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Phagocytic glial cells: sculpting synaptic circuits in the developing nervous system

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In the developing nervous system, synaptic connections are formed in excess and must remodel to achieve the precise synaptic connectivity characteristic of the mature organism. Synaptic pruning is a developmental process in which subsets of synapses are eliminated while the remaining synapses are preserved and strengthened. Recent findings have demonstrated unexpected roles for glial cells in this developmental process. These data demonstrate that phagocytic glia engulf synaptic and/or axonal elements in the developing nervous system and disruptions in this process result in sustained deficits in synaptic connectivity. These new findings highlight the importance of glia for nervous system development and function and may shed new light on mechanisms underlying nervous system disease.

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Introduction

The synapse is a structure fundamental for the transmission of electrical and chemical signals between neurons. In the mature nervous system, synapses form exquisitely precise connections necessary for neural processing and function. In comparison, the developing nervous system is characterized by a crude synaptic wiring diagram that must undergo a significant degree of remodeling. In a process termed synaptic pruning, exuberant synaptic connections formed early in development are selectively eliminated while the remaining synapses are maintained and strengthened [1–5]. This may involve the elimination of an axonal input that overshoots its target and/or the elimination of exuberant axonal collaterals innervating multiple targets [4] (Figure 1). Alternatively, pruning may involve the elimination of local, intact synapses (i.e. juxtaposed pre and postsynaptic elements) (Figures 1 and 2).

Surprisingly, a flurry of recent studies have implicated glia in the remodeling of synaptic connections in the healthy, developing nervous system. In particular, a role for glia possessing high phagocytic capacity has emerged. These cells include microglia, astrocytes, and Schwann cells in mammals and their glial counterparts in *Drosophila*. Here, we review recent findings demonstrating critical roles for phagocytic glia in developmental synaptic pruning.

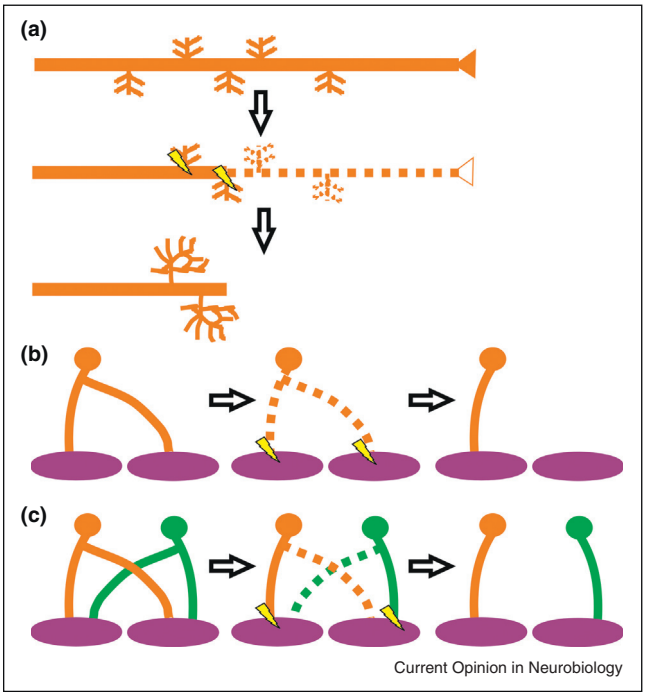
Glia and developmental synaptic pruning: axonal pruning in the CNS

Some of the first evidence suggesting phagocytic glia were involved in synaptic pruning was a light and electron microscopy (EM) study in the developing cat corpus callosum undergoing large-scale axonal pruning (Figure 1) [6•]. Within a developmental pruning window (E53–P39), there was an increase in callosal axon degeneration accompanied by the appearance of microglia and astrocytes containing degenerated axonal material within their cytoplasm.

Since this early work, little is still little known regarding the role of glia in large-scale axonal pruning in the mammalian CNS. However, elegant work in the developing *Drosophila* CNS demonstrated that glia play a key role in axonal pruning during metamorphosis from a larvae to a mature, adult insect [7••,8••,9]. During metamorphosis, γ neuron axons within the larval mushroom body are pruned away and new, adult-specific γ axons grow to their targets. While local axon degeneration mediated by the intrinsic ubiquitin–proteosome system (i.e. ecdysone) is an initiating step, two groups demonstrated that glia participate in this process by engulfing γ axons during the pruning period [7••,8••]. Furthermore, data suggest that these glial cells are not just passively scavenging leftover debris but rather active participants in the pruning process (Figure 3). Glial cells accumulate within the mushroom body lobes before detectable degeneration and engulf axonal varicosities, thought to be synaptic boutons, before these varicosities became fragmented [7••]. In addition, blocking glial phagocytic function during development (i.e. glia-specific *shibire* mutant) resulted in a γ axon pruning deficit; however, it was not clear whether this effect was sustained into adulthood [8••].

