http://dx.doi.org/10.5607/en.2012.21.3.83
Exp Neurobiol. 2012 Sep;21(3):83-93.
pISSN 1226-2560 • eISSN 2093-8144

Review Article

Experimental Neurobiology

Sensory Axon Regeneration: A Review from an *in vivo* Imaging Perspective

Seung Baek Han[1,2], Hyukmin Kim[1,2], Andrew Skuba[1,2], Alan Tessler[1], Toby Ferguson[1,3] and Young-Jin Son[1,2]*

[1]Shriners Hospitals Pediatric Research Center and Center for Neural Repair and Rehabilitation, [2]Department of Anatomy and Cell Biology, [3]Department of Neurology, Temple University School of Medicine, Philadelphia, PA 19140, USA

Injured primary sensory axons fail to regenerate into the spinal cord, leading to chronic pain and permanent sensory loss. Re-entry is prevented at the dorsal root entry zone (DREZ), the CNS-PNS interface. Why axons stop or turn around at the DREZ has generally been attributed to growth-repellent molecules associated with astrocytes and oligodendrocytes/myelin. The available evidence challenges the contention that these inhibitory molecules are the critical determinant of regeneration failure. Recent imaging studies that directly monitored axons arriving at the DREZ in living animals raise the intriguing possibility that axons stop primarily because they are stabilized by forming presynaptic terminals on non-neuronal cells that are neither astrocytes nor oligodendrocytes. These observations revitalized the idea raised many years ago but virtually forgotten, that axons stop by forming synapses at the DREZ.

Key words: dorsal root entry zone, sensory nerve regeneration, NG2 glia, oligodendrocyte precursor cells, *in vivo* imaging, astrocytes

INTRODUCTION

The cell bodies of dorsal root ganglion (DRG) neurons, which relay sensory information into the spinal cord, are located in peripheral ganglia. They emit one process that bifurcates into a peripheral axon branch and another that projects centrally into the spinal cord within the dorsal root. Dorsal roots, the central branches of DRG neurons, mount a far weaker regenerative response than other peripheral nerves [1-4]. Unlike motor nerves and peripheral branches of DRGs, DR axons often fail to regenerate across a transection site [5].

After DR crush, a less severe injury that does not interrupt root continuity, axons regenerate along the root but more slowly than other peripheral nerves. Their regeneration ceases at the dorsal root entry zone (DREZ), the transitional zone between the CNS and PNS. As first shown in the drawing of Ramòn y Cajal (Fig. 1), DR axons turn around and grow back to the PNS (arrow), or stop at (arrowheads) the DREZ. What prevents the regeneration of DR axons across the DREZ remains unclear. Inhibition by CNS glia such as astrocytes and oligodendrocytes makes an important contribution to regenerative failure at the DREZ, but the decisive factor(s) and their mechanisms of action are unknown [6-12]. Also unknown is whether the growth inhibitory activities at the DREZ are the same or different from those elsewhere within the CNS [6, 13, 14].

Although interventions that enhance the regeneration capacity of DR axons by means of neurotrophic factors or that neutralize astrocyte- or myelin debris-associated growth-inhibitors have been partially effective [7, 14-23], no strategies have promoted

Received February 14, 2012, Accepted March 28, 2012

*To whom correspondence should be addressed.
TEL: 1-215-991-8274, 1-215-926-9354, FAX: 1-215-843-9082
e-mail: yson@temple.edu

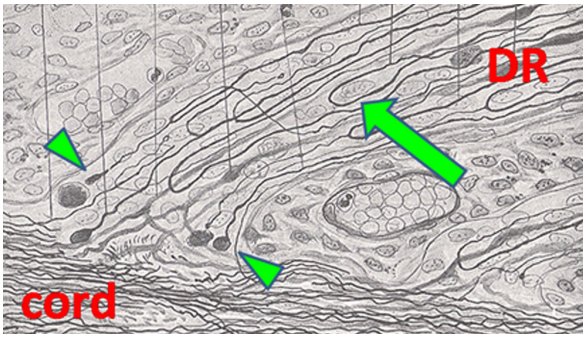

Fig. 1. Cajal's drawing illustrating DR axons growing away from (arrow) or arrested (arrowheads) at the entrance into adult spinal cord.

regeneration by all or most DRG neuron subtypes. A glial cell line-derived neurotrophic factor (GDNF), artemin, induces topographically specific regeneration of most DR axons in rodents [24, 25] but, like other neurotrophic factors, severe side effects preclude its use in clinic trials. The need to develop new treatments is urgent.

