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The role of glial cells in synapse elimination

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Abstract

Excessive synapses generated during early development are eliminated extensively to form functionally mature neural circuits. Synapses in juvenile and mature brains are highly dynamic, and undergo remodeling processes through constant formation and elimination of dendritic spines. Although neural activity has been implicated in initiating the synapse elimination process cell-autonomously, the cellular and molecular mechanisms that transduce changes in correlated neural activity into structural changes in synapses are largely unknown. Recently, however, new findings provide evidence that in different species, glial cells, non-neuronal cell types in the nervous system are critical in eliminating neural debris and unwanted synapses through phagocytosis. Glial cells not only clear fragmented axons and synaptic debris produced during synapse elimination, but also engulf unwanted synapses thereby actively promoting synapse elimination non-cell autonomously. These new findings support the important role of glial cells in the formation and maintenance of functional neural circuits in development as well as in adult stages and neurodegenerative diseases.

Introduction

Proper nervous system function depends on the establishment of precise connectivity between neurons at a specialized site called the synapse. In the developing nervous system, neurons produce exuberant processes and their connections are initially redundant [1–4]. During early postnatal life, these excessive connections are removed by a process called synapse elimination and subsequently, functional neural circuits are formed by strengthening the remaining synapses [1, 5–8]. Beyond early developmental stages, synapse elimination events also persist in the mature nervous system through experience-dependent structural synaptic plasticity, although the number of elimination events may decline with age [9–12]. Therefore, synapse elimination is critical not only in shaping neural circuits during development but also in regulating synaptic plasticity in response to experience and memory.

Synapse elimination generally can be classified into two types of events: “input elimination” where entire axonal arbors and presynaptic terminals are eliminated (Figure 1a), and “individual synapse elimination” where individual synaptic contacts are disassembled without large changes in axonal arbors (Figure 1b) [6, 13]. Whereas synapse elimination accompanying “input elimination” has been extensively studied in developing nervous systems, including the mammalian neuromuscular (NMJ) system [2, 7, 14], the retinogeniculate system [13, 15, 16], the climbing fiber-purkinje cell connection in the cerebellum [3, 8, 17] and invertebrate neuronal remodeling during metamorphosis [5],

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“individual synapse elimination” has been observed in the fly larval NMJ during target muscle expansion [18**] and in mammalian brains during synaptic plasticity induced by learning and memory [19–21*].

How is synapse elimination regulated? Although it has been well documented that activity-dependent competition drives synapse elimination, little is known about the molecular mechanisms that transduce changes in neuronal activity into synapse elimination.

However, studies in various species, including mammals and flies, have discovered that a population of non-neuronal cells known as glial cells play central roles in neural debris and synapse elimination through an engulfment process called phagocytosis. This phagocytic process involves the proper recognition, ingestion, and degradation of neural debris/synapses by glial cells [22, 23]. For example, in the mammalian nervous system, microglia (a resident population of professional phagocytes) in the brain and Schwann cells (glial cells that ensheath peripheral axons) at the NMJ have been suggested to eliminate synapses and fragmented axons. As in the mammalian nervous system, glial cells in flies are the main cell type responsible for eliminating excess axons during development and clearing severed, degenerating axons during injury [24]. Notably, if glial engulfment is prevented, normal developmental input elimination does not occur, strongly implicating an active role for glia cells in triggering input elimination [25].

In this review, we will discuss several models of synapse elimination during development as well as later postnatal stages and summarize recent progress on how synapse elimination is regulated. In particular, we will focus on the non-cell autonomous aspects of synapse elimination, the role of glial cells in regulating synapse elimination.

Synapse elimination in the neuromuscular junction

Due to the simplicity, accessibility and relatively large size of the synapse, NMJ synapse elimination has been characterized in depth in various organisms. In the mammalian NMJ, one motor neuron sends multiple axons to synaptic sites of muscle fibers, so that one NMJ is innervated by multiple axons. However, within the first several postnatal weeks, all but one of the motor inputs to each NMJ are eliminated through activity-dependent competition, leaving a one-to-one match between each motor input and NMJ (Figure 1a) [2, 14]. Activity-dependent NMJ synapse elimination has been clearly demonstrated in many studies. For example, Buffelli et al. showed that by conditional genetic manipulation of choline acetyl transferase in subsets of motor neurons, synapses from more active axonal inputs survive while weaker synapses are eliminated in the same target muscle [26]. Time-lapse imaging suggests that this elimination of excess axons/synapses occurs by retraction of the “loser” axons [2]. The retracting “loser” axons produce axonal fragments, called axosomes, which are eventually engulfed by Schwann cells [14]. Subsequent studies showed an increased lysosomal activity both in retracting axons themselves and Schwann cells during the elimination process, suggesting autophagic and heterophagic mechanisms in input elimination [27]. Although these studies have provided an important framework for understanding the cellular mechanisms of synapse elimination in the mammalian NMJ, several important questions remain to be addressed. What are the molecular players that enable Schwann cells to recognize retracting axons? How can activity-dependent competition induce the disassembly of pre- and post-synaptic connections? Some of these questions are beginning to be addressed in recent studies in the fly NMJ.