To more specifically assess the role of glia in pruning, recent work has genetically targeted phagocytic pathways in *Drosophila* glia [9,10•,11•]. By deleting the glial engulfment receptor Draper and downstream signaling

Figure 1



Axonal pruning in the nervous system. **(a)** A presynaptic input (orange) with interstitial branches overshoots its target or is inappropriately targeted. Subsequently this input and small branches are pruned and remaining interstitial branches are elaborated (e.g. callosal projections). **(b)** A presynaptic input (orange) forms synapses on a postsynaptic target during early development (purple). These synaptic connections are subsequently eliminated and reform to form circuitry necessary for processing in the mature animal (e.g. *Drosophila* mushroom body). **(c)** Axon pruning involving the elimination of an axon collateral (orange and green dotted lines) from the postsynaptic target (purple) (e.g. NMJ). In all cases, axon pruning is driven by neural activity. Those synapses that are more active (lightning bolts) are maintained and strengthened.

molecule dCED-6, glial cell invasion into the larval mushroom body was blocked. Furthermore, while there is still evidence of γ axon degeneration, larval γ axon fibers persist in the adult mushroom body lobes in these mutants. These results demonstrate that, within the context of the developing *Drosophila*, glial cells have a significant and sustained impact on the elimination of axonal inputs. It remains to be determined if similar mechanisms apply to large-scale axonal pruning in the developing mammalian CNS.

Glia and developmental synaptic pruning: axonal pruning in the PNS

Because of its simplicity, large size relative to its CNS counterpart, and accessibility for live imaging, the mammalian neuromuscular junction (NMJ) has served as an exquisite model system for studying pruning in the developing nervous system [1,12]. During early development, a single NMJ is innervated by multiple motor neuron axons (Figure 1). These multiple inputs are subsequently pruned leaving a single remaining axon to be maintained and strengthened. One known regulator of this process is neural activity where, by Hebbian mechanisms, those synaptic inputs that are more active are more likely to retain postsynaptic territory while less active inputs are eliminated [13–16].

Using the postnatal mouse NMJ, live imaging and serial EM revealed a role for perisynaptic Schwann cells, glial cells known to ensheath NMJ synapses, in motor axon pruning [17••]. This study demonstrated that motor axons destined for elimination form large retraction bulbs containing degenerating presynaptic material, which shed small fragments of presynaptic material termed 'axosomes.' During this process, Schwann cells were observed to enwrap retraction bulbs and engulf previously shed axosomes. While intriguing, several questions remain. For example, it was unclear whether Schwann cells were performing an active (i.e. initiating or facilitating retraction and pruning of axons) and/or passive (i.e. cleaning up debris) role during the pruning process (Figure 3). Indeed the molecular mechanisms underlying Schwann cell–presynaptic input interactions were unknown. Last, it remains unknown if Schwann cells are necessary for developmental pruning of motor axons.

To address whether Schwann cells were playing a more active role in the activity-dependent elimination of extranumerary presynaptic inputs at the mammalian NMJ, recent work used simultaneous calcium imaging in perisynaptic Schwann cells and recording from dually innervated NMJs in the developing mouse [18•]. The authors found that purinergic receptor-mediated intracellular calcium fluxes in Schwann cells reflected the relative synaptic strength of nerve terminals competing for postsynaptic 'turf. These data suggest that Schwann cells are able to detect relative strengths of synapses. However, it remains unknown whether these calcium fluxes influence physical associations (e.g. phagocytosis) between the Schwann cell and retracting presynaptic inputs.

Work in *Drosophila* has addressed more specific molecular mechanisms underlying activity-dependent glia–axon interactions at the developing NMJ [19••]. While relative levels of activity between presynaptic inputs will result in a selective elimination of less active inputs and strengthening of more active inputs, globally blocking neural activity in all inputs will result in a reduced capacity to eliminate synapses and globally increasing activity results in an increased rate of synapse elimination [1,13–15]. On the basis of this principle, Fuentes-Medel *et al.* activated motor neurons with Channelrhodopsin in developing larvae and observed an increase in presynaptic debris and unattached presynaptic terminals at the mammalian NMJ [19••]. Similar to the study