DORSAL ROOT INJURY

Dorsal root injuries, which include brachial plexus, lumbosacral plexus and cauda equina injuries, result in permanent loss of primary afferent terminals in the spinal cord. These injuries have profound effects on the spinal cord and evoke chronic, often agonizing, pain and permanent loss of sensation. Brachial plexus injury (BPI), the most common form of DR injury, results from high-energy traction injuries in which the head and neck are forced away from the shoulder. Obstetrical BPI is the most common etiology of a plegic arm in infants, occurring in ~3 per 1,000 live births. In adults, BPI occurs most commonly in motor vehicle accidents, particularly motorcycles, contact sports and falls. Overall, DR injuries are 10~20 times more common than spinal cord injury (SCI) [13, 26-28]. There is an urgent unmet clinical need for effective therapies that can reduce the extent of the initial injury or, at a later stage, enhance repair. The need for effective treatment is increasing due to higher survival rates following severe traumatic injuries and the increasing number of elderly individuals susceptible to these injuries because of falls.

Clinical treatment of brachial plexus injury is often surgical. In children, damaged peripheral components of the plexus may be repaired with donor nerve (usually the patient's own sural nerve) or other graft material, including processed cadaver nerve or tubes containing extracellular matrix [29, 30]. In adults, the distance that regenerating nerves must grow is often too lengthy for effective grafting. Nerve anastomosis to a nearby denervated nerve and muscle may be useful in both children and adults [31, 32]. However, it must be emphasized that recovery after peripheral injury is generally incomplete even with these treatments and that there is no effective therapy for dorsal root avulsions.

CELLULAR CHANGES AT THE DREZ AFTER DORSAL ROOT INJURY

At the DREZ, the glial ensheathment of the axons changes abruptly from Schwann cells in the dorsal root to astrocytes and oligodendrocytes in the spinal cord. Following DR injury, on the PNS side, macrophages rapidly phagocytose myelin and degenerating axons [33], while Schwann cells become activated/dedifferentiated and occupy axon-free endoneurial tubes. By contrast, on the CNS side, astrocytes rapidly undergo reactive changes, which include proliferation and extension of their processes further distally into DR, between the endoneurial tubes (i.e., astrogliosis) [34, 35]. Following DR crush, injured axons regenerate past the crush site, but turn around or stop at the DREZ. DR injury evokes changes similar to those induced by direct spinal cord injury (SCI), without the formation of a dense astrocytic glial scar. Nevertheless, the axotomized DREZ prevents regeneration surprisingly efficiently: Whereas peripheral conditioning lesions promote intraspinal regeneration of their central axons in the dorsal columns [36, 37], the same axons fail to regenerate through the DREZ, as confirmed in our *in vivo* imaging study [5, 6, 38, 39].

ARE ASTROCYTES OR CSPGs DECISIVE FACTORS INHIBITING REGENERATION AT THE DREZ?

What prevents axons from regenerating across the DREZ? The prevailing view in the field is that regeneration is prevented at the DREZ primarily by growth-inhibiting activities associated with reactive astrocytes and/or degenerating oligodendrocytes. Because axons contact astrocytes when they have stopped regeneration at the DREZ [40-42], reactive astrocytes are thought to form the primary regenerative barrier. Consistent with this notion, axons grow through the DREZ that has been depleted of astrocytes by X-irradiation [43], and, in general, (despite exceptions [6, 44]), reactive astrocytes inhibit neurite outgrowth [8, 45] by producing chondroitin sulfate proteoglycans (CSPGs) that collapse or repel neurite outgrowth [46, 47]. Members of the CSPG family of extracellular matrix molecules include neuroglycan 2 (NG2), aggrecan, brevican, neurocan, vesican and phosphacan [45]. These CSPGs are expressed at the DREZ both during development and after dorsal root injury [48].

The differential expression and contribution of individual

http://dx.doi.org/10.5607/en.2012.21.3.83

members of the CSPG family have also been studied. NG2, the most important component, is a major inhibitory proteoglycan for sensory axons [49]. NG2 is expressed by oligodendrocyte progenitor cells, which react rapidly following CNS injury, and by some reactive astrocytes. Virus-mediated knockdown or antibody blocking of NG2 promotes intraspinal sensory axon regeneration [50]. Recently, a transmembrane protein tyrosine phosphatase, PTPσ, was identified as a high affinity receptor of CSPG that mediates its inhibitory effect [51, 52]. Disruption of the PTPσ gene reduced inhibition by CSPG.

Several lines of evidence argue that astrocytes and CSPGs are not the critical determinant of regeneration failure. For example, the same CSPGs are expressed abundantly in tissue that supports regeneration (dorsal roots) and in tissue in which regeneration fails (DREZ and spinal cord) [53]. The inhibitory properties of CSPGs are primarily due to glycosaminoglycan (GAG) side chains; enzymatic removal of GAG chains by chondroitinase ABC (ChABC) promotes intraspinal axon regeneration [46, 54]. However, pharmacological degradation of CSPGs by chondroitinase ABC or Phosphatidylinositol-specific phospholipase C (Pi-PLC), which enhances regeneration in the damaged spinal cord [55], does not promote regeneration across the DREZ [23; but see Cafferty et al., 2007]. How astrocytes prevent regeneration, if they indeed play a crucial role in the regeneration failure, remains uncertain.