Recently, it has been reported that at the *Drosophila* NMJ, normal synaptic growth continuously produces presynaptic debris and ghost boutons (undifferentiated synaptic boutons) in response to changes in growth and activity [18**]. The authors stimulated motor neurons using a light-activated ion channel, Channelrhodopsin-2, and observed significant

production of presynaptic membrane debris and detachment of undifferentiated synaptic boutons. Importantly, glial cells were found to cover the NMJ and extend highly dynamic membrane projections to engulf presynaptic debris (Figure 2a). Glial cells' phagocytic activity was dependent on Draper (a phagocytic receptor that recognizes target cells/debris) and dCed-6 (an adaptor protein in the Draper pathway-an ortholog of mammalian GULP) [22], and specific knock-down of either of the proteins in glial cells caused a significant accumulation of presynaptic debris, resulting in a severely reduced number of synaptic boutons with abnormal growth. Surprisingly, Fuentes-Medel et al. found that muscle cells also express Draper. When Draper and dCed-6 were knocked down in muscle cells, flies showed defects in clearing neural debris. Thus, each cell type seems to have a distinct function during the engulfment process; glial cells primarily engulf presynaptic debris, whereas muscle cells primarily engulf ghost boutons. This observation strongly indicates that muscle cells are not simply postsynaptic target cells, but tissue resident phagocytes that participate in sculpting the *Drosophila* NMJ. Also, this data suggests that Draper-dCed-6, along with other phagocytic pathway components are expressed in Schwann cells or postsynaptic muscle cells, and could also be involved in mammalian NMJ synapse elimination.

Recent studies of Fly NMJ during metamorphosis also provide new insight into how synapse elimination progresses [28*]. Using this system, Liu et al. showed that pre- and post-synaptic elimination are initiated at different time points. Post-synaptic components disappeared and eliminated earlier than pre-synaptic elimination. Moreover, the authors found several mechanistic differences between pre- and postsynaptic elimination suggesting that postsynaptic muscle cells play an instructive role in synapse elimination, and that postsynaptic elimination is independent of presynaptic elimination. Interestingly, the authors suggest that there seems to be no obvious contribution of glial cells in this type of fly NMJ elimination during metamorphosis. However, given the report that muscle as well as glial cells express phagocytic receptors such as Draper in clearing the fly larval NMJ [18**], it is possible that other non-neuronal cell types could participate in eliminating pre- or postsynaptic compartments of the fly NMJ during metamorphosis. These data are consistent with accumulating data supporting the idea that the postsynaptic side plays a critical role in synapse elimination [29, 30].

Synapse elimination in the retinogeniculate system

In the retinogeniculate system in mammals, synapses from retinal ganglion cells (RGCs) are eliminated to form distinct non-overlapping eye-specific domains in their major diencephalic target, the dorsal lateral geniculate nucleus (dLGN). Three phases of RGC afferent pruning in the LGN have been documented [8, 31]. First, early in development, axons from the contralateral and ipsilateral eyes overlap in the LGN. By spontaneous retinal activity, these overlapping connections are eliminated, so that a single LGN cell receives RGC inputs from only one eye. After eye-specific segregation is completed, LGN cells are multiply innervated by many weak RGC axons. Again, by the third postnatal week, spontaneous retinal activity drives elimination of all but one or two RGC axons onto each LGN cell, and the remaining RGC inputs get stabilized. In the third phase, visual experience drives synaptic remodeling and maintenance, such that visual deprivation during this phase causes the weakening of synaptic connections and recruitment of additional inputs. All three phases of RGC refinement involve elimination of inappropriate synapses and maintenance of remaining connections.

