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Challenges and Opportunities for Regeneration in the Peripheral Nervous System

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Abstract

Regeneration in the peripheral nervous system offers unique opportunities and challenges to medicine. Compared to the central nervous system, peripheral axons can and do regenerate resulting in functional recovery, especially if the distance to target is short as in distal limb injuries. However, this regenerative capacity is often incomplete and functional recovery with proximal lesions is limited. Furthermore, regeneration of axons to the appropriate targets remains a challenge with inappropriate reinnervation being an impediment to full recovery. The reviews and selected original research papers in this Special Issue will address some of these challenges and highlight new opportunities for development of effective therapies for nerve regeneration.

Keywords

peripheral nerve regeneration; conditioning lesion; preferential motor reinnervation; chronic denervation; Schwann cell

As outlined elsewhere in this introduction and in subsequent reviews, regeneration in the peripheral nervous system (PNS) offers unique challenges and opportunities to clinical and translational neuroscience. This brief overview outlines these challenges and opportunities in two sections focusing on i) intrinsic and extrinsic determinants of speed and quantity at which nerves can regenerate, and ii) appropriate reinnervation of the correct targets. Clinically relevant functional recovery is dependent on both of these two critical factors.

INCREASING THE SPEED AND AMOUNT OF REGENERATION

Many clinical and pre-clinical animal studies have shown that prompt reinnervation of the end organ is the single most important determinant of good functional recovery. Experience going back to World War II injuries suggests that delay in repairing an injured nerve results in poor functional outcome (Sunderland, 1952, Woodhall and Beebe, 1956). Determinants of such poor recovery after delayed nerve repairs or with proximal injuries are probably multifactorial, but include changes in the end organ with decreased ability to be reinnervated and atrophic changes in the pathway that makes Schwann cells unable to support regeneration (Bishop, 1982, Sunderland, 1952, Sunderland and Bradley, 1950, Terenghi, et al., 1998). If a muscle is not reinnervated in a timely manner, prominent atrophy of the myofibers (Guth, et al., 1964, Romanul and Hogan, 1965) and likely loss of satellite cells is

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seen. This hampers later attempts at reinnervation. Similarly, skin undergoes prominent atrophic changes after denervation and reinnervation is unlikely to occur after prolonged denervation (Hoffer, et al., 1979). In addition to these data, in primate models of nerve injury and repair, the primary determinant of functional recovery is time to reinnervation (Krarup, et al., 2002).

With time to reinnervation being the most important determinant of successful clinical outcome, one strategy to improve it is to speed the rate at which the axons regenerate (reviewed in Hoke, 2006). In mammalians, rate of axonal elongation during regeneration is fairly constant across species and is determined largely by the rate of slow axonal transport, which is 1–4 mm/day (Grafstein, 1971, Hoffman and Lasek, 1980). This rate, however, declines with aging and contributes to poor recovery in older adults (Verdu, et al., 2000). There is, however, experimental evidence that suggests that speed at which axons regenerate can be manipulated. A classical example of this is “conditioning lesion” in rodent sciatic nerve injury models. In this paradigm, if a crush is made in the sciatic nerve, the rate of regeneration after a more proximal second crush is enhanced (Forman, et al., 1980, McQuarrie, et al., 1977). This increased rate of regeneration correlates with increased gene expression and protein synthesis in the neuronal cell body, and an increased rate of slow axonal transport (Hoffman and Lasek, 1980, McQuarrie, 1986, McQuarrie and Jacob, 1991).

Two non-injurious strategies have been experimented with to mimic the conditioning lesion. One of the hallmarks of conditioning lesion is an increase in cAMP levels in DRG neurons; this is similar to high levels of cAMP in embryonic DRG neurons, a time during which intense and rapid axonal growth is taking place (Weill, 1986). Thus, administration of membrane permeable cAMP analogues to DRG would be expected to mimic conditioning lesion and enhance axon regeneration after injury. However, this strategy failed to demonstrate any increase in the speed of peripheral regeneration but allowed central branches of DRG neurons to regenerate better after dorsal column lesions (Qiu, et al., 2002). This observation allows dissociation of two separate effects of conditioning lesion: i) enhanced speed of peripheral nerve regeneration, and ii) overcoming inhibitory signaling and enhancing regeneration in the central nervous system.