ARE OLIGODENDROCYTES OR MYELIN-ASSOCIATED INHIBITORS DECISIVE FACTORS INHIBITING REGENERATION AT THE DREZ?

Oligodendrocyte/myelin-derived inhibitors, such as Nogo-A, myelin-associated glycoprotein (MAG) and oligodendrocyte-myelin glycoprotein (Omgp), may also contribute to regeneration failure at the DREZ [7]. These molecules, except MAG, are distributed exclusively in CNS myelin synthesized by oligodendrocytes and are not found in PNS myelin synthesized by Schwann cells. All three of these myelin inhibitors bind to the glycosylphosphatidylinositol-anchored Nogo-66 receptor (NgR1), which is expressed by many CNS neurons [56, 57]. Other receptors, including NgR2 and the paired immunoglobulin-like receptor B (PirB), have also been implicated in mediating the inhibitory growth signaling [58, 59].

Intrathecal application of the soluble Nogo-receptor peptide fragment of the NgR (sNgR) after DR crush elicited regeneration of myelinated, but not unmyelinated, sensory axons [18]. This result supports the idea that myelin-associated molecules contribute to regeneration failure. It is important to note, however, that myelin-associated molecules are eventually, although slowly, cleared, together with degenerating oligodendrocytes [35, 38, 40]. Thus, their actions are exerted only transiently during the initial phase of inhibition. In addition, it is noteworthy that axons can regenerate along degenerating white matter [60, 61], and that simultaneous elimination of multiple inhibitory molecules does not promote intraspinal regeneration [62, but see, 63]. Whether regeneration is promoted at the DREZ in these triple knockout mice has yet to be determined.

Lastly, but perhaps most importantly, myelin-derived inhibitors and CSPGs act as repellent cues that cause only brief growth cone collapse or retraction [10, 64, 65]. Furthermore, DRG axons can grow despite growth cone collapse [66-68]. Moreover, unlike *in vitro*, regenerating DR axons *in vivo* are accompanied by growth-promoting Schwann cells, which could provide attractive alternative growth pathways for axons with 'transiently' collapsed growth cones to turn around and grow back toward DRG. The myelin-associated growth inhibitory molecules and CSPGs could therefore explain why some axons turn around at the DREZ, but not why others become immobilized. These considerations highlight our current lack of understanding of the molecular and cellular mechanisms that account for regeneration failure at the DREZ. They also call into question whether astrocyte- or oligodendrocyte-associated growth inhibitory molecules are paramount in preventing sensory axon regeneration after dorsal root injury.

'SYNAPTOID': A FORGOTTEN IDEA

One provocative idea raised many years ago that has received little subsequent attention is that astrocytes induce DR axons to form synapses that immobilize them at DREZ. Carlstedt first made this proposal based on his observation of structures at DREZ that he termed synaptoids [40], because of their interesting resemblance to nerve terminals. Other investigators have observed similar nerve terminal-like structures [38,69], and Liuzzi and Lasek also speculated that astrocytes provide a physiological 'stop signal' that prevents regeneration of DR axons at the DREZ [69]. As shown in Fig. 2, these axonal profiles resemble nerve terminals because mitochondria and vesicles are copious whereas microtubules and neurofilaments are sparse or absent. These 'synaptoid' profiles lack some characteristic features of pre- and postsynaptic differentiation, however, and mitochondria and vesicles are also abundant even in non-synaptic dystrophic endings [70]. In addition, none of the early studies provided direct evidence that these synapse-like profiles belong to sensory axon growth tips, rather than to other neuronal elements such as the

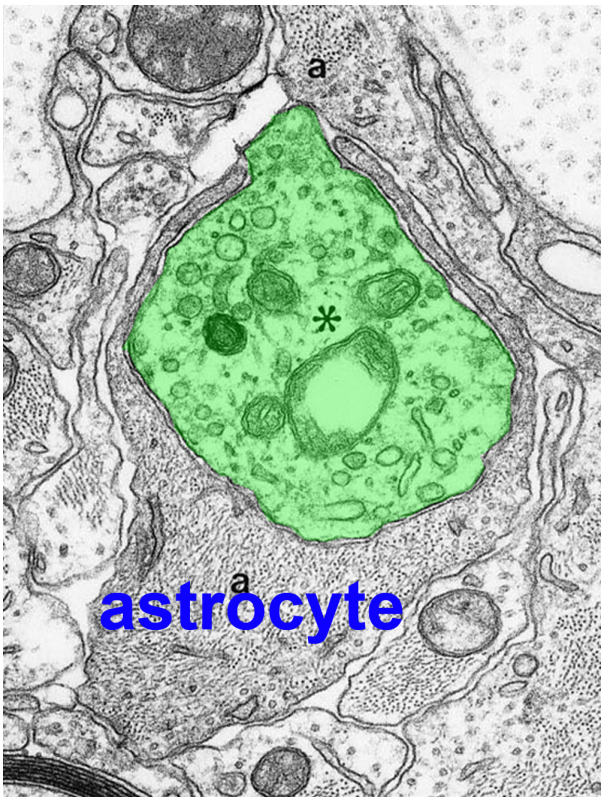

Fig. 2. An example of a "synaptoid". A DR axon surrounded by astrocytic processes in the DREZ, exhibiting nerve terminal-like profile. Adapted from Chong et al., 1999 [38].

chronically remodeled dendrites of dorsal horn neurons. These studies also did not determine whether axon endings became 'nerve terminal-like' as they entered the DREZ or whether the nerve terminal-like appearance developed only after chronic remodeling.