In recent years, several pieces of evidence suggesting the significance of glial cells in LGN synapse elimination have emerged. Stevens et al. found that the classical complement cascade, a well-known player in innate immunity, mediates synapse elimination in the

retinogeniculate system during development [32]. The authors found that C1q, the initiating protein in the classical complement cascade, is expressed in the CNS and often localizes to synapses throughout the postnatal brain. In the immune system, C1q binds and opsonizes the surface of target debris and activates the downstream complement cascade including C3, which results in phagocytosis by macrophages or cell lysis via membrane-attack complex formation [33, 34]. Mice deficient in the complement protein C1q or the downstream complement cascade protein C3 exhibit significant sustained defects in CNS synapse elimination, as shown by the failure of anatomical refinement of retinogeniculate connections and the retention of excess functional retinal innervation by LGN cells. The relevant phagocytic cells are likely to be microglia, since microglia cells are the only cells to express receptors for complement component C3 in the brain, although this hypothesis awaits a definitive proof. These findings add to the growing pool of evidence that glial cells play crucial roles in patterning neural circuits through immune molecules, and support a model in which unwanted synapses are tagged by complement proteins for elimination by glial cells. Moreover, in a mouse model of glaucoma, one of the most common neurodegenerative diseases, C1q becomes highly upregulated and re-localized to retinal synapses of RGCs at early stages of disease progression [35]. Consistent with this finding, Howell et al. have shown by unbiased gene expression profiling that the complement cascade is one of the earliest molecular changes in glaucoma [36*]. Complement component 1a (C1qa) is highly expressed in microglia/macrophages in the optic nerve head during early stages of glaucoma, and mice with a mutation in *C1qa* are protected from glaucoma. Although further studies are necessary to understand the exact roles of microglia/macrophage in neurodegenerative disease such as glaucoma, it is likely that a common mechanism may underlie glia-mediated synapse elimination during development and glia-mediated neurodegenerative disease progression.

In addition to the complement cascade, other immune-related molecules, such as Major Histocompatibility complex class I (MHCI) and neuronal pentraxins (NPs- NP1, NP2 and NP receptor) have been shown to be involved in synapse elimination in the retinogeniculate system [37, 38]. Both MHCI and NPs are localized in the LGN during the period of synapse elimination and upregulated by neuronal activity. A recent study by Datwani et al. shows that H2-K^b and H2-D^b, two specific MHCI molecules out of more than 50, are required for retinogeniculate refinement [39**]. Mice lacking both H2-K^b and H2-D^b showed a failure of RGC axons from the two eyes to segregate fully and an enhanced ocular dominance phenotype, suggesting a critical role of these MHCI molecules in developmental synapse elimination. Similarly, NP1/NP2 mutant mouse retinal axons fail to undergo eye-specific refinement although the defect is transient during early stages [40]. Another recent study also shows that NP1/NP2 mutant mice have severely reduced AMPA receptor-mediated synaptic transmission during a defined developmental period, which can be explained by an increase of silent synapses [41*]. After this critical period, AMPA-mediated transmission is restored, but also shows abnormally enhanced currents in the mutant. These data indicate that in NP1/NP2 mutants, LGN neurons fail to eliminate synapses from RGCs and the resulting excessive inputs can drive aberrantly large currents.

Collectively, immune-related pathways such as the complement cascade, MHCI, and NPs may act as molecular transducers that translate RGC activity into structural changes at retinogeniculate synapses. The involvement of glial cells in MHCI- and NPs- mediated synapse elimination is not known. However, given the link between the complement cascade and microglia in synapse elimination, these immune-related pathways may interact with each other in glial cell mediated developmental synapse elimination [35, 39**].

Synapse elimination in other brain regions

It is becoming increasingly apparent that synapses, especially dendritic spines, turn over rapidly, and that the mechanisms of synapse elimination during early developmental stages persist in the later postnatal stages as well [42–44]. Recent studies using two-photon microscopy with *in vivo* cortical neurons revealed that synapses in the adult brain are highly dynamic [45] (Figure 1b). New motor learning and sensory experience induce not only new spine formation but also rapid elimination of pre-existing synapses established early in life [19–21*].

Importantly, it has been suggested that glial cells again play critical roles in eliminating individual synapses in juvenile brains (Figure 2b). Using electron microscopy and two-photon *in vivo* imaging in the primary visual cortex of juvenile mice, Tremblay et al. demonstrated the close relationship between microglial processes and synapse components [46**]. Consistent with previous findings that show the dynamic motility of microglial processes in uninjured mature brains [47, 48], the authors confirmed that microglial processes are highly motile and form transient contacts with synaptic elements during normal visual experience. Interestingly, microglial processes tend to localize at small and growing spines, which are eliminated eventually over 2 day-periods [46**], suggesting that microglia may actively control the structural states of synapse and participate in synapse elimination. Indeed, cellular inclusions that contain synaptic elements were found inside of microglia and the total number of microglia-associated inclusions was increased after manipulating visual experience through light deprivation and re-exposure (Figure 2b) [46**].

