

Axon Retraction and Degeneration in Development and Disease

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axon elimination, visual system development, cytoskeletal signaling, Rho GTPases, Wallerian degeneration, neurodegenerative diseases

Abstract

The selective elimination of axons, dendrites, axon and dendrite branches, and synapses, without loss of the parent neurons, occurs during normal development of the nervous system as well as in response to injury or disease in the adult. The widespread developmental phenomena of exuberant axonal projections and synaptic connections require both small-scale and large-scale axon pruning to generate precise adult connectivity, and they provide a mechanism for neural plasticity in the developing and adult nervous system, as well as a mechanism to evolve differences between species in a projection system. Such pruning is also required to remove axonal connections damaged in the adult, to stabilize the affected neural circuits, and to initiate their repair. Pruning occurs through either retraction or degeneration. Here we review examples of these phenomena and consider potential cellular and molecular mechanisms that underlie axon retraction and degeneration and how they might relate to each other in development and disease.

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INTRODUCTION

The selective elimination of axons, dendrites, and synaptic connections without death of the parent neurons is a crucial common theme in many biological processes central to normal neural function. Development of precise axonal connectivity between appropriate sets of neurons or to peripheral targets such as muscles and organs requires the elimination of exuberant neuronal processes. Maintenance of functional circuits involves plasticity in their connectivity to allow for growth, learning, and memory. Finally, responses of axons to injury

or disease that remove or stabilize affected circuits can promote their repair.

In both developmental and adult axon elimination, the phenomena can be divided into two general classes on the basis of the relative scale of the events: small-scale events such as elimination of synaptic connections and the local pruning of an axonal or dendritic arbor, and large-scale events such as the elimination of a significant length of the primary axon and major axon collaterals. The first section of this article describes examples of these phenomena during development and

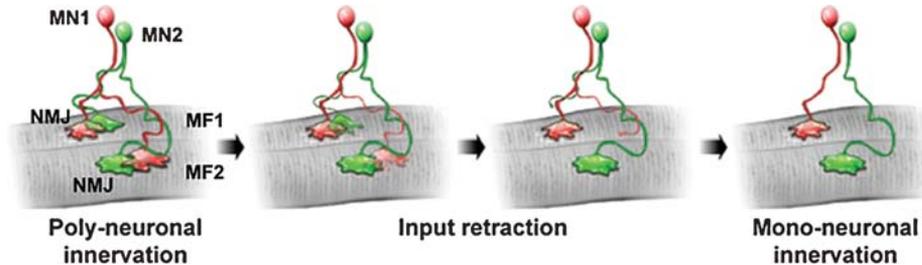


Figure 1

Small-scale axon elimination during development of the vertebrate nervous system. Small-scale axon elimination exemplified by synapse elimination at a “generic” vertebrate neuromuscular junction (NMJ). Input from a motor neuron (MN) to a muscle fiber (MF) forms a neuromuscular junction. In an adult, a typical neuromuscular junction is innervated by an axon from a single motor neuron. However, during development, a junction is multiply innervated by axons originating from two or more motor neurons. Competition between the inputs results in progressive changes in synaptic structure and strength, and the “loser” eventually retracts its terminal ending and the branch that gives rise to it.

mechanisms that selectively pattern developmental axon elimination. The second section focuses on axon elimination in the adult nervous system, which occurs during disease, aging, and injury. Learning and memory are not considered here, although structural changes underlying learning and memory may share mechanisms similar to those described here.

In general, the mode of axon elimination occurs through one of two distinct phenomena that relate to the scale of the event: Small-scale elimination typically occurs by retraction, whereas large-scale elimination appears to occur primarily by degeneration. The final section of the article considers potential cellular and molecular mechanisms that underlie axon retraction and degeneration and how these mechanisms might relate in development and disease.

AXON ELIMINATION DURING DEVELOPMENT

A common feature of the development of neural connections throughout the nervous system, particularly in vertebrates, is that compared with the adult, axons connect to more targets and targets are contacted by more axons during development. This early phase of exuberance in the formation of connections requires that the initial axonal connections be

pruned through retraction or degeneration to develop the precise connectivity found in the adult. These strategies ensure the establishment of proper functional connections and provide a substrate for neural plasticity. Following, we present examples of small-scale (**Figure 1**) and large-scale axon elimination (**Figure 2**).

SMALL-SCALE DEVELOPMENTAL PRUNING OF AXONAL CONNECTIONS

Small-scale events include synapse elimination, reduction of poly-neuronal innervation of a target cell, and local pruning of an axonal arbor. Most if not all neural systems exhibit each of these overlapping phenomena (Katz & Shatz 1996, Lohof et al. 1996, Wong & Lichtman 2003). Classic examples of synapse elimination per se include the neuromuscular junction and climbing fiber inputs to cerebellar Purkinje cells, each of which reduces innervation of the target cell from poly-neuronal to mono-neuronal (**Figure 1**). Classic examples of local arbor pruning include segregation of visual input into eye-specific patterns in the retinogeniculate and geniculocortical projections, each of which reduces input to a target cell from binocular to monocular (**Figure 3**). In each of these instances,

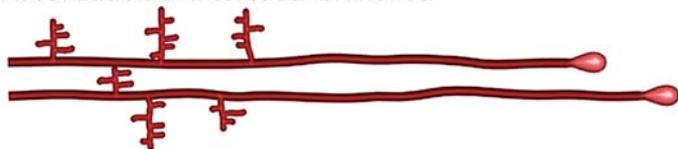
Extension and overshoot of primary axons



Interstitial branching along primary axons



Arborization of interstitial branches



Degeneration of overshooting primary axons and ectopic branches and arbors

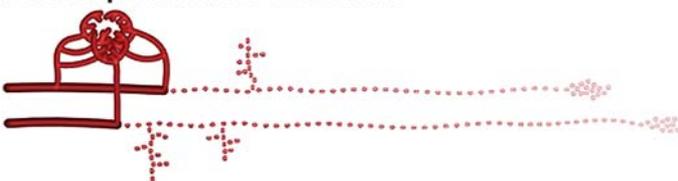


Figure 2

Large-scale axon elimination during development of the vertebrate nervous system. Large-scale axon elimination exemplified by a generic example. In the developing vertebrate brain, the primary axon typically extends well past its targets, which are later innervated by collateral branches that form *de novo* interstitially along the primary axon. Later, excess or inappropriate collaterals as well as a considerable length of the primary axon that overshoots the most distal target are eliminated by a process that appears to rely primarily on degeneration (indicated by broken axons).

synapses are eliminated early in the developmental maturation of the projection system, and the total number of synapses, or the size of the neuromuscular junction (NMJ), exhibits a progressive and substantial increase after the reduction in poly-neuronal input.

Studies that have directly visualized these events, or analyzed systems that mimic them, have provided considerable information about the process and the sequence of changes that underlie them. Time-lapse video imaging of marked axons in numerous systems has shown that developing axons, even after they have established synaptic contacts, are extremely dy-

namic and typically probe their locale through ongoing extensions and retractions of thin processes (O'Rourke et al. 1994, Alsina et al. 2001, Ruthazer et al. 2003, Walsh & Lichtman 2003, Kasthuri & Lichtman 2004). Further, these studies have directly revealed that small-scale elimination events appear to be due to retraction of selective axon segments and terminal endings or selective branches that form an axon arbor (Bernstein & Lichtman 1999). Time-lapse analysis of the developing neuromuscular junction revealed that the vacated postsynaptic site is quickly reinnervated by a persistent axon enhancing that axon's synaptic strength (Walsh & Lichtman 2003); similar events likely occur at CNS synapses (Mariani & Changeux 1981, Chen & Regehr 2000). Both the relative level of neural activity and correlation in activity patterns between axons that innervate the same target cells have pivotal roles in determining the competitive interactions between axons that drive these elimination events (Wiesel 1982, Personius & Balice-Gordon 2001, Feller 2002, Wong & Lichtman 2003). What axons compete for remains ambiguous, but it is widely assumed to be trophic support provided by the target cells.

Synapse Elimination at the Neuromuscular Junction

In the adult, a typical NMJ is innervated by a single motor axon. However, during development, a junction is usually multiply innervated by axons originating from two or more motor neurons (Figure 1). At developing neuromuscular synapses in vertebrates, different motor axons connecting to a muscle fiber compete for maintenance of their synapses. Competition results in progressive changes in synaptic structure and strength that lead to the physiologic weakening and retraction of some inputs, a process termed synapse elimination. At the same time, the remaining, "victorious" input is strengthened as it takes over vacated postsynaptic sites and is subsequently maintained throughout adult life (Colman et al.

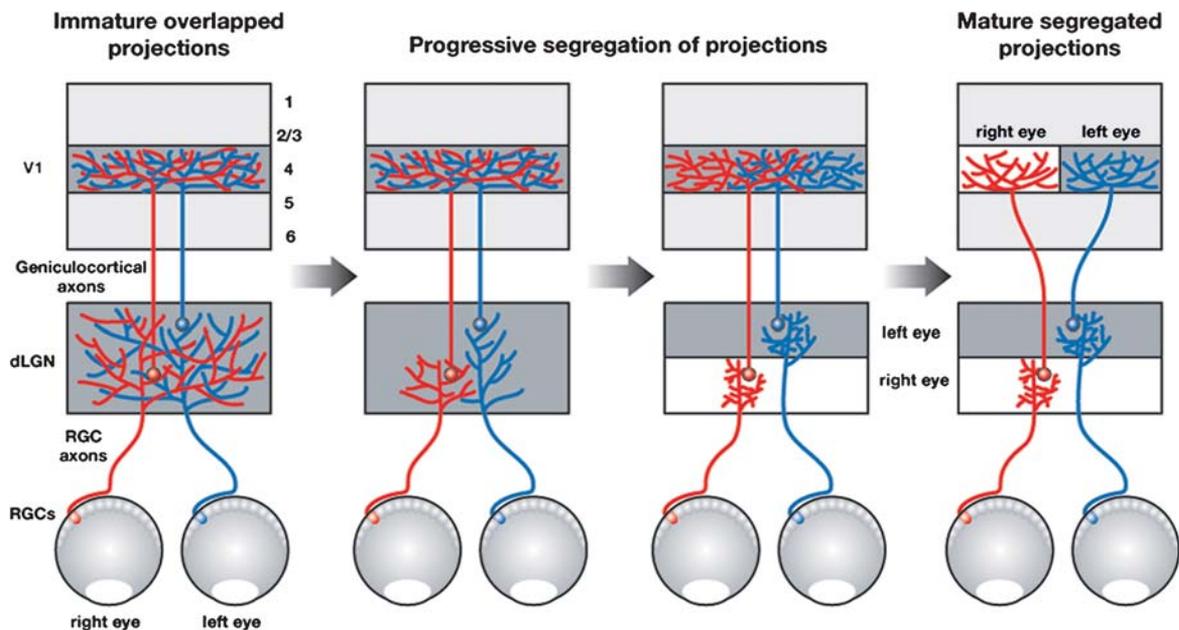


Figure 3

Small-scale axon elimination to develop eye-specific patterned connections. In adult mammals, inputs from the two eyes projecting to the same target are segregated into eye-specific domains. Axons of retinal ganglion cells (RGCs) from each eye are segregated into distinct layers or patches in their major diencephalic target, the dorsal lateral geniculate nucleus (dLGN). In the primary visual area (V1) of the cortex, geniculocortical axons from the eye-specific layers of the dLGN are segregated into eye-specific stripes or columns in layer 4. In each projection system, the two monocular inputs are initially overlapped and gradually segregate from one another by the selective local pruning of overlapping parts of axonal arbors to form the adult pattern of connections through a competitive process driven by correlated neural activity.

1997, Walsh & Lichtman 2003). Postsynaptic sites not reinnervated quickly lose receptors for neurotransmitter signaling, i.e., acetylcholine receptors concentrated at the postsynaptic site on the muscle. Competition between axons at neuromuscular synapses is based on the degree of correlation in their spiking patterns; uncorrelated activity enhances competition, whereas correlated activity diminishes competition (Personius & Balice-Gordon 2001, Kasthuri & Lichtman 2003). In addition, the relative level of activity between competing axons appears to be a critical parameter, where decreasing synaptic efficacy diminishes the competitive edge of the affected synapses (Balice-Gordon & Lichtman 1994, Buffelli et al. 2003). Activity-dependent modulation of axonal

competition is considered in more detail at CNS synapses in the following section.

Axon Pruning in Development of Eye-Specific Retinal Connections

The interdigitation of eye-specific axon connections is a fundamental organization of the mammalian visual system (Reid 2003). Eye-specific connections are formed initially through the direct projections of retinal ganglion cells (RGCs) to diencephalon and mid-brain, and they are subsequently reiterated through higher-order projections in neocortex. Eye-specific connections are patterned to segregate inputs from the two eyes, which allows for binocular inputs to selective sets of higher-order neurons to generate stereopsis.

In the adult brain, inputs from the two eyes projecting to the same target are segregated into eye-specific domains. In the major diencephalic target of RGC axons, the dorsal lateral geniculate nucleus (dLGN), RGC axons from each eye are segregated into distinct layers or patches, whereas in the primary visual area of the cortex, dLGN axons from the eye-specific layers are segregated into stripes or columns within layer 4. In each projection system, the two monocular inputs are initially overlapped and gradually segregate from one another by the selective local pruning of overlapping parts of axonal arbors to form the adult pattern of connections (**Figure 3**). The preferential stabilization of some axonal connections and the elimination of others that result in the segregation of eye-specific inputs were postulated decades ago to be controlled by a Hebbian mechanism (Hebb 1949) on the basis of a postsynaptic detection of correlated (i.e., appropriate) versus noncorrelated (inappropriate) inputs (Stent 1973). Neural activity has since been shown to drive eye-specific segregation; critical features of this activity-dependent process have been identified (Wiesel 1982, Feller 2002, Wong & Lichtman 2003) and the NMDA receptor has been implicated as a postsynaptic detector of correlated activity in some systems (Debski & Cline 2002, Zhou et al. 2003). In the absence of retinal activity, RGC inputs to the dLGN do not segregate into eye-specific layers (Penn et al. 1998, Rossi et al. 2001). Altering spontaneous activity in one retina relative to the other alters the normal pattern of eye-specific connections, which indicates that local arbor pruning is a competitive process between RGC axons from the two eyes (Penn et al. 1998, Stellwagen & Shatz 2002).

Although patterned vision would correlate activity among neighboring RGCs relative to distant ones, segregation is largely complete prior to the onset of visually evoked activity (Wong 1999, Tian & Copenhagen 2003). However, this form of arbor pruning is coincident with retinal waves that spontaneously propagate across the retina in a stochas-

tic manner and are generated by a network of cholinergic amacrine cells that interconnect RGCs expressing nicotinic acetylcholine receptors (nAChRs). Retinal waves strongly correlate patterns of spontaneous activity among neighboring RGCs even before photoreceptors are generated and become synaptically connected to RGCs through bipolar cells (Meister et al. 1991, Wong et al. 1993). Local pruning of RGC axon arbors, which leads to eye-specific layers, is delayed in mice deficient for the $\beta 2$ subunit of the neuronal nAChR that lack cholinergic-mediated retinal waves, and in contrast to wild type mice, $\beta 2$ mutant mice projections from the two eyes to the dLGN remain overlapped at the end of the first postnatal week (Rossi et al. 2001, Muir-Robinson et al. 2002). Subsequently, though, correlated RGC activity due to ionotropic glutamate receptor-mediated waves and patterned vision do drive the pruning of RGC inputs from the two eyes into eye-specific patches, albeit in an abnormal distribution (Muir-Robinson et al. 2002). These findings indicate that correlated RGC activity is required and sufficient to drive the competitive interactions between RGC axons from the two eyes, which leads to small-scale axon elimination and eye-specific segregation of retinogeniculate arborizations.

LARGE-SCALE DEVELOPMENTAL PRUNING OF AXONAL CONNECTIONS

In the vertebrate brain, most neurons innervate multiple, widely separated targets by long axon collaterals extended from a primary axon, and therefore they face a unique problem of target selection during development. This process of target selection is accomplished by a distinct mechanism referred to as delayed interstitial axon branching. As the primary axon elongates, its growth cone makes navigational decisions in response to guidance molecules to direct the axon through its pathway along which its targets are positioned at varying distances, some adjacent to the path

and others at a considerable distance. Later, collateral branches form along the primary axon and extend toward their target, where upon reaching the target the collaterals themselves extend branches that go on to arborize. Initially, the number of collaterals formed and the number of targets to which they project is larger than that found in the adult, but both are later reduced through a process of large-scale axon elimination (**Figure 2**). This mechanism is used to generate the adult patterns of all vertebrate projection systems analyzed, including all major projections of the mammalian neocortex—callosal, intracortical, and subcortical projections (**Figure 4**) (O’Leary 1992, O’Leary & Koester 1993). In addition, large-scale axon elimination is used to remodel projections within a large target, as in, for example, the elimination of the distal component of the primary axon and entire ectopically positioned arbors, as during the development of topographic mapping of RGC axons in the midbrain (**Figure 5**; McLaughlin et al. 2003a).

In a sense, large-scale axon elimination is an effective means of post hoc axonal pathfinding and target selection. Given that during vertebrate development large-scale axon elimination typically involves the rapid pruning of many millimeters of primary axon along with its distal interstitial branches and the arbors that they may have formed, degeneration seems to be a more likely mechanism over retraction. Although limited, morphological evidence obtained using anterograde axon fate labeling that spans the time course of axon pruning indicates that large-scale axon elimination is indeed accomplished by axon degeneration, at least in the development of layer 5 subcortical projections (**Figure 4**) (Reinoso & O’Leary 1989) and retinotopic mapping in the midbrain (**Figure 5**) (Nakamura & O’Leary 1989). In these systems, the portions of the fate-labeled axons undergoing elimination have a blebbed, fragmented morphology coincidentally along their length that closely resembles the appearance of γ neuron axons in the *Drosophila* mushroom body (MB)

pruned via a mechanism of local degeneration, as described in a later section.

Target Selection by Interstitial Axon Branching Followed by Axon Elimination

A classic example of neurons that employ delayed interstitial branching and subsequent axon elimination to develop their functionally appropriate connections are layer 5 neurons in the mammalian neocortex (O’Leary et al. 1990, O’Leary & Koester 1993) (**Figure 4**). Layer 5 neurons form the major output projection of the cortex and establish connections with numerous nuclei in the midbrain and hindbrain, as well as with a specific level of the spinal cord. During development, layer 5 axons extend out of the cortex along a spinally directed pathway; their growth cones ignore all eventual targets as they grow past them and continue to extend caudally through the corticospinal tract (O’Leary & Terashima 1988). Axon collaterals extended by layer 5 axons later innervate the brainstem and spinal targets by a dynamic process that involves the repeated extensions and retractions of short branches before a branch is stabilized and extends as a long collateral into the target where it arborizes (Bastmeyer & O’Leary 1996). Initially, layer 5 neurons project more broadly and form collateral projections to a larger set of layer 5 targets than they will retain in the adult (**Figure 4**). This widespread, or exuberant, projection pattern of layer 5 axons seems to be elaborated according to a specific axonal growth program characteristic of a general class of subcortically projecting, layer 5 neurons. However, the organization of the adult neocortex into specialized areas characterized by unique functions and axonal connections requires that each area establish projections to specific subsets of targets in the brainstem and spinal cord.

The patterns of layer 5 projections characteristic of the adult are pruned from the initial widespread pattern by selective axon elimination, which in rats occurs during the second

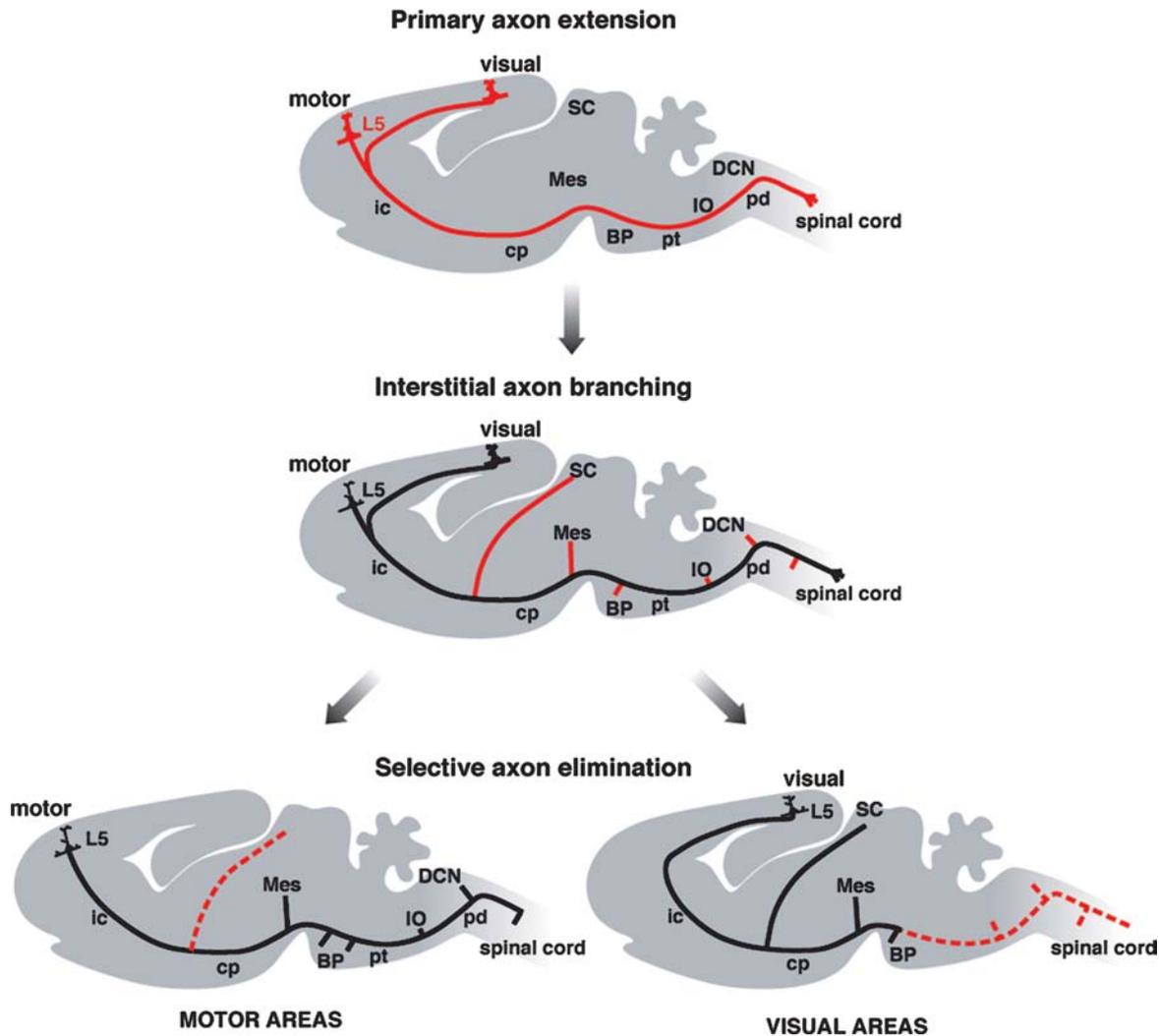


Figure 4

Large-scale axon elimination to develop area-specific subcortical projections of layer 5 neurons of the neocortex. The three major sequential steps in the development of subcortical projections of mammalian layer 5 neurons are illustrated in schematics of a sagittal view of the developing rodent brain. Primary axon extension: Layer 5 neurons (L5) extend a primary axon out of the cortex along a pathway that directs them toward the spinal cord passing by their subcortical targets. Delayed collateral branch formation: Subcortical targets are later contacted exclusively by axon collaterals that develop by a delayed extension of collateral branches interstitially along the spinally directed primary axon. As a population, layer 5 neurons in essentially all areas of rodent neocortex develop branches to a common set of targets. Selective axon elimination: As illustrated for visual and motor areas, specific collateral branches (some up to 2 mm in length) or segments of the primary axon (often more than 5 mm in length) are selectively eliminated, apparently by degeneration (*dashed lines*), to generate the mature projections functionally appropriate for the area of neocortex in which the layer 5 neuron is located. Abbreviations: BP, basilar pons; cp, cerebral peduncle; DCN, dorsal column nuclei; ic, internal capsule; IO, inferior olive; Mes, mesencephalon; pd, pyramidal decussation; pt, pyramidal tract; SC, superior colliculus (adapted from O'Leary & Koester 1993).

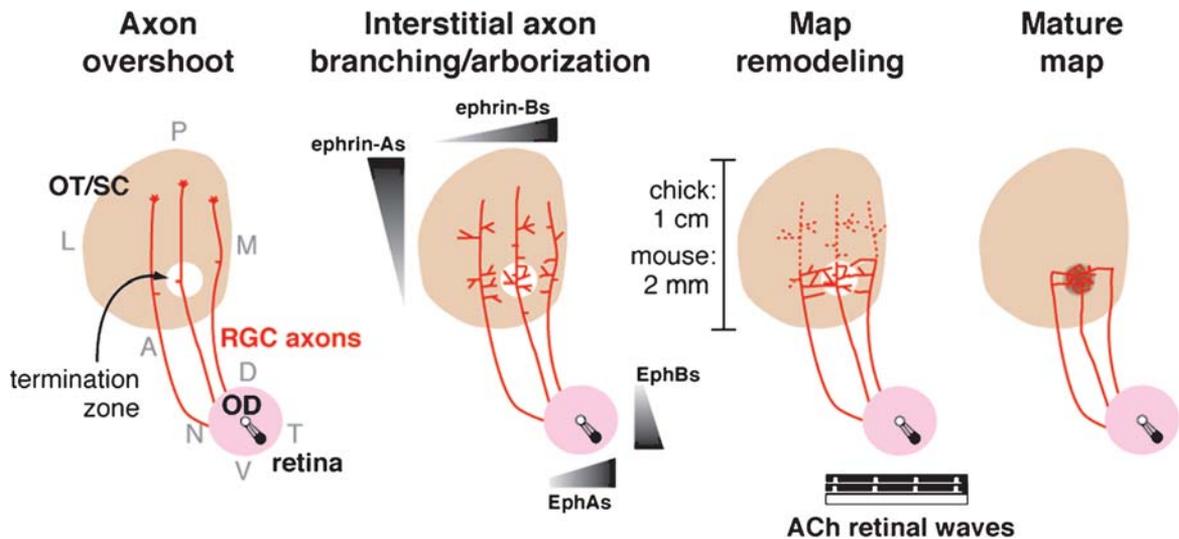


Figure 5

Large-scale axon elimination to develop retinotopic maps. Illustrated are the progressive stages in the development of a retinotopic map in the optic tectum (OT) of chicks or its mammalian homologue, the superior colliculus (SC) of mice. Initially, RGC axons enter the OT/SC and overshoot the location of their future termination zone (white circle), often by several millimeters. In addition, RGC axons are distributed over a broad lateral (L) to medial (M) extent of the target. The low to high anterior (A) to posterior (P) gradient of the axon repellents, ephrin-As, stops the posterior extension of RGC axons and generates topographic specificity in the subsequent interstitial axon branching and branch arborization. RGCs express EphAs in a high temporal (T) to low nasal (N) gradient. Interstitial branches extend laterally or medially toward their future TZ, guided in part by attractant and repellent actions of graded ephrin-Bs in the target mediated by a high ventral (V) to low dorsal (D) expression of EphB receptors by RGCs. Inappropriate arbors and branches, and long segments of the primary axon posterior to a sustained branch and its arbor, are rapidly eliminated over a few days largely by degeneration through a process dependent on spontaneous waves of activity mediated by a network of cholinergic (ACh) amacrine cells that propagate across the retina and strongly correlate to the spiking patterns of neighboring RGCs (white spike trains inside black bar). Ephs and ephrins also likely contribute to aspects of map remodeling independent of correlated activity, including the elimination of overshooting primary axons. The lengths of the A-P axis of the chick OT and mouse SC during map development are indicated. OD, optic disk. Adapted from Hindges et al. (2002).

and third postnatal weeks, after functional identities of cortical areas are established (Stanfield et al. 1982, O'Leary & Koester 1993). The selection of collaterals or distal parts of the primary axon to be eliminated versus retained is dictated by the functional specialization of the cortical area in which the layer 5 neuron is located. For example, layer 5 neurons in motor areas, which control muscle movements, lose their collateral branch to the superior colliculus but retain branches to other targets involved in motor control including the basilar pons, inferior

olive, dorsal column nuclei, and spinal gray matter. In contrast, layer 5 neurons in visual areas, which are specialized to process vision, lose all collateral projections caudal to the basilar pons, and even the entire segment of their primary axon directed to the spinal cord, while retaining branches to the basilar pons and superior colliculus. The elimination process is very effective; for example, for a visual layer 5 neuron, elimination results in the loss of the distal part of the primary axon in its entirety up to the branch point in the axon tract where a "sustaining" collateral

extends from it and arborizes in the basilar pons (O'Leary & Terashima 1988).

Progress has been slow in defining the molecular and cellular mechanisms that control large-scale axon elimination, but some intriguing insights are available, including a role for the homeodomain transcription factor *Otx1* in the pruning of layer 5 axons. *Otx1* protein, which localizes to layer 5 neurons, undergoes a translocation from the cytoplasm to the nucleus coincident with the normal pruning of initially exuberant layer 5 axons (Weimann et al. 1999, Zhang et al. 2002). In adult mice with a targeted deletion of *Otx1*, layer 5 neurons in occipital cortex (e.g., visual areas) retain their normally transient primary axon to the spinal cord (Weimann et al. 1999). In addition, the collateral projection of occipital layer 5 axons to the superior colliculus aberrantly extends into the inferior colliculus, and the collateral projections to the basilar pons resemble those formed by motor areas. These findings suggest that *Otx1* provides a late onset of nuclear transcriptional regulation to turn on genes essential for axon pruning and/or to repress genes that are negative regulators of the pruning machinery.

Another example of plasticity in layer 5 axon pruning, possibly related to *Otx1* function, is changes in the selectivity of area-specific axon elimination by layer 5 neurons following heterotopic transplantation of pieces of developing cortex (Stanfield & O'Leary 1985, O'Leary & Stanfield 1989). Layer 5 neurons transplanted from visual areas to motor areas permanently retain their normally transient spinal axon, and layer 5 neurons transplanted from motor areas to visual areas lose their normally permanent spinal axon and retain their transient axon collateral to the superior colliculus. Thus, the projections retained by the transplanted layer 5 neurons are appropriate for the cortical area to which they are transplanted rather than where they were born. These findings indicate that features associated with the local environment in which a layer 5 neuron matures contribute to selectivity of axon elimination. It

is interesting to speculate that changes in *Otx1* expression or defects in translocation of *Otx1* protein by transplanted layer 5 neurons may contribute to their plasticity in axon pruning.

The mechanisms that control large-scale axon elimination may well differ between axonal projections, but studies of other systems suggest a potential contribution of thalamocortical input or the modality of sensory input received by the transplanted layer 5 neurons to changes in pruning selectivity. As with layer 5 projection neurons, the limited adult distribution of callosal cortical neurons is also pruned from an initially widespread distribution by selective axon elimination during development (Innocenti 1981, O'Leary et al. 1981). The selectivity in the process of callosal axon elimination is perturbed by numerous peripheral manipulations of either visual or somatosensory input that have a common feature of altering patterns of neural activity (e.g., strabismus; Shatz 1977, Lund et al. 1978, Koralek & Killackey 1990) or levels of activity (e.g., diminished activity by dark-rearing or eyelid suture, or silencing of retinal activity by enucleation or pharmacological manipulations; Innocenti 1986, Dehay et al. 1989, Frost et al. 1990, Zufferey et al. 1999). These treatments lead to retention of callosal connections between parts of cortex that would normally lose them. Altering sensory input or neural activity also perturbs selective axon elimination required to develop appropriate patterns of horizontal, intracortical connections within the cortex (Katz & Callaway 1992, Katz & Shatz 1996, Zufferey et al. 1999). Thus sensory input has a crucial role in influencing the selectivity of axon elimination required to develop the adult patterns of callosal and intracortical connections, and possibly layer 5 subcortical projections.

Axon Elimination to Develop Topographic Maps in a Target

Most axonal projections within the CNS establish in their target an orderly arrangement

of connections termed a topographic map, which reflects the spatial order of their parent neurons. In the visual system, this map is retinotopic and projects the visual field from the retina onto targets in the brain, initially through direct RGC projections to dorsal thalamus and midbrain. In birds and mammals, the formation of a retinotopic map in the major midbrain target, the optic tectum or superior colliculus, respectively, involves the establishment of an initial, very coarse map, which subsequently undergoes large-scale pruning to generate a refined map (McLaughlin et al. 2003a) (**Figure 5**). All arbors are established by primary branches that form in a topographically biased manner interstitially along RGC axons that overshoot their correct termination zone along the anterior-posterior axis of the target. The interstitial branches often extend long distances along the medial-lateral axis of the target before forming complex arborizations at the topographically correct location.

Receptors of the Eph family of tyrosine kinases and their ephrin ligands have crucial roles in retinotopic mapping, with EphA-ephrin-A signaling graded along the anterior-posterior axis of the target, and EphB-ephrin-B signaling graded along its medial-lateral axis (McLaughlin et al. 2003a). EphA-ephrin-A signaling controls in part RGC axon mapping along the anterior-posterior axis by inhibiting the formation of primary branches and their arborization posterior to the correct termination zone (Yates et al. 2001). Complementing this action, ephrin-B1 acts bifunctionally as an axon attractant and repellent through EphB forward signaling to direct branches along the L-M axis of the target to their topographically correct site and to delimit arbors through branch inhibition (Hindges et al. 2002, McLaughlin et al. 2003b). Computational modeling predicts that the increase in total ephrin-A repellent, due to the progressive increase of ephrin-As on RGC branches and arbors added to that of ephrin-As expressed by target cells, acts later to prune overshooting axons and aberrant arbors (Yates

et al. 2004). In support of this modeling is a report that in vitro, ephrin-As induce the degeneration of EphA-expressing hippocampal axons, implicating ephrin-As in the pruning of hippocampal axons required to generate topographic order in the hippocamposeptal projection (Gao et al. 1999).

Although computer simulations show that gradients of guidance molecules alone can develop a considerable degree of topographic order and refinement, including axon elimination, they are not sufficient to develop a completely refined projection (Yates et al. 2004). In addition, the generation of a properly refined map requires a parameter that resembles an assumed role for near-neighbor-correlated neural activity. Similar to the local arbor pruning that generates eye-specific connections in the dLGN, the large-scale axon elimination required to develop topographic order in the mouse retinocollicular projection occurs during the first postnatal week before the onset of visually evoked activity (Hindges et al. 2002) but is coincident with cholinergic retinal waves (Wong et al. 1993). Mice deficient for the $\beta 2$ subunit of the nAChR, which selectively lack cholinergic retinal waves (Bansal et al. 2000), fail to develop a refined topographic map and retain an aberrantly broad distribution of ectopic arbors and axons (McLaughlin et al. 2003c). During the second postnatal week, glutamatergic retinal waves correlate RGC firing, and later patterned visual activity does also (Bansal et al. 2000), but the $\beta 2$ -deficient mice fail to develop a refined retinotopic map and retain an aberrant pattern of broadly distributed ectopic arbors and axons into adulthood (McLaughlin et al. 2003c). Thus, the competitive interactions that lead to large-scale axon elimination required to remodel the mammalian retinocollicular projection into a refined map require correlated RGC activity during a critical period spanning the first postnatal week.

Developmental critical periods for changes in axonal connectivity including axon pruning have been defined in many neural

connections; in general, maturational changes that affect the parent neurons and their targets are believed to define the end of a critical period and the diminished capacity of axons for large-scale morphological plasticity at later ages (Hensch 2004). Interestingly, although later-correlated RGC activity patterns are insufficient to generate a refined retinotopic map (McLaughlin et al. 2003c), they are sufficient to drive the local pruning of RGC axon arbors, which leads to eye-specific segregation (Muir-Robinson et al. 2002, Ruthazer et al. 2003). Thus, RGC axon projections to the dLGN retain the capacity for the small-scale axon elimination required for their development of eye-specific patterns in the dLGN after the time that the same axons lose their ability to undergo large-scale axon elimination required to generate a retinotopic map in the midbrain superior colliculus. Although in mice, RGC input to the dLGN is formed by collateral branches of RGC axons that project to the SC (essentially all RGCs project to the SC, and about a third project to the dLGN), the difference in the critical periods between the retinogeniculate and retinocollicular projections may reflect differences in plasticity between classes of RGCs, with the subset of RGCs that forms the retinogeniculate projection retaining a greater capacity for activity-dependent remodeling.

Phylogenetic Differences in Developmental Axon Elimination May Generate Species-Unique Axon Projection Patterns

The developmental phenomenon of transiently exuberant axonal projections may provide a substrate for functional sparing or recovery following neural insults during development (Innocenti et al. 1999). In addition, this mechanism may provide a substrate for evolutionary change and contribute to differences between species in axonal connections (O'Leary 1992, Innocenti 1995, Deacon 2000). A compelling example of this

possibility is provided by studies of a substantial projection that arises in the subiculum of the hippocampal formation, passes through the fornix, a major forebrain axon tract, and terminates in the mammillary bodies of the hypothalamus. In adult rats, subicular axons do not extend caudally beyond the mammillary bodies. However, in developing rats, subicular axons extend through the fornix and continue caudally past the mammillary bodies in dense fascicles for considerable distances into the midbrain and hindbrain tegmentum, and some fascicles cross to the opposite side. Later, these axons form collateral projections to the mammillary bodies and lose the long segment of the primary axon distal to it (Stanfield et al. 1987).

Although the postmammillary component of the fornix is not maintained in adult rats, it is present to varying degrees in adult brains of other mammalian species (O'Leary 1992). In some species, such as the squirrel monkey, the postmammillary component of the fornix is minor, whereas in others—for example, the cat, some strains of rabbits, and the African elephant—it is a major axonal projection that extends prominently into the midbrain tegmentum with only a proportion of the axons appearing to connect to the mammillary bodies. These projection patterns in adults of other species closely resemble the distribution of transient postmammillary axons in developing rats. This similarity suggests that mammals initially develop a common pattern of postmammillary trajectories, but that the postmammillary components of the projection are later differentially elaborated and maintained across species, including complete elimination in some. Thus, major phylogenetic differences in axonal projections, and their functional manifestations, could emerge through the evolution of species-specific pruning (or stabilization) of a developmentally similar projection pattern. This phenomenon is not likely limited to the development of the fornix; differences among adults of various species in the distribution of other homologous axonal

pathways may well be found as the result in part of distinctions in axon elimination.

Neuronal Remodeling During Insect Metamorphosis

Axon and dendrite pruning also occur extensively in the development of invertebrate nervous systems. The most extensively studied example is neuronal remodeling during insect metamorphosis. Holometabolous insects possess two distinct nervous systems at the larval and adult stages. Many neurons are born during larval and pupal stages to function specifically in the larger, more complex adult nervous system. Other neurons are used only in the simpler larval nervous system and die during metamorphosis. But a third class of neurons is morphologically differentiated and likely to function in both the larval and the adult systems; these neurons do so by reorganizing their dendrites and axons during metamorphosis (Truman 1990, Tissot & Stocker 2000). With regard to the scale of pruning, although the absolute length of processes to be pruned is relatively small compared with larger brains of vertebrates, the relative length compared with the size of their neurons or their brains would clearly put these prunings into the large-scale category. Interestingly, at least in one most extensively studied example, the elimination of larval-specific axon branches of MB γ neurons, pruning occurs by a degenerative mechanism (see the section on Mechanisms of Axon Degeneration for more details).

AXON DEGENERATION IN NEUROLOGICAL DISEASES

Axon elimination occurs in many neurological diseases or as a consequence of injury. In many cases it is unclear whether retraction or degeneration is the primary cause of axon elimination, so we follow the terminology in medical literature in which degeneration is more commonly used.

Wallerian Degeneration

Wallerian degeneration (Waller 1850) refers to a series of events that occur in distal axons when they are severed from the cell body. The following reasons highlight the importance of studying Wallerian degeneration: (a) It is a model for spinal cord or other nerve injury; (b) many other forms of axon degeneration in various neurological diseases share similarities with Wallerian degeneration at the final stages; (c) Wallerian degeneration can be studied both in the intact animal and in cultured explant *in vitro*; (d) a mouse mutant that significantly delays Wallerian degeneration has provided an entry point into the mechanisms of axon degeneration (see the section on Mechanisms of Axon Degeneration for more details).

Griffin et al. (1995) provided a detailed description of the events following axon transection. After the axons are transected, there is a variable period of time during which the only visible events are an apparent accumulation of materials at the proximal end of the distal axon stump, presumably owing to a block of retrograde axonal transport. What follows next is a rapid breakdown of the axonal cytoskeleton in an all-or-none fashion with few intermediate stages. Breakdown of neurofilaments via ion-sensitive proteases such as calpain causes cytoskeletal breakdown. Axonal degeneration then triggers a series of responses from surrounding cells, including changes in glial cells surrounding the axons and recruitment of microglia and macrophages to the severed axons, presumably to phagocytose the debris of degenerated axons and myelin (Griffin et al. 1995). This stereotyped sequence of axon degeneration may efficiently remove the damaged tissues, which would facilitate regeneration and repair.

Dying Back Degeneration

A variety of neurological disorders are characterized by initial degeneration of the distal regions of long axons, followed by distal-to-proximal progression, and therefore this

process is termed dying back degeneration (Cavanagh 1964). These disorders include amyotrophic lateral sclerosis (Lou Gehrig's disease), spinal muscular atrophy, spinocerebellar disorders, peripheral neuropathies, nutritional neurological disorders, various intoxications, and AIDS (Cavanagh 1964, Berger & Schaumburg 1995, Raff et al. 2002). The cause and onset of these dying back diseases vary, but the final stage of each disease exhibits axon fragmentation resembling that of Wallerian degeneration. Other diseases described below that exhibit axon atrophies also may share this dying back mechanism, but they have not been studied to sufficient detail.

Wallerian-like degeneration can also be mimicked, at least morphologically, by neurotrophin deprivation. In a classic set of *in vitro* experiments, Campenot (1982) used a culture dish insert with isolated chambers to show that local bath application of nerve growth factor (NGF) to distal portions of axons of sympathetic ganglia was required to maintain the axons. When NGF was withdrawn from the distal chamber, the axons degenerated to their proximal portions that, along with their parent neurons, were maintained by NGF in an environmentally separate chamber.

Neurodegenerative Diseases

Many neurodegenerative diseases including Alzheimer's, Parkinson's, Huntington's, and prion diseases exhibit axonal and dendritic atrophies. The important question is whether these neuritic atrophies precede neuronal degeneration, or whether they are a secondary consequence of neuronal death. As long as axon or dendrite degeneration occurs prior to neuronal death, these neuritic atrophies should contribute significantly to the clinical symptoms whether they are the primary causes of neuronal death. Because it is difficult to determine the sequence of events from postmortem brain, investigators must establish animal models for these diseases. These animal models could then lead to

systematic time-course studies of disease progression with high temporal and anatomical resolution. In the case of Huntington's disease, for instance, Li et al. (2001) showed that, in an animal model, axonal accumulation of the Huntingtin protein, defects of axonal transport, and axonal degeneration all precede neuronal death (Li et al. 2001). *In vivo* imaging also indicated that amyloid deposits in a mouse model of Alzheimer's disease induced breakage of axonal and dendritic branches (Tsai et al. 2004). Delaying or preventing axon degeneration could alleviate the clinical symptoms of some of these diseases (see the section on Mechanisms of Axon Degeneration for more details).

MECHANISMS OF AXON RETRACTION

In principle, axon retraction (as well as axon degeneration, considered in the following section) could be due to (*a*) a direct induction of an intrinsic program for retraction (or degeneration); (*b*) activation of a default retraction (or degeneration) program, owing to its diminished repression; or (*c*) a default, owing to an inadequate maintenance of the axonal cytoskeleton, for example because of diminished trophic support. Compared with axon growth and guidance, relatively little is known about the mechanisms of axon retraction. Because axon retraction is a change of cell shape just as is axon elongation or turning, it is useful to adopt a similar paradigm to consider the process of axon retraction, namely that these changes of cell shapes are based on modifications of the cytoskeleton. These modifications are regulated by signaling pathways that receive input from the extracellular environment or from cell-intrinsic programs that reflect the maturational status of the neurons.

Cytoskeleton

Numerous studies have implicated the integrity of the microtubule cytoskeleton in maintaining the axon stability (e.g., Yamada

et al. 1970). Interestingly, whereas inhibiting microtubule polymerization alone results in axon retraction in cultured neurons, simultaneous inhibition of actin polymerization or deprivation of ATP blocks axon retraction caused by inhibition of microtubule assembly, which suggests that axon retraction is an active process that requires intimate interaction between actin and the microtubule cytoskeleton (Solomon & Magendantz 1981). Recently Ahmad et al. (2000) showed that motor proteins may play important roles in axon retraction. For example, inhibition of microtubule motor dynein caused axon retraction in the presence of intact microtubules; this effect was reversed by depletion of the actin cytoskeleton or inhibition of myosin motors. Thus, myosin-actin and dynein-microtubule cytoskeletons were proposed to provide counter-balance of forces in regulating axon stability.

Eaton et al. (2002) showed that disruption of the dynein/dynactin complex leads to retraction of synaptic terminals in *Drosophila* NMJ in vivo, which indicates that microtubule motors are also essential for stability of presynaptic terminals.

Intracellular Signaling Pathways

Given the importance of cytoskeleton in maintaining axon integrity, signaling pathways that lead to destabilization of microtubules, actin, or interactions between the two cytoskeletal systems could be involved in axon retraction. However, few signaling mechanisms have been investigated thoroughly with the exception of a pathway involving the small GTP-binding protein RhoA.

Activation of RhoA leads to neurite retraction in cultured neuroblastoma cell lines (Jalink et al. 1994, Kozma et al. 1997) or prevention of axon initiation in primary neurons (Bito et al. 2000). Activation of RhoA in maturing neurons in brain slices or in vivo results in retraction of dendritic processes (Li et al. 2000, Nakayama et al. 2000, Wong et al. 2000). Further, activation of RhoA

is also implicated in mediating the activity of myelin-associated inhibitors in blocking axon regeneration (He & Koprivica 2004). RhoA has many downstream effectors. One serine-threonine kinase, Rho kinase (Rok or ROCK), is most critical in RhoA-mediated axon and dendrite retraction because inhibiting this kinase activity completely reverted RhoA-mediated retraction in a number of cases tested in vitro, for example, in cultured hippocampal pyramidal neurons (Nakayama et al. 2000). Many substrates have been identified for Rok, but the most important in the context of axon retraction is the regulation of myosin regulatory light chain phosphorylation (Luo 2002), consistent with the importance of myosin activity in maintaining axon integrity, discussed above.

Does such an axon retraction pathway exist in mature neurons? A genetic study in *Drosophila* MB neurons provided some insight. Inhibition of p190RhoGAP, a negative regulator for RhoA, results in axon retraction of MB axon branches. Genetic analysis indicated that this effect is mediated by RhoA, through activation of Rok and phosphorylation of myosin regulatory light chain (Billuart et al. 2001). These findings suggested that in mature neurons the RhoA-mediated axon retraction pathway is intact but largely repressed by negative regulators such as p190RhoGAP. A recent genome-wide screen of RhoGEFs in *Drosophila* identified two specific RhoGEFs, positive regulators of Rho GTPases, which act to counterbalance p190RhoGAP's effect in regulating axon stability (A. Goldstein, S. Hakeda-Suzuki, A. Maresh, B. Dickson, L. Luo, submitted). Thus, extracellular signals that regulate the activity of these RhoGEFs and RhoGAPs could influence structural plasticity of neurons.

Why should mature neurons maintain an intact axon retraction pathway, only to repress it with negative regulators? One possibility is to use such a pathway for structural remodeling of neurons in response to experience, learning, or injury. For instance, local inactivation of a negative regulator such

as p190RhoGAP could result in removal of specific axonal or dendritic branches. Supporting this structural plasticity hypothesis, p190RhoGAP could be regulated by molecules known to regulate neural plasticity such as integrin or src family tyrosine kinases (Billuart et al. 2001), and regulation of p190RhoGAP and Rok activity has been associated with fear conditioning in mammalian neurons (Lamprecht et al. 2002).

RhoA signaling pathway is most likely one of several signaling pathways whose final readout is destabilization of microtubules or actin or disruption of their interaction, which could lead to axon retraction in mature neurons. Analyses of factors known to regulate microtubule and actin stability could be fruitful entry points for future investigations of signaling pathways that mediate axon retraction. Other potential studies would include testing whether signaling pathways implicated in growth inhibition or repulsion (as in the case of RhoA) play a role in axon retraction in mature neurons.

Extracellular Cues and Their Receptors

Most of the extracellular factors that affect axon behavior are studied in the context of axon guidance, branching, and arborization. Given the potential similarities between repulsive axon guidance and axon retraction, repulsive guidance molecules such as ephrins, Slits, or Semaphorins, all of which signal through RhoA in the context of axon guidance or inhibition of axon growth (Luo 2002, Guan & Rao 2003, He & Koprivica 2004), could also signal axon retraction. Ephrins are strong candidates to promote axon pruning in the context of map refinement, as discussed in a previous section. A recent study also implicates a Semaphorin in regulating axon pruning.

Bagri et al. (2003) found that the stereotypic pruning of a specific component of hippocampal mossy fibers, the infrapyramidal bundle (IPB), is defective in mice mutant for the Semaphorin receptors Plexin-A3 or

Neuropilin-2. Semaphorin 3F is expressed in cells along the path of the IPB when it undergoes pruning, and genetic analysis indicates that Plexin-A3 acts cell autonomously to regulate IPB pruning. IPB pruning appears to occur via axon retraction because no evidence for degeneration was found *in vivo*, and Semaphorins can induce axon shortening and retraction in explant cultures or dissociated neurons in a Plexin A3-dependent manner. Thus, it seems likely that Semaphorins serve as axon retraction signals, and the patterns of Semaphorin expression dictate the extent of axon pruning (Bagri et al. 2003).

MECHANISMS OF AXON DEGENERATION

Axon degeneration, or fragmentation of intact axons into pieces, may involve cell biological mechanisms quite different from axon retraction or any other biological processes. Very little is known about these mechanisms. We focus on two systems for axon degeneration that have given the most insights: developmental axon pruning in insect metamorphosis and Wallerian degeneration of injured axons in mammals. Although these two systems differ in species (invertebrate versus mammal), stage of neuronal maturation, and causes, they exhibit interesting similarities, suggesting the exciting possibility of an evolutionarily conserved common mechanism of axon degeneration.

Degeneration of *Drosophila* Mushroom Body γ Neuron Axons During Development

The γ neurons of the *Drosophila* mushroom body (MB), a structure essential for olfactory learning and memory, form functional circuits in both larvae and adult. During early hours of metamorphosis, γ neurons prune their larval-specific dorsal and medial branches (Lee et al. 1999; see also Technau & Heisenberg 1982). A detailed time course analysis of γ axon pruning at the single-cell level using fluorescence

microscopy revealed that γ axon pruning is achieved via a local degeneration mechanism: Axons appear to break into pieces without obvious distal-to-proximal retraction (Watts et al. 2003) (**Figure 6a**). This degeneration was confirmed by an independent study using a different fluorescent axon marker (Awasaki & Ito 2004) and a genetically encoded EM marker for electron microscopy (Watts et al. 2004). Because of the genetic accessibility, MB γ axon pruning offers a good system to dissect mechanisms of axon degeneration (**Figure 6b**).

MB γ axon pruning requires an intrinsic transcriptional program controlled by the steroid hormone ecdysone. Two complementary experiments concluded that MB γ axon pruning requires an intrinsic transcriptional program controlled by ecdysone. First, single-cell γ neuron clones homozygous mutant for a coreceptor for ecdysone do not prune their axons, despite the fact that all neighboring axons undergo normal pruning (Lee et al. 2000). Second, when the whole brain is mutant for ecdysone receptor (isoform B1), both metamorphosis and γ neuron pruning are arrested. However, if ecdysone receptor is supplied back in γ neurons only, their pruning is restored despite the mutant brain environment (Lee et al. 2000). These experiments demonstrated that γ axon pruning requires a cell-autonomous action of the ecdysone receptor. Recent experiments indicated that ecdysone receptor B1 expression itself is transcriptionally regulated by a TGF- β signaling pathway (Zheng et al. 2003).

MB γ axon pruning requires cell-autonomous action of the ubiquitin-proteasome system (UPS). Watts et al. (2003), who used a gene knock-out in small populations of MB neurons in a wild-type background, concluded that MB γ axon pruning requires cell-autonomous action of the UPS. Remarkably, MB neurons homozygous mutant for E1, the first enzyme in the cascade of the UPS, or either of the

two tested proteasome subunits essential for UPS function, exhibit normal axon growth and guidance in larvae. However, they do not prune their larval-specific axon branches, despite the fact that all neighboring MB axons exhibit normal pruning (**Figure 7b-c**, compared with **Figure 7a**). Dendritic pruning is also blocked by inhibition of UPS (Watts et al. 2003) or ecdysone reception (Lee et al. 2000), suggesting a mechanistic link to axon pruning.

MB γ axon pruning requires glia to engulf degenerating axon fragments. Using a genetically encoded EM marker that allows for the distinction of cellular profiles in electron micrographs, Watts et al. (2004) showed that during early metamorphosis of the fragments of MB γ axons are engulfed by glia for endosome-lysosome-mediated degradation. An interesting question arises as to whether glia simply react to axon fragmentation and serve as scavengers or whether glia actively assist axon fragmentation, perhaps by providing spatial cues that determine which parts of an axon are to degenerate. This question has not been resolved, but some evidence suggests that glial action is not purely passive and that there is extensive interaction between neurons and glia. First, glia cell numbers exhibit a selective increase near degenerating MB axons independent of axon fragmentation (Watts et al. 2004); second, glial infiltration of axon lobes is dependent on an ecdysone-induced signal from γ neurons (Awasaki & Ito 2004); third, transient inhibition of glial function by transiently blocking endocytosis in glial cells arrests axon pruning (Awasaki & Ito 2004). Therefore, glia may actively participate in directing axon pruning.

Mechanisms of Wallerian Degeneration

Lunn et al. (1989) reported a remarkable strain of mice that exhibit much slower Wallerian degeneration when axons are severed. Instead of rapid degeneration in a few days,

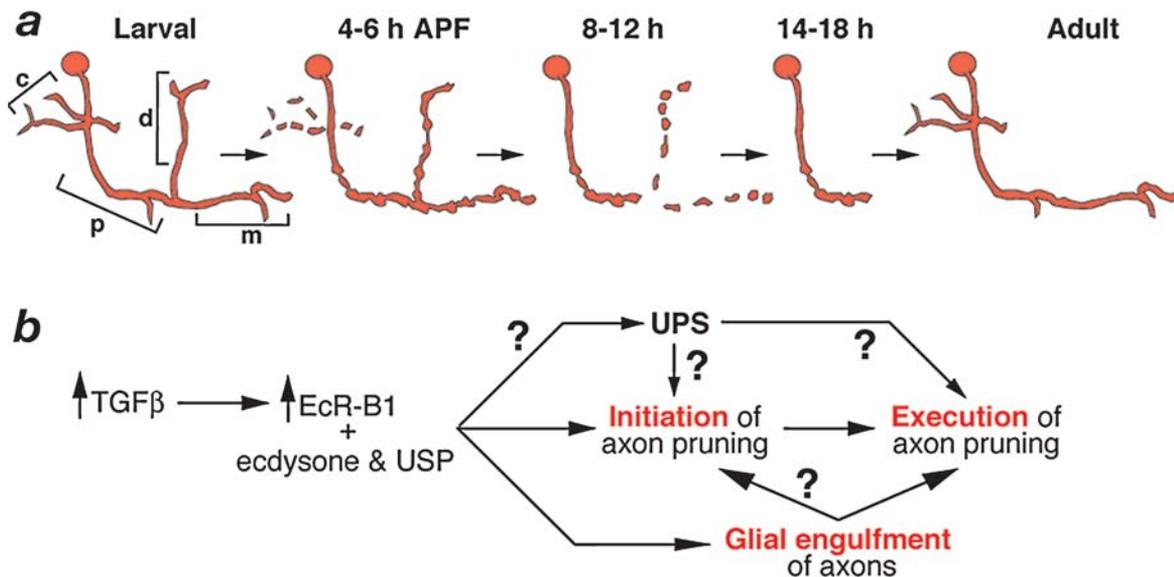


Figure 6

Developmental axon pruning for *Drosophila* mushroom body (MB) γ neurons. (a) Schematic illustration of developmental axon pruning of MB γ neurons. Larval γ neurons consist of dendrites near the cell body in the calyx (c) and axons that extend down the peduncle (p) and branch to form the dorsal (d) and medial (m) axon lobes. Four to six hours after puparium formation (APF), dendrites begin to fragment and axons begin to swell. From 8 to 12 h APF, axons in dorsal and medial lobes undergo fragmentation, whereas dendrite fragments disappear. The remaining axon fragments disappear from 14 to 18 h APF. After 18 h APF, axons re-extend into only the medial lobe. Modified from Watts et al. (2004). (b) A model for molecular pathways involved in MB γ axon pruning. TGF β /Activin signaling is required for the γ neuron-specific expression of ecdysone steroid hormone receptor (EcRB1), which acts as a heterodimer with its coreceptor Ultraspiracle (USP) to initiate a gene expression program for axon pruning in response to ecdysone signaling. Axon pruning requires cell-autonomous action of the ubiquitin proteasome system (UPS) to initiate axon pruning by degrading key regulators of axon pruning and/or to execute the pruning process by degrading essential structural components. In addition to this intrinsic program of axon pruning, glial cells are specifically enriched at axon lobes where they engulf degenerating axon fragments. Question marks denote the links that remain hypothetical.

axons from *Wld^s* (Wallerian degeneration slow) mice survived for 1–2 weeks. This remarkable finding opened doors for mechanistic studies of Wallerian degeneration.

Wallerian degeneration is an active process of axon self-destruction. The notion that distal portions of the transected axons die passively for lack of nutritional support from the soma was shattered by the discovery of *Wld^s* mice, which show that distal axons can survive for a long time after transection. Distal axons degenerate soon after severing most

likely because a degeneration program is triggered and actively executed. The availability of *Wld^s* mice also allowed transplantation experiments showing that severed axons of *Wld^s* genotype surrounded by wild type cells live as long as those in *Wld^s* mice. These findings demonstrate that the neuro-protective effect of *Wld^s* is autonomous to neurons (Glass et al. 1993).

Wld^s acts by regulating NAD metabolism to protect axons. *Wld^s* acts as a dominant mutation and was found to be caused by a

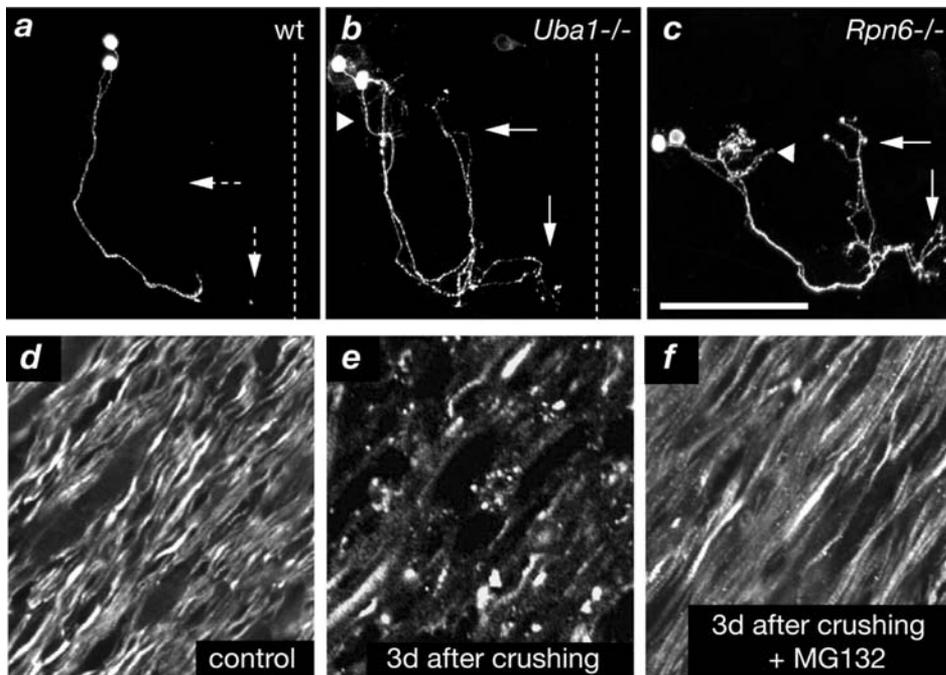


Figure 7

Comparing *Drosophila* MB γ axon pruning and Wallerian degeneration. (*a-c*) At 18 h APF, wild-type γ neurons have pruned their larval specific branches (*a*), but MB γ neurons homozygous mutant for a ubiquitin-activating enzyme *Uba1* (*b*), or a proteasome subunit *Rpn6* (*c*), fail to prune their larval-specific axons, which demonstrates that the ubiquitin proteasome system is cell-autonomously required for MB γ axon pruning. Labeled wild-type or homozygous mutant *Uba1* and *Rpn6* clones were generated by the MARCM (mosaic analysis with a repressible cell marker) technique (Lee & Luo 1999). (*d-f*) Three days after crushing optic nerve in rats, distal axons of retinal ganglion cells undergo characteristic Wallerian degeneration as indicated by broken axon fragments (*e*), compared with uncrushed control (*d*). In the presence of proteasome inhibitor MG132, Wallerian degeneration is significantly delayed (*f*). Axons are stained with anti-tubulin antibody. Panels *a-c* are from Watts et al. (2003), and panels *d-f* are from Zhai et al. (2003).

triplication resulting in the generation of a fusion protein consisting of the first 70 amino acids of UFD2/E4, an evolutionarily conserved protein used in protein polyubiquitination, and the full-length nicotinamide mononucleotide adenylyltransferase (Nmnat), an enzyme involved in NAD metabolism (Conforti et al. 2000). Transgenic expression of *Wld^s* protects distal axon degeneration after severing in a dose-dependent fashion (Mack et al. 2001). Because the first 70 amino acids of UFD2 do not contain the enzymatic domain for polyubiquitination, investigators proposed that *Wld^s* protects axons by either

increasing the activity of Nmnat or by serving as a dominant negative protein that interferes with UFD2 and thereby the ubiquitin-proteasome system (Coleman & Perry 2002, Mack et al. 2001).

A recent report determined that the Nmnat portion of the *Wld^s* fusion protein has axon-protective activity. Using dorsal root ganglia explants as an in vitro model for Wallerian degeneration, Araki et al. (2004) found that expression of *Wld^s* and full-length Nmnat conferred axon-protective activity; expression of the first 70 amino acids or a dominant negative form of UFD2, or Nmnat with

a point mutation in the catalytic domain, did not have an axon protection effect. These findings indicate the importance of a NAD-dependent process in axon protection. Curiously, although overexpression of *Nmnat* did not increase NAD level in the cells, incubating neurons with NAD 24 h prior to severing their axons (but not after the severing) also has the axon-protection effect. These findings, coupled with the predominant nuclear localization of *Wld^s*, suggested that *Nmnat* exerts its neuro-protective function indirectly in the nucleus by regulating gene expression, likely mediated by a Sirt1-dependent chromatin deacytlation, a known effector for NAD metabolism in regulating cellular aging (Araki et al. 2004).

Wallerian degeneration involves regulation of the ubiquitin-proteasome system.

The fact that *Wld^s* fusion protein does not protect axons through interfering with UPS does not exclude involvement of UPS in Wallerian degeneration. In fact inhibition of UPS through proteasome inhibitors, or expression of a deubiquitination enzyme, significantly delayed axon degeneration in an in vitro model of Wallerian degeneration. Application of proteasome inhibitors also delayed the in vivo Wallerian degeneration of the optic nerve after severing (**Figure 7d-f**). Interestingly, to have the protective effector, proteasome inhibitors must be applied to neural explants 1–3 h prior to severing the axons, which suggests that UPS regulates an early step of Wallerian degeneration (Zhai et al. 2003).

Similarities Between MB γ Axon Pruning and Wallerian Degeneration

Cell-autonomous programs for axon self-destruction. As summarized above, both MB γ axon pruning and Wallerian degeneration appear to invoke cell-autonomous action of a genetic program. Available evidence from other systems also supports this notion. For instance, during developmental pruning of layer 5 subcortical projections (see Large-

Scale Developmental Pruning of Axonal Connections), *Otx1* appears to induce transcription of an essential component of the axon pruning machinery or downregulate an inhibitor of axon pruning. In addition, extensive work from *Manduca* and *Drosophila* has shown that the insect metamorphosis hormone ecdysone plays a key role in regulating neuronal remodeling, both pruning of larval-specific axonal and dendritic branches and likely re-extension of adult-specific branches (Levine et al. 1995). Dendritic pruning of peptidergic interneurons also requires cell autonomous action of ecdysone receptors (Schubiger et al. 1998, 2003). Even synaptic partners of a circuit independently require cell-autonomous action of ecdysone receptors to prune their axonal and dendritic branches (Marin et al. 2005).

Microtubule breakdown in early stages of axon degeneration. Systematic search for the sequence of cellular events identified degradation of the microtubule cytoskeleton as the earliest sign of MB axon pruning (Watts et al. 2003). In the in vitro explant model of Wallerian degeneration, breaks in the microtubule cytoskeleton were also identified as the earliest sign (Zhai et al. 2003), preceding the previous earliest sign of neurofilament breakdown. In neither case has a causal relationship between microtubule breakdown and axon degeneration been established. Given the importance of the microtubule cytoskeleton in maintaining axon integrity, this will be an important avenue for future research.

Caspase-independent processes. The self-destructive nature of axon degeneration raises an intriguing possibility that axon degeneration may share mechanisms similar to apoptosis. Caspase activation is a hallmark of apoptosis. However, inhibition of caspase activity using a variety of inhibitors did not prevent or delay distal axon degeneration after transection, even though proximal cell body apoptosis was prevented in the same experiment (Finn et al. 2000). Likewise, deletion of

apoptotic activators or expression of a caspase inhibitor in *Drosophila* MB neurons had no effect on axon pruning (Watts et al. 2003).

Regulation by the ubiquitin-proteasome system. Genetic analysis demonstrated that UPS is cell-autonomously required for MB γ axon pruning and suggested that it may be involved in the initiation of the axon degeneration program by removing one or more negative regulators (Watts et al. 2003). This finding is consistent with an early involvement of UPS in Wallerian degeneration (Zhai et al. 2003). UPS is also critical in structural modification of synapses (DiAntonio & Hicke 2004, Steward & Schuman 2003). If indeed there is a conserved neuro-protective protein that needs to be degraded for the initiation of axon degeneration program, identifying the specific E3 ligase(s) and the neuro-protective substrate(s) will be a major step forward in our understanding of the axon degeneration program and will perhaps offer insights into possible therapies for neurodegenerative diseases.

Although we have discussed the remarkable similarities between certain forms of developmental axon pruning and Wallerian degeneration in both phenomenology and potential mechanisms, a word of caution should be added that they should not be regarded as identical. After all, developmental axon pruning and Wallerian degeneration are triggered by very different events.

Many Degeneration Diseases May Share Similarities with Wallerian Degeneration

The clues from studies of Wallerian degeneration allow researchers to test whether the mechanisms involved in Wallerian degeneration apply to neurodegeneration of other causes. For instance, just like delay of Wallerian degeneration, UPS inhibition could also delay degeneration *in vitro* owing to neurotrophin deprivation (Zhai et al. 2003). Among the most remarkable findings in recent years are those that show the power of

Wld^s in protecting axon degeneration arising from different causes.

Mice homozygous for a naturally occurring recessive mutation *progressive motor neuropathy (pmm)* exhibit muscle weakness, motor axon degeneration in a “dying back” mode, and eventual organismal death by six weeks after birth (Schmalbruch et al. 1991). The cause of the disease is a point mutation in the *tubulin specific chaperone E (Tbce)* locus that encodes a protein essential for folding of α and β tubulin dimers (Bommel et al. 2002, Martin et al. 2002). The symptoms and pathology of *pmm* mice mimic severe forms of human spinal muscular atrophy (SMA), so *pmm* mice have been used as an animal model for studying SMA and other motor neuron diseases. Early work showed that Bcl-2 overexpression or glia-derived neurotrophic factor (GDNF) treatment of *pmm* mice could prevent or delay motor neuron death but could not prevent axon degeneration and did not increase life span (Sagot et al. 1995, Sagot et al. 1996). These studies suggest that axon degeneration, rather than motor neuron death, is the primary cause of the disease. Remarkably, crossing in the *Wld^s* mutation into *pmm/pmm* mice not only delayed axon degeneration and rescued motor neuron death, but also attenuated the symptoms and significantly increased the lifespan of *pmm/pmm* mice (Ferri et al. 2003). This finding suggested the existence of mechanistic similarities between Wallerian degeneration and dying back diseases and demonstrated the utility of inhibition of axon degeneration in treating motor neuron diseases.

The protective effect of *Wld^s* has been demonstrated also in two other cases. Axon atrophy contributes to clinical symptoms of demyelination diseases such as multiple sclerosis (Bjartmar et al. 2003). Mice homozygous mutant for myelin basic protein P0 exhibit myelin-related axon loss. Introduction of *Wld^s* into homozygous P0 mutants reduced axon loss and increased motor neuron survival and function at least at early ages (Samsam et al. 2003). The neuroprotective effect of

dLGN: dorsal lateral geniculate nucleus

IPB: infrapyramidal bundle

MB: mushroom body

nAChRs: nicotinic acetylcholine receptors

NGF: nerve growth factor

pmm: *progressive motor neuropathy*

RGC: retinal ganglion cell

UPS: ubiquitin-proteasome system

Wld^s: Wallerian degeneration slow

Wld^s was examined also in an animal model of Parkinson's disease (Sajadi et al. 2004). Stereotaxic injection of 6-hydroxydopamine (6-OHDA), a catecholaminergic neurotoxin, into the nigrostriatal pathway of wild-type mice results in degeneration of dopaminergic axons and death of dopaminergic neurons. A strong protective effect for dopaminergic axons and their function was seen when similar injections were performed in Wld^s mice. However, this protective effect was limited to injections of distal but not proximal nigrostriatal pathways, and dopaminergic neuron death was not protected. These results suggested that axon segments distal to the toxin injection site may undergo degeneration similar to Wallerian degeneration, which can be protected by Wld^s (Sajadi et al. 2004).

Taken together, these recent studies on animal models suggested that protection of axon degeneration could be an important therapeutic strategy for neurological diseases (Coleman & Perry 2002, Raff et al. 2002). The fact that a delay of axon degeneration can at least alleviate symptoms of motor neuron disease, Parkinson's disease, or myelin disease suggests that axon degeneration is a critical part of their pathogenesis. Finally, these studies support the notion that a common axon degeneration pathway, akin to Wallerian degeneration, is shared in diseases of many different origins.

Potential Connection with Axonal Transport

Defects in axonal transport could be causes of many neurological disorders including Alzheimer's, Huntington's, and motor neuron diseases (Gunawardena & Goldstein 2004). Long-term transport blockade of nutrients, survival factors, or any other proteins essential for neuronal function should lead to neuronal dysfunction and degeneration. Axon transection can be viewed as an extreme case of axonal transport block, and therefore

we speculate that the program for axon self-destruction in Wallerian degeneration may contribute also to degeneration owing to block of axonal transport. Indeed, disruption of the microtubule cytoskeleton appears to be the earliest sign of degeneration, as discussed before, which could further exacerbate the defects in axonal transport. On the other hand, a transport defect could lead to a lack of receiving survival factors that could trigger both developmental axon pruning and dying back diseases.

SUMMARY AND PERSPECTIVE

Pruning of exuberant neuronal connections is a fundamental and widespread mechanism to develop the diversity and specificity evident in axonal connections of the adult vertebrate brain. The phenomenon of developing exuberant axonal projections followed by selective pruning could provide a substrate for plasticity during both development and evolution of the nervous system. Alterations in pruning are a likely source of functional sparing or recovery following insults during development and may contribute to speciation. Large-scale reorganizations of neural circuits through pruning also occur in invertebrates, the best example of which is during metamorphosis of holometabolous insects. Although we have focused on pruning of axons, dendritic pruning is equally prevalent, which also contributes indirectly to synapse or axon elimination.

Developmental axon pruning occurs through retraction, degeneration, or a combination of both processes. Both retraction and degeneration appear to involve cell-intrinsic programs that execute these events. These axon destruction programs likely persist in adult neurons. The existence of these axon-destruction programs in developing and mature neurons requires tight regulation of these programs to maintain the stability of neuronal connections. We speculate that the function of these

axon-destruction programs in the adult is to enable neurons to rapidly change their connections in response to learning, experience, or injury.

Axon degeneration and neuritic atrophies also occur widely in neurological and neurodegenerative diseases, and are the primary causes for some diseases. Protecting axon degeneration could be useful to ameliorate clinical symptoms. Axon degeneration in diseases may utilize mechanisms similar to those used during developmental axon pruning. We speculate that mis-regulation of axon-destruction programs could contribute significantly to the pathogenesis of certain neurological diseases, and interference of the axon self-destruction program may be a fruitful therapeutic approach.

We are only beginning to explore the mechanisms of axon degeneration and retraction. Given their relevance to wiring, plasticity, repair, and dysfunction of the nervous system, we feel that relatively little is known about the molecular and cellular mechanisms that govern these fundamental events, particularly during development. We

hope that our review will inspire talented young scientists to work on these fascinating problems.

NOTE ADDED IN PROOF

A recent technically elegant study of axon elimination at the developing NMJ has revealed that as motor axons retract they shed membrane-enclosed axosomes, which are engulfed by surrounding Schwann cells, leading to a mixing of axonal and glial cytoplasm (Bishop et al. 2004). This mechanism is different from the previous view that axon retraction from a NMJ occurs by a progressive distal to proximal “resorption” of the retracting axon (Bernstein & Lichtman 1999, Walsh & Lichtman 2003) and is also distinct from a classical Wallerian degeneration, where no proximal to distal retraction is evident (see text) but shares some features of both. Whether this provocative finding proves to be unique for synaptic elimination at NMJ, and whether branch retraction occurring in the CNS is also accompanied by such resorption, remains to be determined.

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