Nonetheless, it is notable that these nerve endings clearly differ from the retraction bulbs, originally described by Cajal (Fig. 1), formed by severed dorsal column axons and from the dystrophic endings formed in an *in vitro* model of the glial scar [70], in which fragments of disorganized microtubules or vacuoles are abundant. Moreover, the idea that astrocytes act as a stop signal also receives support from recent studies demonstrating the ability of astrocytes to promote synapse formation and maintenance [71, 72]. Although supported by anecdotal evidence, these intriguing speculations await rigorous, and challenging, investigations with advanced techniques, such as the combined application of *in vivo* imaging, targeted electron microscopy (EM) and fluorescent mouse transgenics.

IMAGING AXONS IN LIVING ANIMALS

Until recently biologic processes could only be analyzed using static images obtained after death from animals euthanized at multiple time points. The temporal and spatial resolution of these analyses was limited because dynamic changes had to be deduced from comparisons of static images. Accordingly, prior studies based on conventional static analyses provided evidence that is often conflicting or inconclusive. Dynamic cellular processes, including axon regeneration and the interactions between axons and their environment that determine the success or failure of regeneration, are best studied with techniques that capture real-time events with multiple observations of each living animal.

Our ability to monitor neurons serially *in vivo* has increased dramatically owing to revolutionary innovations in optics and mouse transgenics. Several lines of thy1-XFP transgenic mice raised by Drs. Josh Sanes and Guoping Feng, in which subsets of neurons are genetically labeled in distinct fluorescent colors, have permitted individual neurons to be imaged *in vivo*, serving as a 21st century upgrading of Golgi staining. These mice have been used extensively for *in vivo* imaging of muscles [73-75] and brain [76-78], in both physiological and pathological conditions, and have provided novel insights into physiological mechanisms that static analyses could not have resolved. Surprisingly, however, imaging studies of living spinal cord have been limited. Lichtman and his colleagues first demonstrated their feasibility by tracking injured dorsal column (DC) axons with wide-field microscopy [60, 79]. Two-photon imaging of DC axons, microglia and blood vessels was also accomplished [80]. Recently, we provided the first study of dorsal root (DR) regeneration in living mice [5]. We used wide-field microscopy and a line of thy1-YFP mice (thy1-YFPH), in which a subset of large-diameter DRG neurons and their axons are brightly labeled by green fluorescence. This approach was more challenging to develop than we had anticipated but it generated unexpected insights in support of the 'synaptoid' hypothesis postulated many years ago but virtually forgotten.

FEASIBILITY AND UTILITY OF *IN VIVO* IMAGING OF DR REGENERATION

Axon sparing has been responsible for conflicting or inclusive observations in studies of spinal cord or root injuries [81]. We consider the power to distinguish between spared and regenerating axons an important asset of *in vivo* imaging studies of DR regeneration. This distinction is possible because *in vivo* imaging allows observation of the spatiotemporal responses of both regenerating proximal stump axons and of the same axons

http://dx.doi.org/10.5607/en.2012.21.3.83

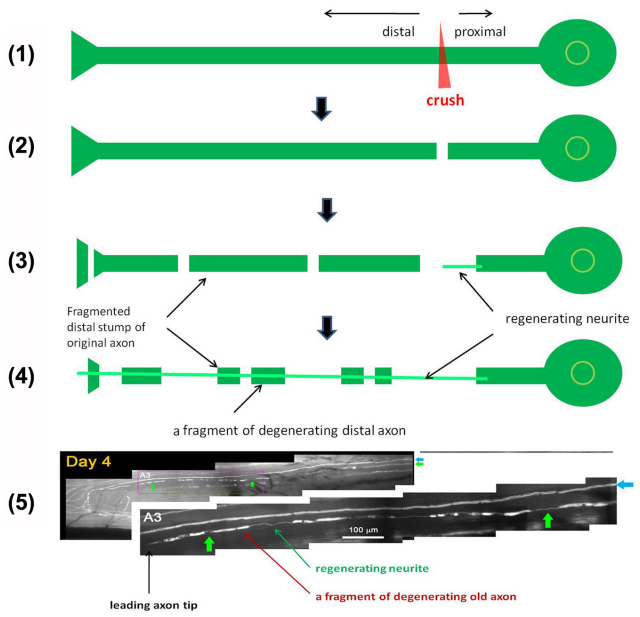

Fig. 3. Schematic illustration of spared, degenerating and regenerating axons observed during *in vivo* imaging of YFP-labeled DR axons following crush injury (1). A non-fluorescent gap is obvious at the site of crush (2), and then in the distal axons undergoing fragmentation (3). Non-fluorescent gaps widen and become more numerous as distal axons degenerate (4). A thin regenerating neurite extends from the proximal stump of the same axon (3), which elongates through the much thicker and brighter fluorescent fragments of a degenerating axon (4, 5). A spared axon (blue arrow) and a crushed axon (green arrow) showing degeneration of an old axon and regeneration of a new neurite from its proximal stump.

degenerating distal to the crush. As illustrated in the cartoon (Fig. 3), several features of crushed axons *in vivo* were very useful in differentiating regenerating axons from axons that had not been crushed or had recovered from crush. Within a few hours after crushing: 1) An ~30 μm wide non-fluorescent portion of the YFP+ axon crushed at the injury site becomes widened rather than narrowed (i.e., narrowed if axons survive the crush due to fluorescent cytoplasm refilling the squeezed/crushed axon site). 2) The distal portions of the same axons beyond the crush begin to degenerate and become fragmented. In addition, within a few days after crushing: 3) Fragments of fluorescent distal axons beyond the crush progressively shorten due to degeneration at both ends of each fragment. Moreover, 4) Regenerating axons or neurites are much thinner, less brightly fluorescent and more undulating than the intact axons (or recovered YFP labeled large diameter axons), and 5) The thin, dimly fluorescent regenerating neurites extend through the degenerating fragments of the original, brightly fluorescent axons. 6) Unlike surviving or spared axons, regenerating axons stop at the DREZ. 7) Spared axons exhibit nodes of Ranvier, whereas regenerating axons do not.

RAPID IMMOBILIZATION OF AXONS AT THE DREZ

If growth-inhibitory molecules prevent regeneration of DR axons at DREZ, in agreement with , the predominant view in the field, then one would predict dynamic responses by DR axons at DREZ. After encountering CSPGs and myelin-associated inhibitors that are produced by reactive astrocytes and degenerating oligodendrocytes in the spinal cord and cause growth cone collapse, axons would be expected to regenerate back into the PNS, where growth-promoting Schwann cells are present, or to continue to try to grow within the DREZ and then to degenerate. Surprisingly, our *in vivo* imaging studies showed that > 95% of YFP-labeled axons rapidly stopped and became completely and permanently immobilized at the DREZ, even after conditioning lesions that enhanced their growth potential [5]. Notably, *in vivo* imaging also revealed that very few axons detoured back into PNS, and that they did so immediately after encountering the border between CNS and PNS ([5, 82, 83] and unpublished data).

PRESYNAPTIC DIFFERENTIATION AT THE DREZ AND THE RESURGENCE OF THE FORGOTTEN IDEA

Light microscopic and ultrastructural analyses targeted specifically to axon tips monitored *in vivo* subsequently demonstrated that almost all axons stopped at the CNS territory of the DREZ [5], and that axon tips and adjacent shafts were intensely immunolabeled with synapse markers such as SV2 and synaptophysin [5]. Numerous axonal profiles demonstrated characteristic features of pre- but not postsynaptic endings [5]; these axonal profiles were filled with mitochondria and ~40-nm vesicles but lacked the vacuoles and disorganized microtubules that are typical of dystrophic endings. Postsynaptically, no indications of differentiation such as postsynaptic densities were observed (Fig. 4; red-pseudocolored).

Our findings conflict with the prevailing view that axons stop at the DREZ by forming swollen dystrophic endings [84]. We have found that surprisingly few stalled axons terminate in large swollen tips and that most end as slender tips that persist [5]. Notably, almost all axons, including those with large swollen endings, display small varicosities along their shafts that are intensely immunolabeled with synapse markers [5]. Our recent EM study confirmed that the large swollen endings are indeed dystrophic and lack synaptic characteristics. Importantly, however, these axon tips are completely surrounded by acellular collagenous fibers (Skuba et al., unpublished data), rather than by non-neuronal cellular elements, and they are located superficially where collagenous scars have formed adjacent to dura. Together, our

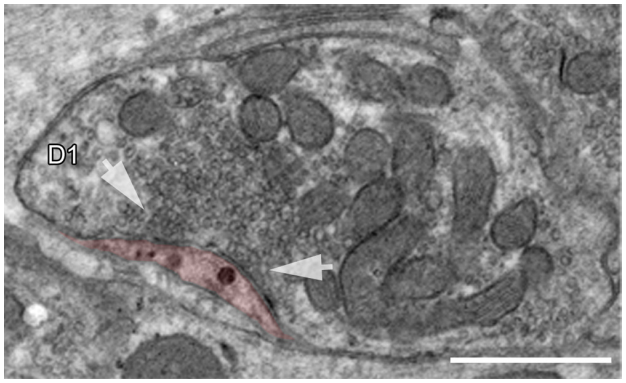

Fig. 4. An electron micrograph of the DREZ 13 days after crush injury showing a presynaptic axonal profile. Vesicles are highly clustered and docked at an electron-dense membrane that resembles an active zone (white arrows). No postsynaptic densities are present on the non-neuronal, postsynaptic cell process, which also lacks the abundant intermediate filaments that are characteristic of astrocytes. Scale bar, 250 nm.

findings show that most, if not all, DR axons become arrested as they enter the CNS territory of the DREZ by forming presynaptic terminals on non-neuronal cellular elements. These observations are also in line with the provocative idea that astrocytes induce DR axons to form 'synaptoids' that cause their arrest at the DREZ [38, 40, 69].