A recent paper also shows that microglia actively engulf synapses in the developing hippocampus [49**]. Small but significant portions of the excitatory postsynaptic density, PSD-95 and the presynaptic protein, SNAP25 were found to be in the microglial cytoplasm suggesting that microglia engulf synaptic components in the normal brain. In mice lacking the fractalkine receptor, *Cx3cr1*, which is expressed by microglia and critical for microglial migration [50*], the density of total PSD-95 and dendritic spines were significantly increased compared to wild-type control mice. The significant increase of synaptic density in the mutant was observed during the second and third postnatal weeks, suggesting transient synaptic elimination defects in *Cx3cr1* mutant mice. Electrophysiology data also shows immature connectivity and increased synaptic release sites in the mutant mice implying delayed synapse elimination. Long-term depression (LTD) was significantly increased and susceptibility to seizures was reduced in the mutant at P13, but not in adulthood, consistent with a delayed synapse elimination and immature brain development in these animals. The authors suggest that a transient reduction in microglia population in *Cx3cr1* mutant mice could account for the delayed synaptic elimination. It is also possible that fractalkine signaling is required transiently for recruiting fractalkine receptor-expressing microglia to the unwanted synapse similar to the proposed model for complement-dependent synapse elimination in the developing retinogeniculate system. It is interesting to note that C1q is required for synapse elimination in cortical layer V pyramidal neurons, as revealed by an increased number, yet weaker connections in *C1q* mutant mice [51*]. These *C1q* mutant mice showed spontaneous and evoked-epileptiform activity. Future studies would be necessary to determine whether microglial-mediated synapse elimination is conserved in other brain regions until adulthood and what other signaling mechanisms besides the complement cascade are involved in recognizing and engulfing synapses by glial cells.

Conclusion

Together, the extensive role of glial cells in synapse elimination is becoming increasingly evident. Failure in synapse elimination by glial cells can result in developmental and neurophysiological dysfunction [18**]. A recent paper showed that the excessive pathological grooming behavior exhibited by *Hoxb8* mutant mice is caused by a defect in microglia [52**]. Currently, it is not clear how impaired microglia results in inducing a specific neurophysiological disorder. However, it is quite possible that an abnormal synaptic connection due to pruning defects is responsible for this unexpected phenomenon.

Glial cells could mediate the synapse elimination process either by clearing synaptic debris produced from the pre- and postsynaptic compartment, as observed in the NMJ synapse elimination by fly glial cells and mammalian Schwann cells (Figure 2a), or by actively promoting synapse disassembly, as observed in synapse elimination in the retinogeniculate system and other brain regions, such as cortex and hippocampus by microglia (Figure 2b). Although these different types of glial cells are fundamentally distinct in their origins and functions, common or specific phagocytic pathways may be expressed and underlie the tissue-resident phagocytic roles of glial cells. In addition to microglia, in mammals, growing evidence suggests that astrocytes, another glial subtype in the brain, may also play a role in clearing neural debris and synapses [53–55]. Astrocytes in the optic nerve head have been recently shown to phagocytose axonal cytoplasm and organelles in the non-injured optic nerve [56*]. It is interesting to note that astrocytes express a plethora of genes that have been implicated in phagocytosis [53]. Those genes fall into at least three partially redundant pathways that activate phagocytosis [22]. The first pathway includes the proteins CrKII, DOCK180, Rac1, and ELMO and controls rearrangement of the actin cytoskeleton, which is required to surround the cellular debris [57]. A recent study has also identified Bai1 as a phagocytic receptor that is expressed in astrocytes and acts upstream of these components [58]. The second pathway includes the c-Mer tyrosine kinase receptor (MerTK), which works with the Integrin pathway to regulate CrKII/DOCK180/Rac1 modules [59, 60]. The last pathway consists of MEGF10 (an ortholog of fly Draper), GULP (an ortholog of fly dCed-6), and ABCA1 (ATP-binding cassette, sub-family A member 1), and participates in cellular debris recognition and engulfment [61]. It is possible that astrocytes may participate in synapse elimination in coordination with professional phagocytes, such as microglia in the CNS. How they coordinate the elimination process of the synapses and whether there is any molecular specificity in recognizing the target synapses are important questions that need to be addressed in the future.

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References and recommended reading

Papers of particular interest, published within the period of review have been highlighted as:

* of special interest

** of special interest

1. Katz LC, Shatz CJ. Synaptic activity and the construction of cortical circuits. *Science*. 1996; 274:1133–1138. [PubMed: 8895456]
2. Walsh MK, Lichtman JW. In vivo time-lapse imaging of synaptic takeover associated with naturally occurring synapse elimination. *Neuron*. 2003; 37:67–73. [PubMed: 12526773]

3. Mariani J, Changeux JP. Ontogenesis of olivocerebellar relationships. I. Studies by intracellular recordings of the multiple innervation of Purkinje cells by climbing fibers in the developing rat cerebellum. *J Neurosci.* 1981; 1:696–702. [PubMed: 7346578]
4. Chen C, Regehr WG. Developmental remodeling of the retinogeniculate synapse. *Neuron.* 2000; 28:955–966. [PubMed: 11163279]
5. Luo L, O'Leary DD. Axon retraction and degeneration in development and disease. *Annu Rev Neurosci.* 2005; 28:127–156. [PubMed: 16022592]
6. Goda Y, Davis GW. Mechanisms of synapse assembly and disassembly. *Neuron.* 2003; 40:243–264. [PubMed: 14556707]
7. Sanes JR, Lichtman JW. Development of the vertebrate neuromuscular junction. *Annu Rev Neurosci.* 1999; 22:389–442. [PubMed: 10202544]
8. Kano M, Hashimoto K. Synapse elimination in the central nervous system. *Curr Opin Neurobiol.* 2009; 19:154–161. [PubMed: 19481442]
9. Gan WB, Kwon E, Feng G, et al. Synaptic dynamism measured over minutes to months: age-dependent decline in an autonomic ganglion. *Nat Neurosci.* 2003; 6:956–960. [PubMed: 12925856]
10. Trachtenberg JT, Chen BE, Knott GW, et al. Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. *Nature.* 2002; 420:788–794. [PubMed: 12490942]
11. Grutzendler J, Kasthuri N, Gan WB. Long-term dendritic spine stability in the adult cortex. *Nature.* 2002; 420:812–816. [PubMed: 12490949]
12. Holtmaat A, Svoboda K. Experience-dependent structural synaptic plasticity in the mammalian brain. *Nat Rev Neurosci.* 2009; 10:647–658. [PubMed: 19693029]
13. Eaton BA, Davis GW. Synapse disassembly. *Genes Dev.* 2003; 17:2075–2082. [PubMed: 12952887]
14. Bishop DL, Misgeld T, Walsh MK, et al. Axon branch removal at developing synapses by axosome shedding. *Neuron.* 2004; 44:651–661. [PubMed: 15541313]
15. Hooks BM, Chen C. Distinct roles for spontaneous and visual activity in remodeling of the retinogeniculate synapse. *Neuron.* 2006; 52:281–291. [PubMed: 17046691]
16. Sretavan D, Shatz CJ. Prenatal development of individual retinogeniculate axons during the period of segregation. *Nature.* 1984; 308:845–848. [PubMed: 6201743]
17. Hashimoto K, Kano M. Postnatal development and synapse elimination of climbing fiber to Purkinje cell projection in the cerebellum. *Neurosci Res.* 2005; 53:221–228. [PubMed: 16139911]
- 18*. Fuentes-Medel Y, Logan MA, Ashley J, et al. Glia and muscle sculpt neuromuscular arbors by engulfing destabilized synaptic boutons and shed presynaptic debris. *PLoS Biol.* 2009; 7:e1000184. This study shows that normal synaptic growth produces synaptic debris in response to changes in growth and activity at the fly larval NMJ. Importantly, glial cells and postsynaptic muscle cells express phagocytic components, Draper and dCed-6, and are critical in clearing synaptic debris. Interestingly, glial cells preferentially eliminate presynaptic debris, while muscle cells preferentially eliminate ghost boutons. Failure of the clearance process results in severe abnormality in synaptic growth. [PubMed: 19707574]
- 19*. Xu T, Yu X, Perlik AJ, et al. Rapid formation and selective stabilization of synapses for enduring motor memories. *Nature.* 2009; 462:915–919. [PubMed: 19946267]
- 20*. Yang G, Pan F, Gan WB. Stably maintained dendritic spines are associated with lifelong memories. *Nature.* 2009; 462:920–924. [PubMed: 19946265]
- 21*. Roberts TF, Tschida KA, Klein ME, Mooney R. Rapid spine stabilization and synaptic enhancement at the onset of behavioural learning. *Nature.* 2010; 463:948–952. Through live-imaging experiments using two-photon microscopy, these three papers describe *in-vivo* evidences showing that synapses are highly dynamic in the adult brains in response to learning and memory. New spines are formed and stabilized rapidly during learning, whereas pre-existing synapses established early in life are preferentially eliminated. [PubMed: 20164928]
22. Mallat M, Marin-Teva JL, Cheret C. Phagocytosis in the developing CNS: more than clearing the corpses. *Curr Opin Neurobiol.* 2005; 15:101–107. [PubMed: 15721751]
23. Sokolowski JD, Mandell JW. Phagocytic clearance in neurodegeneration. *Am J Pathol.* 2011; 178:1416–1428. [PubMed: 21435432]

24. Kuran E. Keeping the CNS clear: Glial phagocytic functions in *Drosophila*. *Glia*. 2011; 59:1304–1311. [PubMed: 21136555]
25. Awasaki T, Ito K. Engulfing action of glial cells is required for programmed axon pruning during *Drosophila* metamorphosis. *Curr Biol*. 2004; 14:668–677. [PubMed: 15084281]
26. Buffelli M, Burgess RW, Feng G, et al. Genetic evidence that relative synaptic efficacy biases the outcome of synaptic competition. *Nature*. 2003; 424:430–434. [PubMed: 12879071]
27. Song JW, Misgeld T, Kang H, et al. Lysosomal activity associated with developmental axon pruning. *J Neurosci*. 2008; 28:8993–9001. [PubMed: 18768693]
- 28*. Liu Z, Chen Y, Wang D, et al. Distinct presynaptic and postsynaptic dismantling processes of *Drosophila* neuromuscular junctions during metamorphosis. *J Neurosci*. 2010; 30:11624–11634. This study provides several lines of evidences supporting the hypothesis that postsynaptic side plays a critical role in synapse elimination. Using the fly NMJ system during metamorphosis, the author clearly shows that elimination of postsynaptic compartment starts earlier than presynaptic elimination. Postsynaptic elimination is independent of presynaptic elimination and can trigger the entire elimination process. Interestingly, glial cells appear to be not involved in this type of synapse elimination. [PubMed: 20810883]
29. Balice-Gordon RJ, Lichtman JW. Long-term synapse loss induced by focal blockade of postsynaptic receptors. *Nature*. 1994; 372:519–524. [PubMed: 7990923]
30. McCann CM, Nguyen QT, Santo Neto H, Lichtman JW. Rapid synapse elimination after postsynaptic protein synthesis inhibition in vivo. *J Neurosci*. 2007; 27:6064–6067. [PubMed: 17537978]
31. Huberman AD. Mechanisms of eye-specific visual circuit development. *Curr Opin Neurobiol*. 2007; 17:73–80. [PubMed: 17254766]
32. Stevens BW, Dolan CM, Anderson JG, Bukrey CD. Custom talar prosthesis after open talar extrusion in a pediatric patient. *Foot Ankle Int*. 2007; 28:933–938. [PubMed: 17697660]
33. Perry VH, O'Connor V. C1q: the perfect complement for a synaptic feast? *Nat Rev Neurosci*. 2008; 9:807–811. [PubMed: 18827829]
34. Schafer DP, Stevens B. Synapse elimination during development and disease: immune molecules take centre stage. *Biochem Soc Trans*. 2010; 38:476–481. [PubMed: 20298206]
35. Stevens B, Allen NJ, Vazquez LE, et al. The classical complement cascade mediates CNS synapse elimination. *Cell*. 2007; 131:1164–1178. [PubMed: 18083105]
- 36*. Howell GR, Macalinao DG, Sousa GL, et al. Molecular clustering identifies complement and endothelin induction as early events in a mouse model of glaucoma. *J Clin Invest*. 2011; 121:1429–1444. The authors use unbiased gene expression profiling in glaucoma mouse model, and find the complement cascade and endothelin signaling pathways as one of the earliest molecular changes in glaucoma. Disrupting either pathway shows significant protection against glaucoma, suggesting aberrant activation of these pathways in neurodegenerative disease can lead to disease progression. [PubMed: 21383504]
37. Shatz CJ. MHC class I: an unexpected role in neuronal plasticity. *Neuron*. 2009; 64:40–45. [PubMed: 19840547]
38. Huh GS, Boulanger LM, Du H, et al. Functional requirement for class I MHC in CNS development and plasticity. *Science*. 2000; 290:2155–2159. [PubMed: 11118151]
- 39*. Datwani A, McConnell MJ, Kanold PO, et al. Classical MHCI molecules regulate retinogeniculate refinement and limit ocular dominance plasticity. *Neuron*. 2009; 64:463–470. This paper shows that H2-K^b and H2-D^b, two specific MHCI molecules out of more than 50, are required for retinogeniculate refinement. Mice lacking both H2-K^b and H2-D^b showed a failure of RGC synapse segregation in LGN and an enhanced ocular dominance phenotype, similar to $\beta 2m^{-/-}TAP1^{-/-}$ mice, which lack cell surface expression of all MHCI molecules. This result suggests a critical role of these MHCI molecules in developmental synapse elimination. [PubMed: 19945389]
40. Bjartmar L, Huberman AD, Ullian EM, et al. Neuronal pentraxins mediate synaptic refinement in the developing visual system. *J Neurosci*. 2006; 26:6269–6281. [PubMed: 16763034]
- 41*. Koch SM, Ullian EM. Neuronal pentraxins mediate silent synapse conversion in the developing visual system. *J Neurosci*. 2010; 30:5404–5414. The authors show that NP1/NP2 mutant mice

have severely reduced AMPA receptor-mediated synaptic transmission during a defined developmental period. After this critical period, AMPA-mediated transmission is restored, but also shows abnormally enhanced currents indicating that in NP1/NP2 mutants, LGN neurons fail to eliminate synapses from RGCs and the resulting excessive inputs can drive aberrantly large currents. [PubMed: 20392962]

42. Bhatt DH, Zhang S, Gan WB. Dendritic spine dynamics. *Annu Rev Physiol.* 2009; 71:261–282. [PubMed: 19575680]
43. Fu M, Zuo Y. Experience-dependent structural plasticity in the cortex. *Trends Neurosci.* 2011; 34:177–187. [PubMed: 21397343]
44. Yu X, Zuo Y. Spine plasticity in the motor cortex. *Curr Opin Neurobiol.* 2011; 21:169–174. [PubMed: 20728341]
45. Xu HT, Pan F, Yang G, Gan WB. Choice of cranial window type for in vivo imaging affects dendritic spine turnover in the cortex. *Nat Neurosci.* 2007; 10:549–551. [PubMed: 17417634]
- 46*. Tremblay ME, Lowery RL, Majewska AK. Microglial interactions with synapses are modulated by visual experience. *PLoS Biol.* 2010; 8:e1000527. Through live *in-vivo* imaging, this paper shows that microglial processes are closely associated with dendritic spines in the normal juvenile brains. Microglial processes tend to localize to small and growing spines which are eliminated eventually within 2 days. Inclusions derived from pre- and postsynaptic elements are found in the microglial processes. Manipulating visual experience by light deprivation and reexposure changes morphology and distribution of microglial processes as well as the amount of phagocytosed inclusions in microglia. [PubMed: 21072242]
47. Nimmerjahn A, Kirchhoff F, Helmchen F. Resting microglial cells are highly dynamic surveillants of brain parenchyma in vivo. *Science.* 2005; 308:1314–1318. [PubMed: 15831717]
48. Wake H, Moorhouse AJ, Jinno S, et al. Resting microglia directly monitor the functional state of synapses in vivo and determine the fate of ischemic terminals. *J Neurosci.* 2009; 29:3974–3980. [PubMed: 19339593]
- 49*. Paolicelli RC, Bolasco G, Pagani F, et al. Synaptic Pruning by Microglia Is Necessary for Normal Brain Development. *Science.* 2011 This paper nicely shows that, using STED, postsynaptic PSD-95 and presynaptic SNAP25 immunoreactivity are found in microglial processes in the developing mouse hippocampus. In mice lacking the fractalkine receptor (*Cx3cr1*), the authors find transient synapse elimination defects in the hippocampus due to the delayed appearance of microglial population. The density of total PSD-95 and dendritic spines are significantly increased in the mutant mice. Electrophysiology data also shows immature connectivity and increased synaptic release sites in the mutant mice implying delayed synapse elimination.
- 50*. Fuhrmann M, Bittner T, Jung CK, et al. Microglial Cx3cr1 knockout prevents neuron loss in a mouse model of Alzheimer's disease. *Nat Neurosci.* 2010; 13:411–413. Live *in-vivo* imaging in the intact brains of Alzheimer's disease mice reveals that microglia is involved in neuron elimination. Microglia in *Cx3cr1* mutant mice show the defective migration velocity and fail to be recruited near neurons, which result in prevention of neuronal loss. [PubMed: 20305648]
- 51*. Chu Y, Jin X, Parada I, et al. Enhanced synaptic connectivity and epilepsy in C1q knockout mice. *Proc Natl Acad Sci U S A.* 2010; 107:7975–7980. The authors show that C1q is required for synapse elimination in cortical layer V pyramidal neurons, as revealed by an increased number, yet weaker connections in *C1q* mutant mice. These C1q mutant mice show spontaneous and evoked-epileptiform activity. [PubMed: 20375278]
- 52*. Chen SK, Tvrdik P, Peden E, et al. Hematopoietic origin of pathological grooming in Hoxb8 mutant mice. *Cell.* 2010; 141:775–785. This paper describes an unexpected finding that excessive pathological grooming defect in Hoxb8 mutant mice originates from a defect in microglia. The authors show that Hoxb8 cell lineage gives rise to brain microglia and disruption of Hoxb8 function results in the reduction of the total number of microglia. The excessive grooming defects in the mutant can be rescued by normal bone marrow transplantation and specific deletion of Hoxb8 in the hematopoietic system recapitulate the excessive pathological grooming defect. [PubMed: 20510925]

53. Cahoy JD, Emery B, Kaushal A, et al. A transcriptome database for astrocytes, neurons, and oligodendrocytes: a new resource for understanding brain development and function. *J Neurosci.* 2008; 28:264–278. [PubMed: 18171944]
54. Bechmann I, Nitsch R. Astrocytes and microglial cells incorporate degenerating fibers following entorhinal lesion: a light, confocal, and electron microscopical study using a phagocytosis-dependent labeling technique. *Glia.* 1997; 20:145–154. [PubMed: 9179599]
55. Tansey FA, Cammer W. Differential uptake of dextran beads by astrocytes, macrophages and oligodendrocytes in mixed glial-cell cultures from brains of neonatal rats. *Neurosci Lett.* 1998; 248:159–162. [PubMed: 9654333]
- 56*. Nguyen JV, Soto I, Kim KY, et al. Myelination transition zone astrocytes are constitutively phagocytic and have synuclein dependent reactivity in glaucoma. *Proc Natl Acad Sci U S A.* 2011; 108:1176–1181. This paper shows that astrocytes in the optic nerve head myelination transition zone (MTZ) express MAC-2, a phagocytic component usually expressed in phagocytic cells. Astrocytes in MTZ constitutively internalize axonal evulsions. During glaucoma, astrocytes upregulate MAC-2 and this upregulation is dependent on γ -synuclein. [PubMed: 21199938]
57. Kinchen JM, Cabello J, Klingele D, et al. Two pathways converge at CED-10 to mediate actin rearrangement and corpse removal in *C.elegans*. *Nature.* 2005; 434:93–99. [PubMed: 15744306]
58. Park D, Tosello-Tramont AC, Elliott MR, et al. BAI1 is an engulfment receptor for apoptotic cells upstream of the ELMO/Dock180/Rac module. *Nature.* 2007; 450:430–434. [PubMed: 17960134]
59. Wu Y, Singh S, Georgescu MM, Birge RB. A role for Mer tyrosine kinase in alphavbeta5 integrin-mediated phagocytosis of apoptotic cells. *J Cell Sci.* 2005; 118:539–553. [PubMed: 15673687]
60. D'Cruz PM, Yasumura D, Weir J, et al. Mutation of the receptor tyrosine kinase gene *Mertk* in the retinal dystrophic RCS rat. *Hum Mol Genet.* 2000; 9:645–651. [PubMed: 10699188]
61. Yu X, Lu N, Zhou Z. Phagocytic receptor CED-1 initiates a signaling pathway for degrading engulfed apoptotic cells. *PLoS Biol.* 2008; 6:e61. [PubMed: 18351800]

Highlights

Synapse elimination is critical not only in shaping neural circuits during development but also in regulating synaptic plasticity in response to experience and memory.

Glial cells play central roles in neural debris and synapse elimination through an engulfment process called phagocytosis.

Failure in synapse elimination by glial cells can result in developmental and neurophysiological dysfunction.

(a) Input elimination

(b) Individual synapse elimination

Figure 1. Schematic drawing illustrating input elimination and individual synapse elimination
 (a) Input elimination at the mammalian NMJ. Axons from motor neurons form connections with muscle fibers. Initially, each NMJ has multiple inputs from two or more motor neurons. Through activity-dependent intercellular competition, the “loser” axon retracts and is eventually eliminated, leaving a one-to-one match between each motor input and NMJ. (b) Individual synapse elimination in the brain. During learning and memory formation, new spines (red arrowheads) form rapidly (light red circles) and are stabilized, whereas synapses that existed previously (light black circles) are preferentially eliminated (black arrowheads).

Figure 2. Roles of glial cells in synapse elimination

(a) At the *Drosophila* NMJ, glial cells clear synaptic debris produced during synaptic growth [18**]. A single arbor from the motor neuron (red) innervates a muscle fiber (light blue) and forms synaptic boutons (dark blue). In response to changes in growth and/or activity, the addition of new synaptic connections with the muscle cell involves significant production of presynaptic debris and ghost boutons. The presynaptic debris and ghost boutons are engulfed and eliminated by glial (green) and muscle cells (light blue), respectively. Knocking down Draper or dCed-6 function in glia results in the accumulation of presynaptic debris, whereas blocking muscle-mediated phagocytosis causes the accumulation of ghost boutons. Disruption of clearing either one of the neural debris is sufficient to interfere with proper formation of synaptic boutons and lead to severely compromised synaptic growth. (modified from Ref. [18]) (b) In the mammalian brain, microglia (red) actively promote synapse disassembly (arrowheads) [35, 46**, 49**, 51*]. In the normal brain, microglial processes localize at small and growing dendritic spines, which are eliminated eventually (black arrowheads). Inclusions derived from pre- and postsynaptic elements (green in light yellow circles) are found inside of microglial processes. Studies have suggested that mice with defects in the complement cascade [35, 51*] or fractalkine signaling [49**] show the increased total synaptic density due to the failure of microglial-mediated synapse elimination.