Another strategy to enhance speed of regeneration has utilized an observation that ATF3 (activating transcription factor 3) is rapidly and highly upregulated after peripheral nerve injury and remains elevated until reinnervation is complete (Tsujino, et al., 2000). Overexpression of ATF3 in cultured adult rat DRG neurons induces neurite outgrowth (Seijffers, et al., 2006) and transgenic mice overexpressing ATF3 regenerate their sensory axons faster after sciatic nerve crush (Seijffers, et al., 2007). This observation, however, has not been extended to motor axon regeneration and awaits further confirmation.

Studies with conditioning lesion and ATF3 overexpression suggest that in order to increase the speed of regeneration we need to increase the intrinsic rate at which axons elongate during regeneration. This, however, is not the only rate-limiting step that impedes functional recovery. At the site of injury, regenerating axons have to overcome the growth inhibitory environment of the scar tissue that forms. Unlike the CNS axons, peripheral axons can and do overcome this inhibitory environment but this process can probably be enhanced with approaches aimed at modifying the inhibitory environment (Groves, et al., 2005, Hoke, 2005). Often at the site of injury the axonal sprouts have another challenge to overcome. There is often multiple branches with growth cones that are trying to regenerate and many neurotrophic factors seem to enhance this multiple branching behavior (e.g. nerve growth factor); but, what is needed is directed longitudinal elongation of a main axonal branch in order to enhance the speed and success of regeneration. Molecular mechanisms underlying this branched versus directed outgrowth are not well understood, but cultured adult DRG

neurons after a conditioning lesion do display a directed outgrowth of a main axonal branch so studies aimed at dissecting the molecular pathways activated by conditioning lesion could yield molecular targets to exploit for therapeutic purposes.

SUSTAINING REGENERATION

One of the challenges in translating nerve regeneration studies from small animal models to humans has been the issue of degenerative changes that take place in the denervated segments of the pathway and target tissues. Going to back to extensive experience with war injuries during World War II, we know that for a nerve repair to be effective it had to be done in a timely manner as delayed repairs yielded poor functional recoveries (Woodhall and Beebe, 1956). The issue of whether this is an intrinsic limitation of the neuron or extrinsic to the neuron and due to changes in pathway (i.e. Schwann cells) or target (e.g. muscle) has been answered in an elegant study by Tessa Gordon (Fu and Gordon, 1995, Fu and Gordon, 1995). In a rat model of peripheral nerve regeneration after prolonged period of denervation, she asked if chronically axotomized neurons (i.e. not connected to their target muscles) could regenerate into a freshly denervated pathway and target. Although the number of axons successfully regenerating and innervating muscle declined with prolonged axotomy up to 12 months, there was still adequate number of axons regenerating and innervating the target muscle enough to generate near normal muscle strength (Fu and Gordon, 1995). In a reverse experiment she asked if the pathway (i.e. Schwann cells) remains denervated without axonal contact for a prolonged period, could freshly axotomized neurons regenerate into such a nerve. The answer was a resounding no; almost no axon, even though they were freshly transected and ready to regenerate, could regenerate into a chronically denervated nerve segment where the Schwann cells had atrophied (Fu and Gordon, 1995). In a later study her group showed that this decline in regenerative capacity starts to occur at 1 month and by six months there is absolutely no glial support for axonal regeneration in a chronically denervated rat sciatic nerve (Sulaiman and Gordon, 2000). Although the structural changes are known, molecular events that lead to Schwann cell atrophy, dissolution of the basal lamina and exposure of regenerating axons to an inhibitory environment (e.g. chondroitin sulphate proteoglycans) in the endoneurium are largely unknown. We know that there is a decline in regeneration associated markers in Schwann cells, p75 (You, et al., 1997) and erbB3 (Li, et al., 1997), in addition to a decline in expression of neurotrophic factors such as glial cell line derived neurotrophic factor (Hoke, et al., 2002) (reviewed in Hoke, 2006)).

What we don’t know is what are the molecular regulators of this decline? What mediates Schwann cell atrophy? A careful examination of gene expression changes over a prolonged period of denervation may yield clues to the identity of key regulators and offer new therapeutic targets for maintaining Schwann cell “health” in chronic denervation so that they can support regeneration more effectively.

PROMOTING REINNERVATION OF FUNCTIONALLY APPROPRIATE TARGETS

Successful peripheral nerve repair depends not only upon delivery of axons to the periphery, but also upon re-establishment of appropriate connections between the periphery and the central nervous system. The consequences of non-specific regeneration are often clinically obvious. Faulty localization of sensory stimuli was first described by John Mitchell in a review of his father’s Civil War patients (Mitchell, 1895), and has long been recognized as a hallmark of nerve regeneration. The consequences of misdirected motor axon regeneration are most obvious after repair of the facial nerve, when attempts to smile are often met with gross distortion of the face (Kimura, et al., 1975). Other forms of misdirection may be less

obvious and therefore harder to quantify. Nearly a century ago, Langley and Hashimoto noted, “Some fibers of the central end, though they grow into the peripheral end, are unable to make functional nerve endings. This occurs when efferent fibers grow into afferent, or afferent into efferent” (Langley and Hashimoto, 1917). Sensory and motor axons misdirected in this fashion would fail to establish functional contacts, and could exclude appropriate axons from the pathways they occupy.

Analysis of misdirected regeneration requires a theoretical framework to account for the types of regeneration specificity that would be needed to restore normal function after nerve injury (Brushart, 1991). The most general specificity occurs at the tissue level, nerve regenerating to nerve as opposed to other tissues. Once this is achieved, the next goal is to separate afferent and efferent systems, or sensory/motor specificity. Within both sensory and motor systems, it would then be necessary to establish topographic and end organ specificity. Topographic specificity returns axons to the muscle or area of skin they served initially; end organ specificity matches regenerating axons with end organs of the sensory modality or muscle type to which they were connected originally.

The specificity of axon regeneration is determined primarily at the site of nerve repair; once a regenerating axon is confined to a Schwann cell tube in the distal nerve stump, it usually follows that tube to its peripheral termination (Brown and Hardman, 1987, Brown and Hopkins, 1981). Neurotropism, neurotrophism, and mechanical alignment are factors that may influence the specificity of distal Schwann cell tube reinnervation. Neurotropism, guidance of regenerating axons up a concentration gradient of a substance diffusing from the target, is clearly involved in generating specificity at the tissue level (Lundborg, et al., 1986). Neurotropic guidance to specific Schwann cell tubes is suggested by recent morphologic studies (Witzel, et al., 2005), but has not been confirmed. Neurotrophism, the selective support of axons that have randomly encountered a correct target, is the most plausible explanation for the generation of sensory/motor specificity. Preferential motor reinnervation is the tendency for motor axons regenerating in mixed nerve to selectively reinnervate muscle or the nerves leading to it (Brushart, 1993). Motor neurons initially innervate sensory and motor Schwann cell tubes at random, with many projecting collaterals to both (Brushart, 1990). Pruning of motor axon collaterals from incorrect sensory pathways then occurs, most likely in response to differential trophic support from the two environments. Topographic specificity is not an inherent property of either sensory or motor regeneration, but depends upon the mechanical alignment of axons in the proximal and distal nerve stumps (Brushart, et al., 1983). Precise alignment, obtainable only in the laboratory, increases specificity (de Medinaceli, et al., 1983), while imposition of a gap between stumps progressively degrades specificity as the gap increases (Brushart, et al., 1995). End organ specificity, which could conceivably be generated at the nerve repair or within the target tissue, does not appear to shape either sensory (Koerber, et al., 1989) or motor regeneration (Miledi and Stefani, 1969).

The relative failures of end organ and topographic specificity present opportunities to improve clinical outcomes by enhancing these aspects of regeneration. Surgically demanding techniques of identifying and matching fascicles in the proximal and distal nerve stumps have resulted in modest improvement in small clinical series (Deutinger, et al., 1993, Kato, et al., 1998), but have not been accepted for general use. In response to these difficulties, surgeons moved in the opposite direction, leaving a gap between nerve ends to “increase the possibilities for neurotrophic and neurotropic mechanisms to act” (Lundborg, 2000). This technique produced good results in the digital nerve (Weber, et al., 2000), where a small topographic area is occupied by a large number of receptors, and random axon behavior can still result in functional reinnervation. In mixed nerve, however, this approach improved neither specificity in the laboratory (Brushart, et al., 1995, Valero-Cabre, et al.,

2004) nor functional outcome in the clinic (Lundborg, et al., 2004). Given the failure of both refined surgical technique and inherent neurotropism to improve outcomes significantly, the current challenge is to artificially enhance modality-specific pathway recognition. Growth factor expression by denervated Schwann cells is modality-specific (Hoke, et al., 2006), and the growth cones of adult DRG neurons can turn in response to trophic gradients (Webber, et al., 2008), so this approach is at least theoretically possible.

Abbreviations used

PNS	Peripheral Nervous System
DRG	Dorsal Root Ganglion
ATF3	activating transcription factor 3

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