ARE NG2 CELL OR SYNAPTOGENIC MOLECULES DECISIVE FACTORS INHIBITING REGENERATION AT THE DREZ?

The identity of the postsynaptic cells has yet to be determined. The absence of postsynaptic densities excluded the possibility that these profiles are axon-axon synapses [85], and the cells lack the intermediate filaments that are abundant in astrocytes [5]. Some evidence suggests that they may be NG2 cells forming synapses with neurons. NG2 cells are proteoglycan NG2 (nerve/glial antigen 2)-expressing oligodendrocyte progenitors which constitute a fourth major glial cell in the mammalian CNS, distinct from astrocytes, oligodendrocytes and microglia [86-93]. They are widely distributed in both gray and white matter, and have a complex stellate morphology with highly branched fine processes [86, 94-97]. They have also been reported to form a cellular net in the CNS territory of the DREZ [98, 99], proximal to the astrocyte-Schwann cell border, where our *in vivo* imaging showed that axons stop and become immobilized (Kim et al., unpublished observation). Many investigators have confirmed that NG2 cells form functional glutamatergic and GABAergic synapses with CNS neurons [100, 101], expressing a complex set of voltage-gated channels with AMPA/kainate and/or GABA receptors [86-93].

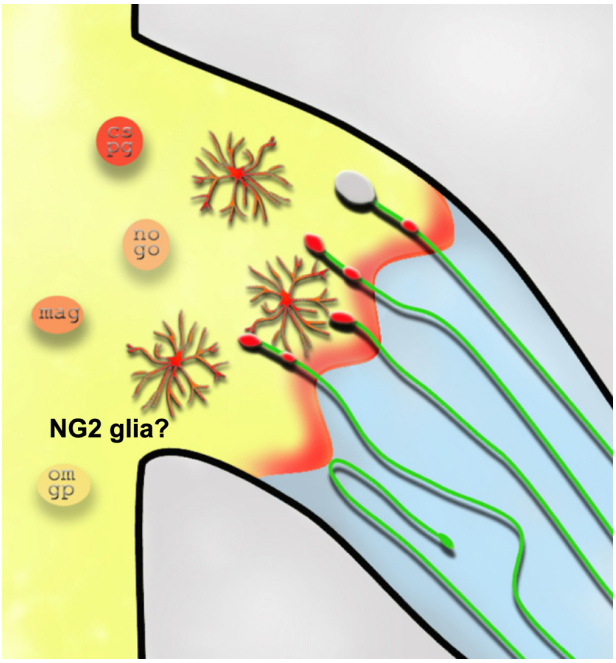

Fig. 5. Schematic illustration of the proposed model of regeneration failure at the DREZ. In addition to various growth inhibitory molecules, the CNS territory of the DREZ contains synaptogenic molecules associated with non-neuronal glial cells (possibly NG2 glia). Growth inhibitors fail to repel most axons at the interface. Most axons therefore invade the CNS territory of the DREZ and then cease their growth by forming presynaptic nerve terminals (red-colored swellings on axon tips and shafts). The few axons that are stopped and exhibit dystrophic endings (e.g., axon with grey-colored tip) may also be immobilized by forming presynaptic terminals on their axon shaft.

Interestingly, the presynaptic active zones observed in DR axons stopped at the DREZ are thinner than at neuron-neuron synapses and resemble those reported at neuron-NG2 cell synapses [100, 101]. Indeed, we have found that NG2 immunolabels are almost always colocalized with the synaptic label SV2 in DR axons stopped at the DREZ (Kim et al., unpublished data). An intriguing possibility therefore is that DR axons fail to regenerate into spinal cord primarily because they form synapses with NG2 glia, which transforms the axons from growth to differentiation mode (Fig. 5).

CAVEATS

Several caveats concerning the imaging studies, however, make our interpretation tentative and justify a more thorough investigation. A particular concern is that the limited spatiotemporal resolution of our axon growth tip imaging might have caused us to overlook subtle local motility. In addition, because we imaged only a few large-diameter axons in a small number of mice, we have little information about possible

http://dx.doi.org/10.5607/en.2012.21.3.83

variability in the responses of the heterogeneous populations of DRG neurons. Imaging more DR axons of all different types at higher spatiotemporal resolution will therefore be necessary. Direct demonstration *in vivo* of the consequences of selectively ablating NG2 glia and astrocytes and eliminating individual or combinations of growth-inhibiting molecules will also be extremely useful to identify the molecular and cellular factor(s) that play a definitive role in preventing regeneration at the DREZ.

CONCLUSION

Both intrinsic and extrinsic factors, particularly growth-inhibitory molecules associated with astrocytes and oligodendrocytes, are thought to contribute to regeneration failure at the DREZ. Several observations previously reported in the literature, however, make it unlikely that these factors are the critical determinant(s) of the regeneration failure: 1) Even after myelin-associated inhibitory cues have disappeared due to oligodendrocyte degeneration, the DREZ continues to prevent regeneration; 2) CSPGs associated with astrocytes and myelin-associated inhibitory cues collapse growth cones transiently, but do not immobilize or chronically stabilize growth cones; 3) Although central processes of DRG neurons in the DC and DR derive from the same neurons and encounter the same inhibitory cues, conditioning lesions enhance regeneration of DC axons [36, 102] but not DR axon regeneration across DREZ [38]; 4) CSPGs are expressed abundantly in the regenerating DR [53] and pharmacological degradation of CSPGs by ChABC does not promote regeneration across DREZ [23]. The first application of *in vivo* imaging to the DREZ provides further support. These studies showed that DR axons rarely turn around or retract at DREZ but quickly become immobile within its CNS territory, and then remain in place for long periods of time, perhaps permanently, even without target innervation. These observations are better explained by mechanisms that stabilize rather than continuously inhibit axon growth and, intriguingly, axons immobilized at the DREZ show numerous features of presynaptic terminals. What causes them to form presynaptic nerve endings and whether they form functional synapses remain to be determined. Future in-depth investigations will determine whether this novel idea, raised many years ago, applies to all the different subtypes of DRG neurons and whether it provides a decisive explanation for failed regeneration at the DREZ and perhaps also within the spinal cord.

ACKNOWLEDGEMENTS

Our laboratory has been supported by grants from: Craig H. Neilsen Foundation, Muscular Dystrophy Association, National Institute of Neurological Disorders and Stroke, and Shriners Hospitals for Children. We also thank Srishti Bhagat, Derron Bishop, Alessandro Di Maio, Tim Himes, and Amy Kim for their contributions to successful application of *in vivo* imaging to monitoring regenerating dorsal roots.

REFERENCES

1. Chong MS, Reynolds ML, Irwin N, Coggeshall RE, Emson PC, Benowitz LI, Woolf CJ (1994) GAP-43 expression in primary sensory neurons following central axotomy. J Neurosci 14:4375-4384.
2. Oblinger MM, Lasek RJ (1984) A conditioning lesion of the peripheral axons of dorsal root ganglion cells accelerates regeneration of only their peripheral axons. J Neurosci 4:1736-1744.
3. Wujek JR, Lasek RJ (1983) Correlation of axonal regeneration and slow component B in two branches of a single axon. J Neurosci 3:243-251.
4. Griffin JW, Price DL (1981) Schwann cell and glial responses in beta, beta'-iminodipropionitrile intoxication. I. Schwann cell and oligodendrocyte ingrowths. J Neurocytol 10:995-1007.
5. Di Maio A, Skuba A, Himes BT, Bhagat SL, Hyun JK, Tessler A, Bishop D, Son YJ (2011) In vivo imaging of dorsal root regeneration: rapid immobilization and presynaptic differentiation at the CNS/PNS border. J Neurosci 31:4569-4582.
6. Golding JP, Bird C, McMahon S, Cohen J (1999) Behaviour of DRG sensory neurites at the intact and injured adult rat dorsal root entry zone: postnatal neurites become paralysed, whilst injury improves the growth of embryonic neurites. Glia 26:309-323.
7. Ramer MS, Duraisingam I, Priestley JV, McMahon SB (2001) Two-tiered inhibition of axon regeneration at the dorsal root entry zone. J Neurosci 21:2651-2660.
8. Silver J, Miller JH (2004) Regeneration beyond the glial scar. Nat Rev Neurosci 5:146-156.
9. Rhodes KE, Fawcett JW (2004) Chondroitin sulphate proteoglycans: preventing plasticity or protecting the CNS? J Anat 204:33-48.
10. Yiu G, He Z (2006) Glial inhibition of CNS axon regeneration. Nat Rev Neurosci 7:617-627.
11. Oertle T, Schwab ME (2003) Nogo and its paRTNers. Trends Cell Biol 13:187-194.
12. Smith GM, Falone AE, Frank E (2012) Sensory axon regene-

ration: rebuilding functional connections in the spinal cord. Trends Neurosci 35:156-163.
13. Ramer MS, McMahon SB, Priestley JV (2001) Axon regeneration across the dorsal root entry zone. Prog Brain Res 132:621-639.
14. Tessler A (2004) Neurotrophic effects on dorsal root regeneration into the spinal cord. Prog Brain Res 143:147-154.
15. Gavazzi I, Kumar RD, McMahon SB, Cohen J (1999) Growth responses of different subpopulations of adult sensory neurons to neurotrophic factors in vitro. Eur J Neurosci 11:3405-3414.
16. Ramer MS, Priestley JV, McMahon SB (2000) Functional regeneration of sensory axons into the adult spinal cord. Nature 403:312-316.
17. Andrews MR, Czvitkovich S, Dassie E, Vogelaar CF, Faissner A, Blits B, Gage FH, ffrench-Constant C, Fawcett JW (2009) Alpha9 integrin promotes neurite outgrowth on tenascin-C and enhances sensory axon regeneration. J Neurosci 29:5546-5557.
18. Harvey PA, Lee DH, Qian F, Weinreb PH, Frank E (2009) Blockade of Nogo receptor ligands promotes functional regeneration of sensory axons after dorsal root crush. J Neurosci 29:6285-6295.
19. Cafferty WB, Yang SH, Duffy PJ, Li S, Strittmatter SM (2007) Functional axonal regeneration through astrocytic scar genetically modified to digest chondroitin sulfate proteoglycans. J Neurosci 27:2176-2185.
20. Ma TC, Campana A, Lange PS, Lee HH, Banerjee K, Bryson JB, Mahishi L, Alam S, Giger RJ, Barnes S, Morris SM Jr, Willis DE, Twiss JL, Filbin MT, Ratan RR (2010) A large-scale chemical screen for regulators of the arginase 1 promoter identifies the soy isoflavone daidzeinas a clinically approved small molecule that can promote neuronal protection or regeneration via a cAMP-independent pathway. J Neurosci 30:739-748.
21. Ramer MS, Bishop T, Dockery P, Mobarak MS, O'Leary D, Fraher JP, Priestley JV, McMahon SB (2002) Neurotrophin-3-mediated regeneration and recovery of proprioception following dorsal rhizotomy. Mol Cell Neurosci 19:239-249.
22. Cafferty WB, Duffy P, Huebner E, Strittmatter SM (2010) MAG and OMgp synergize with Nogo-A to restrict axonal growth and neurological recovery after spinal cord trauma. J Neurosci 30:6825-6837.
23. Steinmetz MP, Horn KP, Tom VJ, Miller JH, Busch SA, Nair D, Silver DJ, Silver J (2005) Chronic enhancement of the intrinsic growth capacity of sensory neurons combined with the degradation of inhibitory proteoglycans allows functional regeneration of sensory axons through the dorsal root entry zone in the mammalian spinal cord. J Neurosci 25:8066-8076.
24. Wang R, King T, Ossipov MH, Rossomando AJ, Vanderah TW, Harvey P, Cariani P, Frank E, Sah DW, Porreca F (2008) Persistent restoration of sensory function by immediate or delayed systemic artemin after dorsal root injury. Nat Neurosci 11:488-496.
25. Harvey P, Gong B, Rossomando AJ, Frank E (2010) Topographically specific regeneration of sensory axons in the spinal cord. Proc Natl Acad Sci U S A 107:11585-11590.
26. Malessy MJ, Pondaag W (2009) Obstetric brachial plexus injuries. Neurosurg Clin N Am 20:1-14, v.
27. Hale HB, Bae DS, Waters PM (2010) Current concepts in the management of brachial plexus birth palsy. J Hand Surg Am 35:322-331.
28. Pham CB, Kratz JR, Jelin AC, Gelfand AA (2011) Child Neurology: Brachial plexus birth injury: What every neurologist needs to know. Neurology 77:695-697.
29. Waters PM (2005) Update on management of pediatric brachial plexus palsy. J Pediatr Orthop B 14:233-244.
30. Moore AM, MacEwan M, Santosa KB, Chenard KE, Ray WZ, Hunter DA, Mackinnon SE, Johnson PJ (2011) Acellular nerve allografts in peripheral nerve regeneration: a comparative study. Muscle Nerve 44:221-234.
31. Fox IK, Mackinnon SE (2011) Adult peripheral nerve disorders: nerve entrapment, repair, transfer, and brachial plexus disorders. Plast Reconstr Surg 127:105e-118e.
32. Mehta SH, Gonik B (2011) Neonatal brachial plexus injury: obstetrical factors and neonatal management. J Pediatr Rehabil Med 4:113-118.
33. Avellino AM, Hart D, Dailey AT, MacKinnon M, Ellegala D, Kliot M (1995) Differential macrophage responses in the peripheral and central nervous system during wallerian degeneration of axons. Exp Neurol 136:183-198.
34. Bignami A, Chi NH, Dahl D (1984) Regenerating dorsal roots and the nerve entry zone: an immunofluorescence study with neurofilament and laminin antisera. Exp Neurol 85:426-436.
35. Fraher JP, Dockery P, O'Leary D, Mobarak MS, Ramer MS, Bishop T, Kozolva E, