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Axon pruning: an essential step underlying the developmental plasticity of neuronal connections

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Regressive events play a key role in modifying neural connectivity in early development. An important regressive event is the pruning of neuronal processes. Pruning is a strategy often used to selectively remove exuberant neuronal branches and connections in the immature nervous system to ensure the proper formation of functional circuitry. In the following review, we discuss our present understanding of the cellular and molecular mechanisms that regulate the pruning of axons during neuronal development as well as in neurological diseases. The evidence suggests that there are several similarities between the mechanisms that are involved in developmental axon pruning and axon elimination in disease. In summary, these findings provide researchers with a unique perspective on how developmental plasticity is achieved and how to develop strategies to treat complex neurological diseases.

Keywords: axon pruning; axon degeneration; axon retraction; Wallerian degeneration

1. INTRODUCTION: REGRESSIVE EVENTS DURING NEUROGENESIS

The progressive growth of the vertebrate nervous system during development results in an overabundance of connections, which are removed later to ensure that a functional organization of circuitry is established. Within the past 30 years, it has become clear that developing vertebrate nervous systems use several diverse mechanisms to remove or modify the exuberant connections (Cowan *et al.* 1984; Luo & O'Leary 2005), an event often referred to as developmental brain plasticity. Programmed cell death (or apoptosis), synapse disassembly and process elimination have become synonymous with phenomena that are associated with this regressive phase of nervous system development (Purves & Lichtman 1980; Cowan *et al.* 1984; Burek & Oppenheim 1996; Lichtman & Colman 2000; Meier *et al.* 2000; Raff *et al.* 2002; Yuan *et al.* 2003; Luo & O'Leary 2005).

It is well known that approximately 50% of the postmitotic neurons during development do not survive till adulthood. Many of these neurons die by a natural process of programmed cell death, which results in the loss of the cell and all its synapses and processes (Burek & Oppenheim 1996; Yuan *et al.* 2003). Programmed cell death has been observed in almost every part of the developing nervous system and attributed to the removal of a large percentage of the excess projections seen in early development (Cowan *et al.* 1984; Yuan *et al.* 2003). The dying cells and their processes are subsequently removed by nearby glial cells. What is the cause for the increase in cell death in early development? Previous studies have established a temporal correlation between programmed cell death and the progressive formation of synapses in early development (Oppenheim 1981). These findings, together with results demonstrating that a number of surviving cells could be affected by altering the size of their potential synaptic targets, led to the idea that cells were competing for a factor that was released in limited amounts from the target region (Oppenheim 1981). This ultimately resulted in the discovery of the nerve growth factor (NGF) and several related growth factors, neurotrophins, which are necessary for neuronal survival during development (Levi-Montalcini 1987; Oppenheim 1989). Additionally, *in vitro* studies have shown that neurotrophins are required not only for cell viability, but also they contribute to the growth and maintenance of neuronal processes, which might be required for ensuring synapse formation (Campenot 1982). In the presence of NGF in culture, stimulated developing neuronal processes are given a competitive growth advantage over unstimulated competing processes (Singh & Miller 2005). Presumably, unstimulated processes are more sensitive to growth inhibitors in the surrounding environment. Although neurotrophins are necessary for the growth and sustenance of a developing neuron, it is still unclear whether such molecules actively sustain synapse formation, or on the contrary, whether neurotrophins can prevent synaptic and process elimination events from occurring.

The disassembly of synapses is an alternative strategy to programmed cell death that neurons use to adjust the number of connections they make with postsynaptic targets. Unlike programmed cell death, synapse disassembly is the removal of only a small subpopulation of synaptic connections (Lichtman & Colman 2000; Eaton & Davis 2003; Goda & Davis 2003). Most of the facts known about how synapses dissemble have been attributed to studies on synapse disassembly at the neuromuscular junction (NMJ; Lichtman & Balice-Gordon 1990; Sanes & Lichtman 1999; Lichtman & Colman 2000). The process is extremely dynamic in nature. Synapses that appear to be regressing in size can

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alter their synaptic area to take over a competing input at the same target (Walsh & Lichtman 2003). Furthermore, inputs that are relatively stronger than their neighbouring competitors tend to maintain their innervation at the synaptic target while competing inputs withdraw their synapses (Colman *et al.* 1997; Gan & Lichtman 1998; Buffelli *et al.* 2003). The weaker inputs have been shown not only to disassemble their synapses, but also to withdraw their short arbors as well. This fine-tuning of circuitry is common to almost every region of the developing nervous system.

Process elimination is a second alternative strategy to cell death, which is used by nervous systems during early development to remove exuberant or misguided connections. Process elimination can occur in different levels and its association with synapse disassembly is not mutually exclusive, as in some cases, process elimination might occur in the absence of synapse formation (Cowan *et al.* 1984; Luo & O'Leary 2005). The elimination of neuronal processes during regressive phases of development range from the small-scale pruning of dendrites, as observed in the neocortex (Yuste & Bonhoeffer 2001; Bonhoeffer & Yuste 2002; Segal 2002; Ethell & Pasquale 2005) and the fine-tuning of subsets of axon terminal arbors at the NMJ (Sanes & Lichtman 1999; Lichtman & Colman 2000), to the large-scale pruning of long axon collaterals of layer V cortical projections, which might extend up to millimetres in length (O'Leary 1987; O'Leary *et al.* 1990; Luo & O'Leary 2005). How are the processes removed during development? Recent evidence suggests that several different cellular mechanisms from retraction to degeneration might be involved in regulating developmental process elimination—the mechanism that is chosen seems to vary from one region of the nervous system to another and across various species (Luo & O'Leary 2005). Our aim is to highlight some of the cellular and molecular mechanisms that regulate process elimination in the developing vertebrate nervous system.

2. AXON PRUNING: THE REMODELLING OF AXONS DURING NEUROGENESIS

During development, neurons extend axons to more targets than are required for normal function in adulthood. Several long axon collaterals often branch off from the primary projection to seek target areas that are separated by long distances and which contain distinct cell populations (Luo & O'Leary 2005). At the axon terminal, numerous short axonal arbors might seek multiple cells within the same target area (Nguyen & Lichtman 1996; Sanes & Lichtman 1999). The fine-tuning of short axonal arbours and elimination of long axon collaterals both fall within the context of developmental axon pruning. Advances in analytical techniques in the past decade have uncovered some of the molecular and cellular processes that regulate axon pruning—the recent evidence suggests that several similarities exist between the mechanisms that control the removal of small terminal arbors and those that control the elimination of long axon collaterals (Luo & O'Leary 2005).

(a) *Insights into the molecular mechanisms of axon pruning from studies in* Drosophila

Identifying the molecular pathways which are involved in process elimination in the vertebrate nervous system is difficult. However, the refinement and the remodelling of projections are also crucial features in the development of many simpler organisms. For example, in *Drosophila*, modern technical advances in gene manipulation and imaging approaches have revealed some of the molecular pathways that regulate the pruning of both long and short projections (Lee *et al.* 2000*a*,*b*; Watts *et al.* 2003; Marin *et al.* 2005).

