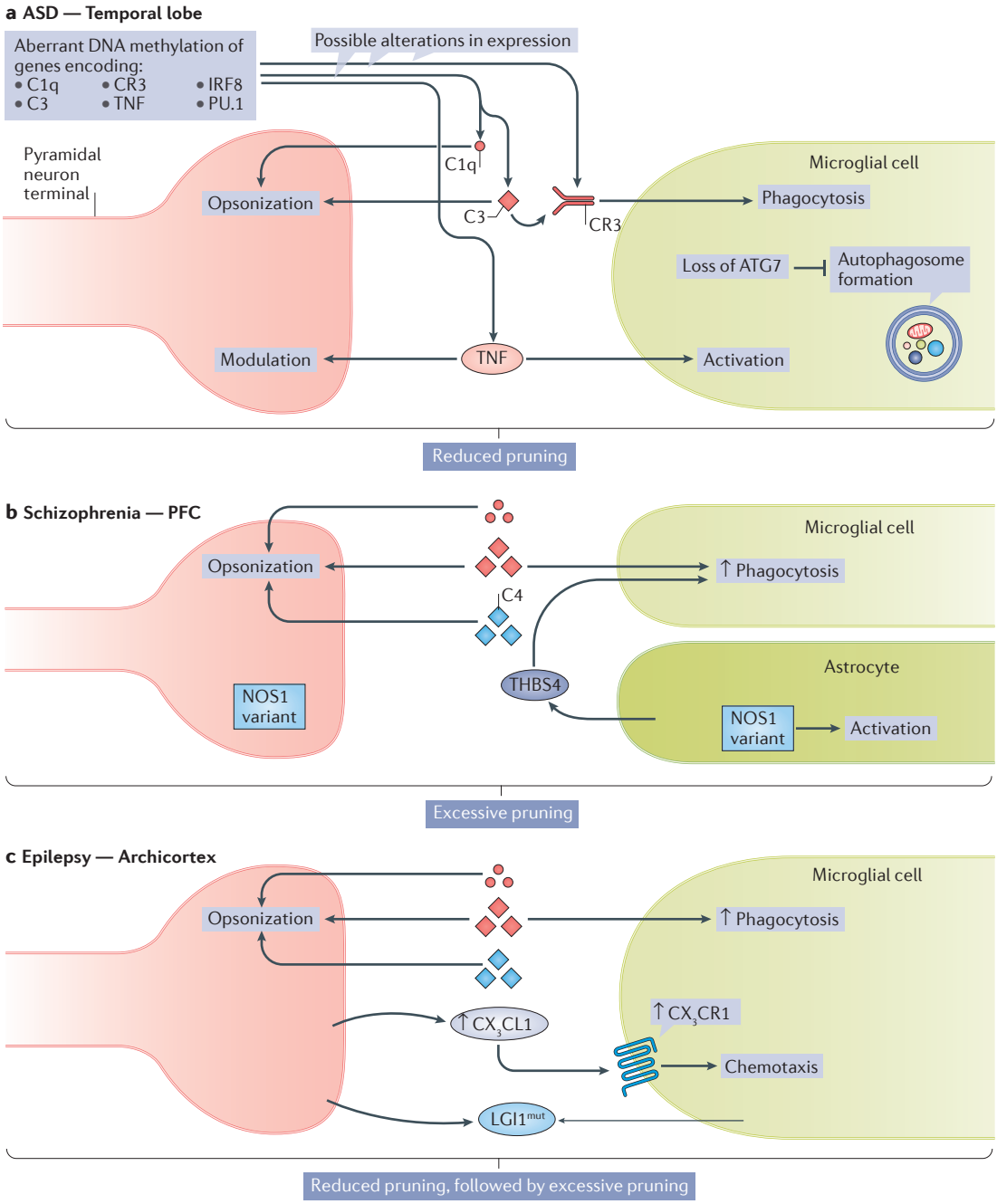


Figure 2 | **Putative molecular components of aberrant synaptic pruning by glia in disease. a** | Autism spectrum disorder (ASD) is associated with decreased DNA methylation, and dysregulated (over)expression, of several genes that have been implicated in glial-cell-dependent synaptic pruning, including those encoding the complement opsonins C1q and C3 and the complement receptor CR3, tumour necrosis factor (TNF) and the transcription factors for microglial activation, interferon regulatory factor 8 (IRF8) and PU.1 (which is encoded by *SPI1*)[73]. Mice lacking ATG7 (which is required for autophagosome formation in autophagy) specifically in microglia exhibit some ASD-like features, including social recognition and social interaction deficits[71]. Overall, individuals with ASD generally exhibit less pruning in regions such as the temporal lobe than do typically developing individuals. **b** | The risk of schizophrenia is associated with genetic variants of nitric oxide synthase 1 (NOS1), which is expressed by neurons and astrocytes, and thrombospondin 4 (THBS4), which promotes phagocytosis[85]. Increased levels of the complement opsonins C1q, C3 and C4 are observed in the brains and serum of patients with schizophrenia[20,90–92]. **c** | Loss-of-function mutations in the gene encoding leucine-rich glioma-inactivated protein 1 (LGI1), which is secreted by neurons and by glia and is required for normal postnatal maturation of excitatory synapses, have been associated with autosomal dominant temporal lobe epilepsy[96]. The complement opsonins C1q, C3 and C4 are upregulated in rodent models (in microglia) and in the brains of patients with temporal lobe epilepsy (in microglia, astrocytes, and neurons)[102], as are CX3C-chemokine ligand 1 ($CX_3CL1$) and its receptor, CX3C-chemokine receptor 1 ($CX_3CR1$)[103,104]. PFC, prefrontal cortex.

factor PU.1 (encoded by *SPI1*)[73,74]. These changes may be reflected in the excessive microglial activation observed in ASD brains[60] and in findings that minocycline can rescue social deficits in oxytocin-receptor-deficient mice with ASD-related behavioural phenotypes[75].

Further evidence for microglial-cell-dependent pruning in ASD comes from mice lacking autophagy-related protein 7 (ATG7; a critical component of cellular autophagy pathways that is significantly impaired in ASD patients) selectively from myeloid cells[68]. These mice show signs of impaired synaptic refinement in the temporal cortex, including increased density of dendritic spines, elevated levels of synaptic markers, altered functional connectivity, and social behaviour deficits and increased repetitive behaviour — all hallmarks of ASD in humans[71]. However, as these mice had *Atg7* eliminated from all macrophages, the specific contribution of microglia to the phenotype remains to be selectively demonstrated. Intriguingly, another study showed that selective deletion of *Atg7* from neurons was associated with similar ASD-like behavioural phenotypes and reduced synaptic pruning during later stages of development[68], suggesting that both microglial and neuronal autophagocytic pathways make important contributions to synaptic pruning. The possibility that these mechanisms are differentially active during early and late pruning remains to be explored.

***Schizophrenia.*** Unlike ASD, the onset of the symptoms of schizophrenia typically occurs between the ages of 15 and 25 and coincides with later stages of synaptic pruning in the adolescent PFC[76]. Appropriate pruning and maturation of PFC circuitry are thought to be required for the emergence of executive function and for the behavioural inhibition that ensures the refined goal planning and impulse control necessary for adult social and cognitive behaviour[77]. As schizophrenia is associated with reduced PFC grey-matter volume[78], it has been proposed to be a disorder of excessive pruning. Moreover, functional imaging indicates weakened connectivity across brain regions in schizophrenia, possibly consistent with excessive elimination of mature synapses[79,80].

Adolescent synaptic pruning has been much less studied in animal models than has early developmental pruning, and how the functional consequences of synaptic pruning in mature circuits differ from those observed during periods of active synaptogenesis remains unclear. For example, adolescent pruning seems to shows some selective differences: during adolescence in NHPs (2–4 years), layer 3 cortical synapses undergo considerably more synaptic pruning than do those in layers 5 and 6 (REF. 52) In line with these data, post-mortem studies in humans revealed that schizophrenia is associated with decreased spine density in layer3 but not in layers 5 or 6 (REFS 81,82). Moreover, as the pruned synapses appear to be exclusively excitatory[83,84], aberrant adolescent pruning may alter excitatory–inhibitory balance, a feature proposed to be central to circuit deficits in schizophrenia[76].

Excessive glial-cell-dependent synaptic pruning in schizophrenia is further supported by genetic studies[85,86]. Genetic variants that are significantly associated with schizophrenia include those in genes related to microglial and astrocytic activation (including *NOS1*, which encodes nitric oxide synthase 1) and phagocytosis (including *THBS4*, which encodes thrombospondin 4)[85] — pathways implicated in synaptic pruning in the cortex and thalamus (see above). There is also a considerable association between schizophrenia and variants of genes involved in neuroinflammation[86], in line with the microgliosis that is observed in some schizophrenic brains[87,88]. These data have prompted the proposal that drugs that suppress microglial activation, such as minocycline, might mitigate excessive synaptic pruning and be therapeutic in schizophrenia[89]. In support of this hypothesis, minocycline can reduce synaptic pruning in the retinothalamic system in wild-type mice[7].

Considerable excitement has surrounded the identification of links between components of the complement cascade and schizophrenia. Several groups have reported increased activity of C1q, C3 and C4 in schizophrenia[20,90–92]. The C3*F variant[90,93] and decreased serum levels of C4b[91] are associated with reduced and increased risk of schizophrenia, respectively, and structural variants of C4 that show higher expression are associated with a dose-dependent increase in risk[20]. Mouse studies confirmed that C4 is required for synaptic pruning in the developing retinothalamic system; there, overexpression of C4 could act upstream of C3 to promote excessive synapse elimination during adolescent synaptic pruning[20]. Finally, at least one study has associated elevated maternal serum C1q levels during pregnancy with increased risk of schizophrenia in adult offspring[92], suggesting that an indirect, maternal programming of complement function could be an alternative route for these pathways to influence the risk of schizophrenia.

***Epilepsy.*** Epilepsy is defined by recurring, unprovoked seizures owing to abnormal, synchronized neuronal firing in the brain. Interestingly, epilepsy is often a comorbid condition in individuals with other neurodevelopmental disorders, such as ASD and schizophrenia[94], suggesting these disorders may have common or overlapping aetiological substrates. In general, two stages are recognized to contribute to epilepsy. During the initial stage, brain hyperexcitability dictates the risk of seizure onset; during the later stage, neural remodelling induced by the initial seizures can further increase susceptibility, driving a vicious cycle of recurring seizures[95]. Evidence suggests a role for glial-cell-mediated synaptic pruning in both of these phases.

Studies on the gene *LGI1* (encoding leucine-rich glioma-inactivated protein 1), which is mutated in individuals with autosomal dominant lateral temporal lobe epilepsy (TLE), show that its gene product is required for the normal postnatal maturation of excitatory synapses[96]. Mice expressing the pathological human form of LGI1 show reduced synaptic elimination and increased excitatory, but not inhibitory, neurotransmission in the hippocampus[96]. Furthermore, mice lacking LGI1 show deficient synaptic pruning of retinogeniculate synapses, providing a potential explanation for the aberrant sensory processing reported in patients with TLE[97,98].

Autophagy
An intracellular self-degradative process for orderly degradation and recycling of cellular components and balancing sources of energy at critical periods.

Microgliosis
An intense inflammatory activation of microglia in response to insults to the CNS (such as infection, trauma or neuronal damage).

Glial cells in some regions of the mouse brain have been reported to express LGI1 (REF. 99); however, the role of glia in LGI1-mediated pruning remains to be described. At the same time, mice lacking C1q, which is also required for retinothalamic pruning, also develop an increased density of cortical synaptic connections, as well as epileptiform activity and spontaneous seizures[100,101]. Again, inhibitory connections appear to be unaffected by mutations in the genes encoding LGI1 and C1q, and a similar specificity has been reported for pruning-deficient animals lacking *Cx3cr1* (REF. 18). Together, these reports suggest that synaptic pruning, at least in cortical structures, may not be necessary for the maturation of inhibitory synapses[18,100,101].

In rodent models, during the neural remodelling phase of epilepsy, complement components C1q, C3 and C4 are upregulated in microglial cells of the hippocampus, suggesting that glial-cell-mediated pruning may be recruited during initial seizures, possibly as an inflammatory mechanism to compensate for growth-induced hyperactivity[95,102]. Furthermore, seizures are associated with upregulation of signalling via neuronal $CX_3CL1$ and its microglial receptor $CX_3CR1$, which both have roles in developmental synaptic pruning and maturation[8,17,103,104]. Increased $CX_3CL1$ signalling through overexpression of $CX_3CL1$ and $CX_3CR1$ is associated with microglial migration to the site of inflammation, with neuronal death and with reduced GABAergic function, thereby exacerbating the excitatory–inhibitory imbalance in response to initial seizures[104,105]. However, whether these changes are accompanied by increased glial-cell-dependent synapse elimination is not known.

## Perspectives

Evidence suggests that synaptic pruning is a fundamental feature of developing neural circuits. In several simple sensory and motor circuits, it has a dual role in reducing an initial exuberance of weak synaptic inputs and strengthening the remaining inputs. Whether and how this dual role manifests more generally in brain circuits remains unexplored. The recent work elucidating the signalling pathways controlling glial-cell-dependent synaptic pruning has enabled further examination of the molecular and cellular mechanisms of pruning and its contribution to disease. Moreover, the field of synaptic pruning has made important recent advances. First, neuron–glial-cell signalling has been shown to play an important part in neural circuit refinement across several brain regions and is likely to be a widely recruited mechanism for synapse pruning. Second, genetic studies have emerged that link deficient or aberrant neuron–glial-cell signalling with neurodevelopmental disorders — particularly in ASD, schizophrenia and epilepsy. These findings provide strong support for earlier speculations that glia play a crucial and widespread part in synaptic pruning[37].

However, the field faces several important questions going forward. Most crucially, whether glial cells directly engulf intact synapses or instead phagocytose synaptic debris remains unclear. In co-cultures of dissociated neurons and microglia, evidence suggests that they do directly engulf synapses[106]; however, when removed from the brain, microglia become activated and thus may engage phagocytic pathways that are not functional under physiological conditions. Phagocytic engulfment of intact synapses has so far not been captured by *in vivo* time-lapse imaging despite a large number of such studies in mice[45,107–109]. However, such interactions might have been missed, as most of these imaging studies were carried out in adulthood (when pruning is reduced) and on relatively small brain volumes, possibly precluding capture of rare phagocytic events. The best evidence for phagocytosis comes from electron microscopy images of fixed brain samples, in which both larger inclusions and clathrin-coated vesicles were found in microglia and near neuronal contacts[8,12,45,110]. In addition, in at least one case, the number of microglial-cell–neuron contacts was found to correlate with synaptic pruning capacity, as measured by the number of engulfed inclusions in microglial cells or the ocular dominance index[12]. However, most studies have not used serial-section reconstruction to rule out the possibility that putative inclusions are simply encapsulated neuronal processes, and the contents of inclusions have not yet been immunologically verified[12,45] (although see REF. 8). The application of multicolour fluorescence time-lapse imaging approaches to long-term brain explant culture systems will probably be necessary to more directly test whether microglia engulf intact synapses. It remains entirely possible, for example, that synapses are eroded in a 'nibbling'-like process through microglial clathrin-mediated endocytosis or that microglia have only trophic effects on synapse elimination and formation, as has been demonstrated in the adult brain[111] (BOX 3). For example, microglial BDNF (brain-derived neurotrophic factor) is required for learning-induced synaptic remodelling in juvenile (P19–P30) mice, and induced loss of microglial trophic support leads to deficits in several learning tasks[111].

A key to unravelling the molecular physiology of glial-cell-dependent pruning will be the identification of the synaptic 'eat me' and 'spare me' signals that respectively trigger and prevent pruning. $CX_3CL1$ could be such a signal, as its expression in neurons is markedly upregulated during synaptogenesis in the developing brain, and it can activate its receptor on microglia in either its membrane-tethered form or its cleaved form[112]. However, most data suggest that $CX_3CL1$ is a chemoattractant, and whether it serves only as a global regulator of microglial tissue infiltration or also as a synapse-specific chemotactic signal remains unknown. Overall, the neuronal 'eat me' signals that trigger complement system opsonization and stimulate microglial phagocytosis remain poorly defined. Several candidates required for macrophage phagocytosis of dying cells, including mannose residues of glycoproteins and glycolipids, as well as the lipid phosphatidylserine, have also been proposed to be crucial for synaptic pruning, but whether they are present on eliminated synapses and what their role may be in pruning remain unknown.

Ocular dominance index
The difference between contralateral response and ipsilateral response divided by the sum of contralateral and ipsilateral responses.

Box 3 | **Synaptic pruning during adulthood — in health and disease**

**Synaptic pruning in adult plasticity**

Even after circuits have matured, synaptic pruning continues to maintain brain plasticity in adulthood — particularly in relation to learning and memory, which are associated with microglial-cell-dependent synaptic plasticity[111].

During motor learning, new synapses are rapidly formed in the motor cortex, and a subset of these synapses is subsequently eliminated to return synaptic density to original levels[111]. Although the causal role of microglial phagocytosis during motor learning has not been directly demonstrated, genetic depletion of microglia in postnatal day 19 (P19) and P30 mice impaired learning-dependent elimination of dendritic spines in motor cortex[111]. Microglia seem to be involved in sensory plasticity as well. In the primary visual cortex of P30 mice undergoing altered sensory experience by visual deprivation, microglia contact a subset of structurally dynamic dendritic spines[45]. These spines are subsequently eliminated, and microglial phagocytosis of synaptic elements is seen[45]. Another type of experience-related remodelling of neuronal circuits is associated with chronic stress. Chronic unpredictable stress reduced both short-term and long-term plasticity in the hippocampus of 2–4-month-old mice and resulted in more phagocytic inclusions of both pre- and postsynaptic origin within hippocampal microglia, suggesting that phagocytic pruning may occur during this circuit remodelling[118]. Stress-related plasticity also seems to be dependent on CX3C-chemokine ligand 1 ($CX_3CL1$)–CX3C-chemokine receptor 1 ($CX_3CR1$) signalling, as *Cx3cr1*-knockout mice did not exhibit any of the aforementioned changes[118].

Continuous synaptic plasticity throughout the lifetime also provides a neuroanatomical substrate for maladaptive learning and memory, such as addiction. Synaptic densities in the nucleus accumbens, amygdala and dorsomedial striatum markedly increase with exposure to addictive substances (such as alcohol or opiates) and decrease upon withdrawal[119]. This withdrawal-associated synaptic pruning is suggested to induce negative affective states during withdrawal from drug and alcohol use[119].

**Synaptic pruning in ageing and disease**

Synaptic pruning has also been suggested in neurodegenerative pathology. Age-related hippocampal decline seems to be mediated by complement proteins, and mice lacking complement component C3 are protected from synaptic loss during ageing[120]. Although a role for microglia was not directly investigated in this study, the involvement of C3 is strongly suggestive of a phagocytic mechanism. Inhibition of the complement proteins C1q and C3 and of the microglial complement receptor CR3 is protective in a genetic mouse model of Alzheimer disease — probably by limiting microglial phagocytosis of synaptic material[121] — and in a mouse model of frontotemporal dementia[122]. In mouse models of ageing-related loss of audition and vision, microglia in the corresponding primary sensory cortices proliferated with age and exhibited accumulations of phagocytic inclusions that displayed structural features of synaptic elements[110].

In addition, synaptic pruning might be the driving force of the pathological circuit remodelling that occurs as a consequence of certain viral diseases. For example, in a murine model of the neuroinvasive form of West Nile virus, infection of adult hippocampal neurons induces complement-mediated elimination of presynaptic terminals[123]. After acute infection, recovered mice exhibit impaired spatial learning and sustained phagocytic microglial engulfment of presynaptic terminals[123]. The loss of presynaptic CA3 terminals is accompanied by upregulation of C1q subunit A in microglia and neurons, and deficiency of C3 or of the C3a receptor is protective against virus-induced synaptic terminal loss[123].

One possible clue to the neuronal signalling pathways that trigger pruning comes from evidence that apoptotic signalling may induce synaptic pruning in the absence of cell death. For example, local caspase 3 activation within neurons stimulates the elimination of dendritic spines without promoting neuronal death, and juvenile (~7 weeks old) and adult (3 months old) mice lacking caspase 3 exhibited increased spine density in CA1 spines[113]. How activation of caspase 3 in neurons may promote neuron–microglial-cell interactions, and whether microglia are required for caspase-induced elimination of spines, remain to be described.

The identification of the neuronal signalling pathways that trigger glial-cell-dependent pruning will be crucial to begin to understand what distinguishes a synapse destined to be eliminated from one that will survive. Moreover, it should enable a mechanistic understanding of pruning that will eventually reveal why this phenomenon is an essential feature of neural circuit formation. Defining the molecular repertoire of glial-cell-dependent synaptic pruning will also facilitate the translation of data from human genetics into molecular pathways and will help to establish the importance of aberrant synaptic pruning in brain diseases.


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