Extensive remodelling of nervous system structures and their connections is a characteristic feature of insect metamorphosis. The *Drosophila* mushroom body (MB), a brain structure implicated in learning and memory, is an area where axonal and dendritic processes undergo considerable remodelling (Lee *et al.* 2000*a*,*b*; Watts *et al.* 2003; Awasaki & Ito 2004). The MB is organized into two distinct populations of neurons, the γ- and α′/β′-MB neurons. Pruning during metamorphosis is selective for γ-MB neurons only. Before the mid-third instar stage of development, γ-MB neurons have extended dendrites near the cell body in the calyx and an axon that bifurcates dorsally and medially at the axon peduncle. During metamorphosis, the dendrites and dorsal and medial segments of the axon are pruned away by a degenerative mechanism (described in detail in §2*b*; Awasaki & Ito 2004; Watts *et al.* 2004). When the γ-MB neurons mature, only the dendrites and medial segments re-extend their processes (Lee *et al.* 2000*a*,*b*; Watts *et al.* 2003).

Studies on MB remodelling have provided important clues about molecular pathways involved in pruning. MB pruning is tightly coupled to the activation of the ecdysone hormone receptor. Loss of function analyses in flies deficient in the ecdysone receptor exhibit profound pruning defects (Lee *et al.* 2000*a*,*b*). Recent studies have also implicated transforming growth factor β (TGF-β) signalling as an important regulator of ecdysone receptor expression (Zheng *et al.* 2003). Unfortunately, it is not known how TGF-β signalling, or for that matter how signalling further downstream from the ecdysone receptor, affects the cytoskeleton of γ-MB axons during pruning.

When axons degenerate in the *Drosophila* MB, there is a sequence of elimination that begins with microtubule and neurofilament degradation, synapse disassembly and the break-up of the axonal membrane into fragments (Awasaki & Ito 2004; Watts *et al.* 2004). Presumably, these events are associated with an elaborate degree of protein turnover (Watts *et al.* 2003). In eukaryotic cells, proteins are disassembled and degraded by the ubiquitin proteosome system (UPS; Voges *et al.* 1999). Protein substrates that are polyubiquitinated by ubiquitin ligases are targeted for destruction by the UPS. Recently, this pathway has been found to play a critical role in regulating stereotyped axon pruning in the *Drosophila* MB (Watts *et al.* 2003). Separate loss of function mutations for molecules that function in polyubiquitination such as the ubiquitin-activating enzyme, *Uba*1, and in subunits for the 19S proteosome regulatory particle,

*Mov*34 and *Rpn*6, all result in defects in γ-MB stereotyped axon pruning (Watts *et al.* 2003). Overexpression of a deubiquitinating enzyme, UBP2, in γ-MB neurons, which led to similar pruning defects, provided additional support that protein degradation was necessary for proper γ-MB stereotyped axon pruning (Watts *et al.* 2003). Since γ-MB stereotyped axon pruning in *Drosophila* is accompanied by glial cell engulfment of axon material, it is interesting to note that molecules which have been linked to programmed cell death pathways do not seem to participate in degenerative modes of pruning. Accordingly, mutations for mediators of apoptosis, such as *grim*, *hid* or *rpr*, did not produce a pruning defect (Watts *et al.* 2003).

Potential substrates for the ubiquitin proteosome pathway in stereotyped pruning might include molecules involved in branch retraction or extension. The Rho family of small GTPases are attractive candidate molecules because they have been shown to be important regulators of cytoskeletal dynamics (Luo 2000; Billuart *et al.* 2001; Ng *et al.* 2002; Govek *et al.* 2005). Some studies have even shown that Rho GTPases are necessary for the stabilization of dendrites and spines in mature neurons. However, since the numbers of known Rho GTPases are fewer than the molecules that regulate their activity, recent findings have suggested that the regulatory molecules might play a more significant role in determining changes at the cytoskeleton, which ultimately can influence the morphology of developing processes (Luo 2000; Govek *et al.* 2005).

The family of guanine nucleotide exchange factors (GEFs) and GTPase-activating proteins (GAPs) regulate Rho GTPase activity positively and negatively, respectively (Luo 2000; Govek *et al.* 2005). Ideally, it has been hypothesized that regulating the balance between Rac versus Rho GTPase activity may decide whether branch-retraction or -stabilization occurs. In support of this idea, p190RhoGAP is a GAP that has been shown to negatively regulate RhoA as a requirement for branch stabilization in α′/β′-MB neurons (Billuart *et al.* 2001). Activation of RhoA and its effector kinase Drok, and a key output for *Drok*, the regulatory phosphorylation of the myosin regulatory light chain, unveiled a pathway that induces branch elimination via regulation of myosin II activity (Billuart *et al.* 2001). The pathway affects branch stability in α′/β′ only and not in γ-MB neurons; hence it may be more important in other remodelling events in development. In contrast, γ-MB neurons are the only population to express Trio, a RacGEF, during peak stages of metamorphosis (Awasaki *et al.* 2000; Bateman *et al.* 2000). Targeted *DRacGAP* loss of function, which tips the balance in favour of activating a Rac GTPase in γ-MB neurons presumably by Trio, results in overextension of dorsal branches which is opposite to the phenotype observed by knocking out p190RhoGAP (Awasaki *et al.* 2000).

However, not all remodelling events in *Drosophila* are associated with hormone-dependent events and axon degeneration. At the *Drosophila* NMJ, a rapid disassembly of synapses and the retraction of axonal arbors occur concurrently with a proliferative phase of synaptic growth (Eaton *et al.* 2002; Hebbar & Fernandes 2004). Eaton and colleagues developed an elegant assay to detect the small-scale pruning events. During synapse formation at the NMJ, the pre- and postsynaptic regions of the synapse can be positively identified by the co-localization of pre- and postsynaptic markers, synapsin and discs-large (Dlg, a PSD-95 like protein), respectively, at opposing areas of the synapse (Eaton *et al.* 2002). Since it takes days to assemble the postsynaptic component at the NMJ, the authors hypothesized that this portion of the synapse would take longer to disassemble compared to presynaptic machinery. Remarkably, the authors were able to detect 'synaptic footprint' scenarios in roughly 20% of cases at the NMJ, where positive postsynaptic labelling with Dlg was no longer directly opposed to the presynaptic labelling of synapsin—this suggested that the presynaptic terminal had withdrawn from the synapse (Eaton *et al.* 2002). Moreover, synapse disassembly occurred only near specific areas, suggesting that the process of synapse and branch elimination was locally restricted. In addition, the authors found ultrastructural evidence demonstrating that the disassembly of synapses and their axon branches at the NMJ of *Drosophila* proceeds by retraction (Eaton *et al.* 2002). The results implied at the least that the disassembly of the synapse occurs via the following sequence of organized events—the break-up of the cytoskeleton, the loss of presynaptic release machinery and the eventual retraction of the axon arbor precede the removal of the postsynaptic scaffolding.

Importantly, synapse disassembly at the *Drosophila* NMJ was not directly correlated with hormone signalling events (Eaton *et al.* 2002; Eaton & Davis 2003). In fact, it was found that other molecules that regulate cytoskeletal dynamics were crucial for ensuring proper synapse disassembly. In their genetic screen, Eaton *et al.* (2002) discovered that intact dynactin function was required for synapse stability. Recent investigations on the same system have revealed an importance of the actin cross-linking protein, spectrin, in stabilizing synapses at the NMJ (Pielage *et al.* 2005). Spectrin acts presynaptically and was required for the normal organization of cell-adhesion molecules, neuroglian and fasciclin II, at the plasma membrane. The loss of spectrin presynaptically results in microtubule retraction, disorganization of the membrane-associated cell-adhesion molecules and the eventual disassembly of the NMJ (Pielage *et al.* 2005).

Synapse growth and remodelling are believed to be coordinated together and are influenced to some degree by the expression of trophic factors at the synapse. At the *Drosophila* NMJ, the canonical bone morphogenic protein (BMP)-signalling system regulates synapse growth and stability. Recently, Eaton & Davis (2005) have discovered that BMP can activate two parallel presynaptic signalling pathways at the NMJ. Downstream activation of the Smad signalling cascade regulates growth and development of the synapse, whereas LIM kinase I signalling acts parallel to Smad signalling to stabilize synapses. This conclusion was supported by observations that overexpression of LIM kinase I completely restores synaptic stability in the absence of Smad signalling and that LIM kinase I binds separately to a region of the BMP receptor, *wishful*

thinking, that is not required for Smad-mediated synaptic growth (Eaton & Davis 2005).

Recently, a small-scale pruning event has been discovered among the neurites of developing AIM interneurons of *Caenorhabditis elegans*. The pruning of excessive AIM neurites occurs presumably by retraction and is regulated by a novel transcription factor, MBR-1 (Kage *et al.* 2005). It will be interesting to see whether the future genetic screens on this system can identify additional molecules that regulate neurite pruning.

(b) *Insights into the cellular mechanisms of axon pruning from studies in* Drosophila

The transition from larva to adult stage during metamorphosis in *Drosophila* is characterized by considerable structural reorganization of areas involved in learning and memory. The *Drosophila* MB (as described earlier) is one region where large-scale remodelling of axons occurs. Interestingly, during metamorphosis, the γ-MB neurons undergo a stereotypical process of axon elimination that is characterized by fragmentation of the dorsal and medial segments of its axon (Watts *et al.* 2003; Watts *et al.* 2004). Ultrastructural analysis of fragmenting axons revealed that the γ-MB axon segments were indeed degenerating. The earliest evidence for γ-MB pruning is the dissolution of the microtubules followed by the break-up of neurofilaments (Watts *et al.* 2003, 2004). Coincidentally, a similar sequence of events is observed during early signs of axonal degeneration in vertebrates (Zhai *et al.* 2003).

Glial cells are important mediators of cell-proliferative events and cell-wide regression phenomena (Barres 1999; Ullian *et al.* 2004; Mallat *et al.* 2005). They also play a significant role in the stereotyped removal of axons during development in the *Drosophila* MB (Awasaki & Ito 2004; Watts *et al.* 2004). Glia processes are prominent near γ-MB degenerating axons and in many circumstances glia have been observed to engulf the degenerating axon fragments (Awasaki & Ito 2004; Watts *et al.* 2004). Whether glia act as active mediators of axon degeneration or passively engulf the leftover axonal debris, remains unresolved. However, some evidence strongly suggests that without glial cells, γ-MB axon pruning is markedly inhibited. Glia numbers increase before γ-MB axons degenerate and their migration towards γ-MB axons is dependent on ecdysone expression (Awasaki & Ito 2004; Watts *et al.* 2004). Furthermore, genetically suppressing glia activity also prevents γ-MB axon pruning (Awasaki & Ito 2004; Watts *et al.* 2004).

The degenerative pruning of processes in *Drosophila* is not limited to long axonal processes (Lee *et al.* 2000*a*,*b*). Dendritic remodelling is also characteristic to γ-MB neurons during metamorphosis. γ-MB dendrites lose their small arbors in early development, but regrow them after metamorphosis. It has been shown that these dendritic arbors undergo considerable fragmentation, a process that is also dependent on ecdysone receptor signalling (Lee *et al.* 2000*a*,*b*; Watts *et al.* 2003). Similar results have also been reported for the pruning of dendritic arbors of *Drosophila* sensory neurons during metamorphosis (Kuo *et al.* 2005; Williams & Truman 2005*a*). During pruning, branch retraction and degeneration can occur simultaneously at different areas of the dendrite. Interestingly, the degeneration of dendritic arbors in sensory neurons is regulated by the metamorphosis-associated hormone ecdysone, while branch retraction is not (Williams & Truman 2005*a*,*b*).

Although we have placed an emphasis on degenerative mechanisms for pruning of some large- and small-scale remodelling events in *Drosophila*, process elimination by retraction seems to be an alternative mechanism that has been conserved for some small-scale pruning events. At the NMJ, Eaton *et al.* have reported a situation where the pruning of small motor axon arbors proceeds by retraction (Eaton *et al.* 2002). Since pruning at the *Drosophila* NMJ is a simultaneous event involving synapse proliferation and synaptic regression (Eaton *et al.* 2002; Hebbar & Fernandes 2004), it seems less likely to be regulated by hormonal signalling events. Furthermore, unlike γ-MB pruning, where the fragmentation of the axon occurs without a directional preference, motor axon retraction at the *Drosophila* NMJ begins with the sequential disassembly of the presynaptic components of the axon prior to axon withdrawal.

(c) *Cellular and molecular mechanisms of axon pruning in the vertebrate nervous system*

Originally, it was believed that axon pruning could occur through one of two mechanisms. Axon branches could just retract in a distal to proximal fashion, as axonal contents are recycled to other parts of the axon; this retraction hypothesis was initially proposed based on the evidence that motor axons did not degenerate during periods of widespread synapse disassembly (Riley 1981). However, as in most documented cases of axon pruning in the vertebrate nervous system, most studies cite retraction in situations where degeneration is not observed. Unfortunately, a detailed analysis and understanding of axons that retract *per se* has not been determined. Alternatively, axon branches might be pruned by local axon degeneration. In support of this hypothesis, denervation studies at the vertebrate NMJ have shown that axon branches in the process of synapse disassembly degenerate (Bixby 1981). Axon degeneration was found to closely resemble the classical Wallerian degeneration, which is observed in injured axons following nerve transection (Rosenthal & Taraskevich 1977; Bixby 1981). However, caution should be taken for some of these earlier findings on degenerative axon pruning, as more sophisticated imaging techniques have revealed that pruning at the NMJ can occur via a mechanism other than pure axon retraction or degeneration (Bishop *et al.* 2004).

(i) *Terminal arbor pruning during synapse disassembly at the vertebrate neuromuscular junction*

The peripheral NMJ is perhaps the most understood and studied synapse in the vertebrate nervous system (Sanes & Lichtman 1999). The events that follow synapse disassembly at the vertebrate NMJ provide a classic example of how terminal arbors are pruned during development. During early postnatal development, muscle fibres are innervated by multiple short axon branches or terminal arbors, which have

extended from several competing motor neurons (Sanes & Lichtman 1999). By the time the animal matures, the muscle fibres are innervated by only one terminal arbor from a single motor neuron.

Earlier studies have established that a majority of arbors are removed by synapse disassembly and axon pruning, rather than programmed cell death (Riley 1981; Sanes & Lichtman 1999). How does this small-scale refinement of processes occur? In the earliest stages of synapse development, the competing terminal arbors occupy relatively equal synaptic areas and have comparable synaptic strengths (Colman *et al.* 1997; Gan & Lichtman 1998). Later in development, the process becomes more dynamic, since the relative activity of competing motor neuron inputs at the muscle fibre determines the final winner—stronger inputs are preserved while weaker inputs lose their synapses and are pruned away (Colman *et al.* 1997; Walsh & Lichtman 2003).

Terminal arbor pruning at the NMJ is characterized by several morphological changes in the axon. In general, synapse disassembly at the NMJ has been synonymous with the term 'axon retraction', which has been used often to describe the withdrawal of the terminal arbor from the NMJ (Sanes & Lichtman 1999; Lichtman & Colman 2000). As an axon withdraws, previous labelling studies have shown that the axon becomes thinner in appearance than neighbouring unpruned axons (Riley 1981; Gan & Lichtman 1998). In addition, the distal ends of the axon form a unique bulb-like structure, a retraction bulb (Riley 1981; Gan & Lichtman 1998; Bernstein & Lichtman 1999). Retraction bulbs were first reported by Ramon y Cajal in lesion studies of the nervous system (De Felipe & Jones 1991). Similar structures were also observed in target-deprived studies of the peripheral nervous system (PNS; Pearson *et al.* 1992; Rossi *et al.* 1993; Marty & Peschanski 1994; Marty *et al.* 1994). Ultrastructurally, retraction bulbs appear as rounded structures that are filled with intact vesicles, disorganized neurofilaments and microtubules and damaged mitochondria (Marty & Peschanski 1994). However, it was unclear at the time how retraction bulbs were formed or what led to thinning and withdrawal of the axon after synapse disassembly. The mechanism seemed to be different from the degeneration of axons observed in *Drosophila*, and the presence of disorganized material in the distal end of the axon did not represent a pure retraction event.

A recent study by Bishop *et al.* (2004) has disproved the myth that axon pruning occurs only by axon retraction or degeneration (Koirala & Ko 2004). *In vivo* time-lapse imaging together with electron microscopy was used to examine retreating axons from the NMJ during synapse disassembly. In their analysis, the authors discovered that withdrawing terminal arbors were pruned by piecemeal removal of distal portions of the axon which were left behind as 'axosomes' (Bishop *et al.* 2004). Axosomes were separate membrane-bound components of the axon which contained numerous intact vesicles and mitochondria that were not degenerating. However, pruning by axosome shedding is probably not a pure retraction event either. Normal neurofilament and microtubule networks are required for retrograde transport of materials from a retracting axon to other parts of the neuron. This is not true for axosome shedding, since analysis of terminal arbors that were pruning demonstrated the presence of disrupted neurofilament and microtubule organization in concentrated areas at the retraction bulb and along longitudinal segments of the axon (Bishop *et al.* 2004).

Similar to the pruning of axons by axon degeneration, axosome shedding is aided by supporting cells in the environment. During synapse development at the NMJ, Schwann cells are closely opposed to terminal arbors (Sanes & Lichtman 1999). They are necessary for synaptic support and maintenance. Without them, the terminal arbors will withdraw from the NMJ (Reddy *et al.* 2003). During axosome shedding, however, Schwann cells surprisingly play an alternative role in synapse development. Their processes are observed to be surrounding and engulfing axosomes from terminal arbors that have disassembled from the NMJ (Bishop *et al.* 2004). An important issue that needs to be addressed in future studies is whether Schwann cells actively disassemble the synapse and engulf pieces of the withdrawing arbors or motor neurons possess an intrinsic self-destruct mechanism that allows them to disassemble inappropriate connections on their own.

(ii) *Terminal arbor pruning during climbing fibre synapse disassembly in the cerebellum*

Few studies have reported the cellular aspects of axon branch elimination in the developing vertebrate central nervous system (CNS) in detail. As an exception, the remodelling of climbing fibre inputs to the cerebellum has served as a classic model for studying synapse disassembly and removal of short terminal arbors in the CNS (Mariani 1983; Ito 1984; Mason & Gregory 1984; Lohof *et al.* 1996). During early postnatal development, Purkinje cells are multiply innervated by competing climbing fibre terminal arbors. Climbing fibre inputs that are positioned on the same Purkinje cell are initially equal in strength; however, at later postnatal ages, the stronger input wins out while the weaker inputs lose their synapses and are pruned away until the Purkinje cell is innervated by only one climbing fibre terminal arbor (Hashimoto & Kano 2003). During climbing fibre synapse disassembly, there is a prevalence of double membrane structures resembling axosomes that were observed at the vertebrate NMJ (Eckenhoff & Pysh 1979). Although more detailed analyses need to be performed, the finding suggests that perhaps at least terminal arbor pruning events that resemble mechanisms of pruning at the NMJ are conserved throughout the CNS.

(iii) *Stereotyped elimination of long axon collaterals in the developing central nervous system*

The remodelling of axons in the vertebrate nervous system is not restricted to small-scale refinement of terminal arbors. During development in the CNS, the removal of axon collaterals can be stereotyped and occur on a much grander scale than terminal arbor pruning (Innocenti 1981; Cowan *et al.* 1984; O'Leary 1987; O'Leary *et al.* 1990; Luo & O'Leary 2005). We have already described a similar event that occurs at the

developing MB of *Drosophila* (Lee *et al.* 2000*a*,*b*), but additional cases have been observed in the vertebrate CNS, where entire neural pathways have been restructured or completely removed.

Evidence for the stereotyped removal of long axon collaterals was first reported by Innocenti (1981) for the remodelling of axons that project callosally to the contralateral side of the brain in cats. In the immature cat brain, visual callosal projections from layer III, IV and VI neurons cover a broad region of the visual cortex on the opposite hemisphere corresponding to cortical areas 17 and 18 (Innocenti & Clarke 1984; Innocenti 1995). Some of these initial projections are transient and the cells that are associated with them eventually die (Innocenti 1995). By contrast, the projections that are maintained continue to proliferate and form collaterals throughout the contralateral visual cortex. During the second and third postnatal weeks of development, there is a waiting period, whereby the axons further extend and arborize into the grey matter (Aggoun-Zouaoui & Innocenti 1994; Houzel *et al.* 1994). At the same time, there is considerable remodelling of the callosal axons via the stereotyped pruning of axon collaterals that extended beyond their proper termination zones. While some reports suggest that these transient collaterals undergo atrophy, it is still unclear which cellular mechanisms lead to their removal (Innocenti 1995). However, the removal of excess callosal projections does seem to rely on normal visual input to the appropriate callosal neurons. Altering normal patterns of visual activity or the level of activity feeding into callosally projecting neurons leads to profound defects in stereotyped pruning (Shatz 1977; Lund *et al.* 1978; Dehay *et al.* 1989; Frost *et al.* 1990; Koralek & Killackey 1990; Zufferey *et al.* 1999). Additional studies have yielded similar results for other callosal neurons, which send projections to the contralateral soamatosensory cortex.

Another well-studied example of stereotyped elimination of long axon collaterals occurs during the refinement of subcortical processes arising from layer V cortical neurons (Stanfield *et al.* 1982; Stanfield & O'Leary 1985*a*,*b*; O'Leary 1987; O'Leary *et al.* 1990). Axons originating from layer V neurons of the motor cortex and visual cortex are guided initially to subcortical targets that overlap in the brainstem and spinal cord. Later in development, only the collateral branches that are functionally appropriate for each cortical region are retained. For example, neurons from layer V of the visual cortex prune away their branches from targets that are specified for motor function (branches that extend to the mesencephalic nucleus, caudal pons, inferior olive, dorsal column nuclei and spinal cord). By contrast, neurons from layer V of the motor cortex prune their branches that are necessary for visual function (branches that extend to the superior colliculus). Unfortunately, there has been little progress in understanding the molecular and cellular regulations of these phenomena. In the past 10 years, only Otx1 has been found to regulate the pruning of layer V subcortical axon collaterals (Weimann *et al.* 1999). Otx1 is a homeodomain transcription factor that is localized in layer V neurons throughout the cortex. During the formation of exuberant collaterals, Otx1 translocates to the cell nucleus, and presumably regulates the transcription of downstream molecules that control axon collateral pruning (Weimann *et al.* 1999). Since Otx1 is not regionally specific among layer V neurons within the visual or motor cortex, it is unclear how the selective pruning of motor subcortical projections is regulated differently from the selective pruning of visual subcortical projections.

Some studies have suggested that the final patterning of layer V subcortical projections might depend on local cues which the layer V cells receive in the cortex. Evidence that factors from the surrounding cortical regions could lead to selective differentiation of layer V pyramidal cells was first observed in heterotopic cortical transplant experiments (Stanfield & O'Leary 1985*a*,*b*; O'Leary & Stanfield 1989). In early development, when visual cortical areas are transplanted into motor areas of cortex or vice versa, the transplanted cortex retains only the projections that are functionally appropriate to the region they replaced. Thus, visual cortex transplanted to the motor cortical areas retains subcortical projections targeting nuclei responsible for controlling motor function, whereas motor cortex transplanted to visual cortical areas retains only the projections which are responsible for normal visual function.

The removal of long subcortical axon collaterals originating from layer V neurons seems to be an extremely efficient process that occurs within the first two to three weeks of postnatal development in rodents. Indeed, several millimetres of an axon are removed without any remnants of the process left behind. While some experiments on the pruning of callosal projections suggest that long axon collaterals undergo axonal atrophy, there is relatively little information concerning the cellular processes taking part in the stereotyped removal of subcortical layer V projections. An earlier anterograde labelling study has suggested that the presence of fragmented axons could imply that axon collaterals were degenerating (Reinoso & O'Leary 1989). It will be interesting to see if additional ultrastructural analyses on these projections during phases of stereotyped axon pruning are consistent with the earlier findings. In addition, the small-scale pruning of axon terminal arbors described at the NMJ and among cerebellar climbing fibres is coupled with synapse disassembly. Relative levels of activity from inputs onto their targets seem to play a major role in determining what branches will stay (Colman *et al.* 1997; Hashimoto & Kano 2003; Luo & O'Leary 2005). In the stereotyped pruning of subcortical long axon branches, however, no evidence has yet been established that the pruning of these collaterals is associated with synapse formation or that the elimination of excessive inputs is activity-dependent.

(iv) *A role for stereotyped axon pruning in the topographic mapping of retinal ganglion projections*

In the adult nervous system, several areas of the brain contain connections that are organized in a functional pattern called a topographic map. For example, retinal ganglion cells (RGCs), which are organized along the temporal–nasal axis of the retina pattern their projections along the anterior–posterior (AP) axis of

the chick tectum (Yates *et al.* 2001; McLaughlin *et al.* 2003). In the adult, the organization of connections is retinotopic. By contrast, in early development, the map is less refined as RGCs overextend their axons beyond their intended targets. This is followed by interstitial branching of axon collaterals to the proper targets in the tectum. Stereotyped pruning of the overextended axon branches is regulated by a gradient of signalling by the Eph–ephrin family of tyrosine kinases (Cheng *et al.* 1995; Nakamoto *et al.* 1996; Yates *et al.* 2001). Eph–ephrin signalling restricts overextended axons to more anterior targets along the AP axis of the tectum. The cellular mechanism of axon pruning has not been elucidated; however, it is interesting to note that during the Eph–ephrin-dependent phase of remodelling, axon-labelling studies have revealed an increase in the blebbing of axonal processes in the posterior tectum, which is reminiscent of axonal degeneration (Nakamura & O'Leary 1989). After the stereotyped pruning of overextended axon branches, the retinotopic map is still not completely refined. Presumably, normal patterns of correlated activity are required to further refine the broad distribution of secondary and tertiary arbors that branch-off from the primary axon (McLaughlin *et al.* 2003; Yates *et al.* 2004). It is unclear whether the branches which overshoot form synapses and what cellular mechanisms regulate the fine-tuning of these projections.

(v) *Extrinsic factors signal the fate of transient axon collaterals in the hippocampus*

The hippocampus is another area in the CNS where considerable remodelling of projections occurs (Bagri *et al.* 2003). In recent years, our studies have suggested that the stereotyped pruning of long axon collaterals could be regulated by the ability of the axon to sense pruning signals from the environment (Bagri *et al.* 2003). In the immature hippocampus, granule cells of the dentate gyrus extend two bundles of mossy fibre axons—a main bundle that courses adjacent to the apical dendrites of CA3 pyramidal cells and a transient infrapyramidal bundle (IPB) of axon collaterals that course adjacent to the basal dendrites a few days later (Amaral & Dent 1981). During the stereotyped pruning of the IPB, granule cells express Plexin-A3, a receptor for secreted Sema3F (Cheng *et al.* 2001). Sema3F is expressed in a spatially restricted manner along the areas coursed by the IPB projections, and along with Plexin-A3, it has been shown to be crucial for regulating IPB stereotyped axon pruning (Bagri *et al.* 2003). Knockouts for either Sema3F or Plexin-A3 result in profound pruning defects in the IPB (Bagri *et al.* 2003; Sahay *et al.* 2003).

Interestingly, an *in vitro* analysis of mossy fibre axon pruning suggested that stereotyped IPB pruning occurs by axon retraction (Bagri *et al.* 2003). Our recent ultrastructural analysis indicated that mossy fibres did not degenerate or undergo axosome shedding when they were pruned away, which further supported the idea that branch elimination was mediated by axon branch retraction (Liu *et al.* 2005). In this study, we showed that the IPB mossy fibre collaterals formed immature synaptic complexes before pruning. As mossy fibres were pruned from the IPB, the size of the synaptic complexes regressed and the number of synapses reduced prior to the retraction of mossy fibre axons (Liu *et al.* 2005). In other regions of the CNS, the remodelling of axons depends on normal correlated patterns of activity. Coincidently, the length of the IPB has been previously shown to be affected by relative levels of activity in the hippocampus (Adams *et al.* 1997). However, it remains to be seen whether Sema3F expression is affected by neurotransmission.

3. IMAGING STUDIES OF PROCESS REFINEMENT DURING DEVELOPMENT AND IN THE ADULT

Imaging developmental plasticity in the vertebrate CNS has traditionally been challenging, given some of the obvious technical hurdles that need to be surpassed. The advent of time-lapse two-photon microscopy has made imaging possible at levels below the surface of the neocortex. In combination with the use of transgenic mice that express fluorescent molecules in only a tissue-specific subpopulation of neurons in the cortex, several studies have addressed some of the key issues regarding cellular mechanisms of process elimination in the CNS.

(a) In vivo *time-lapse imaging of developmental plasticity in the vertebrate central nervous system*

During the first three weeks of postnatal development, several neurons that project to and within the neocortex reorganize many of their processes. Unfortunately, little is known about the cellular mechanisms that control the development of these processes. Using time-lapse two photon imaging on mice expressing green fluorescent protein (GFP) in a subset of thalamocortical (TC) neurons and Cajal–Retzius (CR) cortical neurons, Portera-Cailliau *et al.* (2005) were able to compare both the spatial and the temporal aspects of small- and large-scale remodelling events in the neocortex. TC and CR axons develop in the same overlapping regions of the cortex, but their patterns of growth and regression are strikingly different. TC axons, which originate in the thalamus, tend to project in a straight line to the cortex and end in small bulbous growth cones. By contrast, CR axons, which are formed within the cortex, take a nonlinear path to their targets and end in large growth cones with long filopodial extensions. In both cases, axon collaterals are formed by interstitial branching of secondary axons along the primary axon shaft.

Regression of TC versus CR axons by axon pruning was remarkably similar. In a majority of pruning events observed, small-scale CR pruning of short axon branches and large-scale pruning of TC long axon branches occurred by retraction rather than degeneration (Portera-Cailliau *et al.* 2005). Axon degeneration, which resembled classic Wallerian degeneration, was observed only in the pruning of TC axon collaterals and occurred in only 5% of all pruning events. Axon degeneration in TC axon collaterals was not induced by axonal injury; hence it remains to be seen why, in some instances, TC axons choose to prune their projections by axon degeneration rather than retraction. In addition, the factors that decide the fate of the axon collaterals might be regulated intrinsically, provided

that TC and CR axon collaterals overlap in similar areas of the cortex.

(b) In vivo *time-lapse imaging of adult plasticity in the vertebrate central nervous system*

A central issue in neurobiology is the understanding of the mechanisms involved in regulating changes in the structural organization of circuitry in the adult neocortex in response to alterations in sensory experience from the environment. In early development, structural remodelling is a common and extremely dramatic event—axon and dendritic branches, and dendritic spines can lose and regrow processes in the order of minutes. The situation is quite the opposite in adult cortex, where structural reorganization is restricted mostly to the small-scale turnover of dendritic spines. Long-term *in vivo* time-lapse imaging studies in the neocortex of GFP- or yellow fluorescent protein (YFP)-expressing mice have reported that a majority of plastic changes in the adult brain are regressive in nature and the kinetics are much slower than the comparable cases seen in early development (Lendvai *et al*. 2000; Trachtenberg *et al*. 2002; Zuo *et al*. 2005). Dendritic spines in the adult brain are more stable and are pruned or sprout in new areas only after a period of days. In addition, the small-scale pruning of dendritic spines occurs by retraction (Trachtenberg *et al*. 2002; Zuo *et al*. 2005).

Changes in experience (as a result of increased sensory stimulation or deprivation) tend to have significant effects on the level of plastic occurrences in the neocortex. For example, Trachtenberg *et al*. (2002) tracked changes in the rate of dendritic spine turnover in the barrel cortex before and after altering the sensory experience of mice. The barrel cortex contains a somatotopic representation of the location of all sensory stimuli that are received from the whiskers of a mouse, which are arranged in an orderly pattern on a mystacial whisker pad. The ability to sense the location and the intensity of stimuli from the environment is changed by whisker trimming. As a result of whisker trimming, there is a significant increase in the level of plastic changes in the barrel cortex. Existing spines retract, whereas others sprout in different locations along the dendritic shaft. Importantly, the growth of new spines or the retraction of old spines is accompanied by synapse formation and elimination, respectively (Trachtenberg *et al*. 2002). Thus, while large-scale structural rearrangements of axons and dendrites are rarely observed, the results suggest that small-scale synaptic reorganization might contribute to the majority of experience-induced plastic changes in the adult brain.

(c) *Lessons learned from the* in vivo *time-lapse imaging of developmental plasticity in* Drosophila

Extensive structural remodelling is a common feature of insect morphogenesis. Few studies have examined the cellular remodelling events in detail. However, two recent time-lapse imaging studies were successful in recording many of the critical cellular events associated with the patterning of dendrites of GFP-labelled dendritic arborizing (da) sensory neurons in *Drosophila* (Williams & Truman 2004, 2005*a*,*b*; Kuo *et al*. 2005). During development, some da sensory neurons die and the dendritic processes associated with them degenerate and undergo fragmentation. Phagocytic blood cells invade the area of the dying cell and then engulf the leftover debris.

In contrast, some da sensory neurons survive and their dendritic arbors are remodelled in an elegant way (Williams & Truman 2005*b*). The pruning of the dendritic branch begins with the dismantling of the microtubule network, which coincides with the thinning of the dendrite. As the dendritic branch thins, it detaches or 'severs' itself from the thicker unpruned dendrite; this is immediately followed by degeneration and fragmentation of the detached dendritic branch. Phagocytic blood cells seem to play an active role in dendritic pruning as they are found closely apposed to thinning dendritic branches prior to severing and appear to actively engulf the detached dendritic segments after severing (Williams & Truman 2005*b*).

Importantly, some dendritic branches are pruned by retraction. It is interesting that branch-retraction and -degeneration can occur in separate locations along the same dendritic scaffolding. It is not clear how the fate of the dendrite is specified, but interestingly, it was found that pruning by degeneration rather than pruning by retraction was dependent on ecdysone signalling, as degenerative pruning was markedly reduced when ecdysone receptor signalling was perturbed (Kuo *et al*. 2005; Williams & Truman 2005*b*). In additional studies (Kuo *et al*. 2005), the initial severing of dendrites from the dendritic scaffold was found to rely heavily on the normal function of the ecdysone receptor and the degradation of proteins via the ubiquitin proteosome pathway in da sensory neurons. On the other hand, matrix metalloproteases, which normally aid in tissue remodelling during morphogenesis were important for later stages of pruning. After dendritic severing, matrix metalloproteases in the surrounding environment are required to clear the dendritic debris (Kuo *et al*. 2005).

4. PROCESS ELIMINATION IN CHRONIC NEUROLOGICAL DISEASE

During development, axon collaterals seem to be primarily remodelled by axon retraction or degeneration. During chronic neurological disease or when axons are injured, axon segments distal to the injury site are often eliminated. In §4*a–c*, we explore some of the mechanisms that regulate axon elimination during disease and injury. What we observe are several similarities between axon degeneration in disease and injury versus degenerative modes of axon pruning.

(a) *Wallerian degeneration after axonal injury*

When an axon is cut in the CNS or PNS, the distal segment of the axon degenerates over a period of days by a process termed Wallerian degeneration (Waller 1850). During Wallerian degeneration, many changes occur along the injured axon—the myelin sheath is disassembled, neurofilament and microtubule networks break up, mitochondria swell, the diameter of the axon is thinner than the intact proximal axon segment and the

membrane undergoes fragmentation which is accompanied by phagocytosis (Griffin *et al.* 1996). Recent studies have shown, however, that after axons are cut, there is an acute axonal degeneration (AAD) event at the injury site that precedes classic Wallerian degeneration (Kerschensteiner *et al.* 2005). AAD affects axons within minutes rather than days after axonal injury. The injured areas of the intact proximal axon segment and detached distal segment degenerate into fragments in bi-directional fashion during AAD. After AAD is complete, the proximal axon segment retracts further, while the remaining portion of the distal axon segment undergoes Wallerian degeneration. It is interesting that AAD and Wallerian degeneration share many of the same features. The kinetics of axonal fragmentation as well as the size of the fragments that are formed is similar in both cases. Furthermore, calpain proteases, which mediate the breakdown of the axonal cytoskeleton in Wallerian degeneration seem to be involved in AAD as well, since calpain protease inhibitors slow down both processes effectively (Kerschensteiner *et al.* 2005).

When axons are cut in the PNS, the cell survives along with the intact proximal axon, which eventually regenerates after axonal injury. These findings together with earlier studies have suggested that Wallerian degeneration and apoptosis are regulated by different mechanisms. First, the overexpression of *bcl-2*, which acts as an inhibitor of apoptosis has no effect on inhibiting axotomy-induced Wallerian degeneration in RGCs (Dubois-Dauphin *et al.* 1994). Second, increased caspase activation is common during apoptosis, but seems to be absent during Wallerian degeneration (Finn *et al.* 2000). As further support for this finding, blocking caspase activity does not seem to slow down or prevent Wallerian degeneration from occurring either. Third, apoptosis is unaffected in mice, where Wallerian degeneration is severely slowed (*Wld*^{s}; Kerschensteiner *et al.* 2005). An important caveat to the final observation is that AAD is also slowed in *Wld*^{s} mice, but axon retraction occurs normally, suggesting that perhaps axon retraction events are controlled by a separate mechanism. Additional evidence for this hypothesis was observed during synapse disassembly at the NMJ in *Wld*^{s} mice, where the pruning of axon terminal arbors was unaffected compared to wild-type animals (Gillingwater & Ribchester 2001).

(b) *Wallerian degeneration can be actively regulated by intrinsic signals within the neuron*

The first evidence that Wallerian degeneration was mediated by an intrinsically activated axon self-destruct mechanism came with the accidental discovery of the *Wld*^{s} mouse in regeneration studies (Lunn *et al.* 1989). Injured axons in *Wld*^{s} mice are protected from Wallerian degeneration. Efforts to clone and sequence the *Wld*^{s} gene were successful in identifying an 85 kb tandem triplication that resulted in the overexpression of a chimeric protein containing full-length nicotinamide mononucleotide adnenylyl transferase (NMNAT), an enzyme involved in nicotinamide adenine dinucleotide (NAD) synthesis fused to the first 70 amino acids of an E4 ubiquitin ligase, Ufd2a, which functions in ubiquitinating protein substrates for proteolysis by the UPS (Coleman *et al.* 1998; Conforti *et al.* 2000; Mack *et al.* 2001). Notably, the UPS has been shown to be important for regulating the degenerative pruning of axons in *Drosophila* and in Wallerian degeneration after axons are transected (Watts *et al.* 2003). A recent study demonstrating that increased NMNAT activity, rather than Ufd2a, conferred a neuroprotective effect on injured axons was unexpected given these earlier findings (Araki *et al.* 2004). Further analysis showed that the extrinsic application of NAD by itself in culture could also protect injured axons, but only if NAD was applied prior to severing an axon (Araki *et al.* 2004). This suggested that the axon protective effect incurred by NAD was likely due to an indirect signal that was initiated at the cell body. In a siRNA screen for NAD-dependent molecules that protect against axon degeneration, NAD-dependent Sir2 histone deacetylase, SIRT1, was shown to be required for axon protection from Wallerian degeneration. SIRT1 is localized primarily in the cell nucleus, and in the previous studies, it has been shown to promote longevity in yeast and *C. elegans* (Araki *et al.* 2004).

The conclusion that the axon protective effects of SIRT1 are non-local is puzzling, considering that the extent of the protective effects of *Wld*^{s}, NMNAT and NAD on injured axons is unchanged in wild-type versus SIRT1 knockout mice (McBurney *et al.* 2003). Furthermore, another study by Wang *et al.* (2005) has shown that NAD can act locally at the axon even after it has been transected from the cell body. NAD levels were shown to decrease in degenerating axons, but preventing the NAD concentration from decreasing by applying NAD exogenously had a protective effect on injured axons. At the level of the axon, NAD can participate in a bioenergetic pathway that fuels ATP synthesis. Consistent with this hypothesis were the observations by Wang *et al.* (2005) that: (i) ATP levels decrease along with NAD in degenerating axons and (ii) the external application of a membrane permeable form of pyruvate (to prevent ATP decline) protects against axonal degeneration similarly to NAD.

(c) *Models for studying axon pathology in disease*

Several discoveries in the past decade have increased our knowledge on the various molecular and cellular mechanisms of developmental plasticity in the nervous system. Perhaps the greatest rewards from these discoveries are on understanding how similar phenomena contribute to the neuropathology in neurological disease. In §4*c*(i, ii), we address this issue in the context of axonal degeneration and its prevalence in diverse forms in chronic neurological disease.

(i) *Dying back degeneration in development and disease*

Developing axons that are locally deprived of NGF tend to degenerate in a unique way. Degeneration begins at the distal end of the axon and spreads retrogradely. In the early 1980s, Campenot was the first to demonstrate this phenomenon in compartmentalized cultures containing NGF, where sympathetic axons were grown in chambers which separated them from their cells (Campenot 1982*a*,*b*). When NGF was removed from the chamber with axons, several distal ends of axons atrophied and underwent fragmentation

while the cells survived. Ironically, it has been reported that dying back degeneration is slowed in *Wld*^{s} mice, suggesting that it is regulated by some of the same pathways involved in Wallerian degeneration (Deckwerth & Johnson 1994).

Dying back degeneration is common in many diseases affecting the PNS (Cavanagh 1964). Examples include polyneuropathies associated with alcoholism, diabetes, AIDS and chronic exposure to various toxic agents (Coleman & Perry 2002). Dying back degeneration is also seen in motor neuron diseases of the CNS, such as amyotrophic lateral sclerosis and spinal muscular atrophy (Coleman & Perry 2002). In motor neuron diseases, there is extensive neuronopathy as neurons undergo apoptosis in addition to axon degeneration and so it is unclear which event, apoptosis or axonal degeneration, takes precedent in the disease.

The *pmn*/*pmn* mouse has served as a useful animal model for studying progressive motor neuron diseases affecting the CNS (Schmalbruch *et al*. 1991*a*,*b*). The mice exhibit a progressive form of motor neuronopathy—a dying back degeneration that spreads to the cell body and is associated with cell death. The clinical symptoms are dramatic; mice become progressively weak and survive for only six weeks. When *pmn*/*pmn* mice are crossed with a transgenic line of mice that overexpress *bcl-2*, which has been shown to perturb apoptosis, the motor neurons survive, but no effect is observed on the degenerating axons and the mice eventually die (Dubois-Dauphin *et al*. 1994; Sagot *et al*. 1995). In contrast, when *pmn*/*pmn* mice are crossed with the *Wld*^{s} mouse line, they survive for up to a week longer than the single *pmn*/*pmn* knockouts (Ferri *et al*. 2003). In these double knockouts, Wallerian degeneration is attenuated and the motor neuron numbers are increased. In summary, the results indicate that axon degeneration rather than cell death takes precedence in causing weakening and death of *pmn*/*pmn* mice.

(ii) *Neurodegeneration in neurological disease*

There are many additional diseases affecting the CNS that are associated with both neuronal cell death and a non-dying back form of axonal degeneration. Some of these neurodegenerative disorders include, but are not limited to, Parkinson's, Alzheimer's and Huntington's diseases (Coleman & Perry 2002). Although different factors might contribute to axon pathology in each case, what is clear is that the end result is always the same—the axon degenerates in a process that resembles Wallerian degeneration (Coleman & Perry 2002). Furthermore, in a mouse model for Parkinson's, the *Wld*^{s} mutation has a profound effect on slowing down axon degeneration in dopaminergic fibres (Sajadi *et al*. 2004). The result serves as an important link between processes that regulate Wallerian degeneration, degenerative forms of axon pruning in development and the various modes of axon degeneration observed in neurological disease. Our knowledge of studies on mechanisms of Wallerian degeneration has served as a useful starting point for analysing how axons degenerate in neurological disease. The road ahead looks promising as more studies are finding similarities between the two modes of degeneration, which will aid in determining potential avenues of clinical treatment and early diagnosis of neurological diseases in the future.

5. PERSPECTIVES ON THE FUTURE OF STUDIES ON DEVELOPMENTAL PRUNING AND AXON ELIMINATION IN DISEASE

The holy grail of all research on developmental pruning and elimination of processes in disease is the planning and designing of a workable scheme of treatments to regenerate the complex organization of circuitry in the adult nervous system, which has been damaged as a result of chronic disease or injury. As we have seen in chronic neurological disease, neurons die and have to be replaced. In other cases, neurons do not die, but their processes need to regrow and then locate the proper targets. While our emphasis throughout this review has been on diverse aspects of developmental and disease-related forms of regional axon elimination, we will also briefly discuss the lessons we have learned from these studies and how they might apply to future treatments for axon regeneration.

When axons are injured, the environment that they experience is different from what they have encountered during development (Horner & Gage 2000). Therefore, the first challenge is getting the processes to regrow. The efforts in the past 20 years have shown that the limited regenerative capacity of axons could actually be dramatically improved by either changing their intrinsic growth ability or blocking extrinsic inhibitors in the environment (Horner & Gage 2000; Neumann *et al*. 2002). However, axons that regenerate are rarely targeted to the appropriate functional areas. Some grow too many connections while others are simply mistargeted. Thus, another challenge to axon regeneration involves axon guidance and remodelling after axonal re-extension (Horner & Gage 2000; Tessier-Lavigne 2002).

A lesson that can be learnt from our understanding of remodelling events in early development is the fact that the immature brain contains an environment that is conducive for large-scale changes in axon structure. In the adult brain, the story is very different as the plasticity of axonal remodelling seems to be limited primarily to the reorganization of simple synaptic connections. Thus, many cues in the developmental environment that favour process elimination could be absent in the adult environment, which would complicate the remodelling of regenerated axons. We know from studies on Wallerian degeneration that mature neurons contain an intrinsic axonal self-destruct programme. Turning on such a pathway may be required for remodelling axons in the adult nervous system, but this first requires identifying more molecular regulators of axon elimination.

Our understanding of how axon degeneration is regulated will be significantly improved by recent efforts to identify cellular and molecular regulators of the process. However, the studies in invertebrate model organisms also provide us with invaluable information about the mechanisms. Interestingly, a recent report has demonstrated that axon regeneration can be modelled to some extent in *C. elegans* (Yanik *et al*.

2004). When a single axon is cut by laser-mediated excision, the proximal and distal ends of the axon withdraw much as seen in AAD, but what is amazing is the observation of the axon regrowing back towards its original target area. Thus, along with present studies in more complex systems, the powerful genetics of *C. elegans* might provide additional hope for uncovering novel regulatory factors in regeneration that are presently unknown to researchers in the regeneration field.

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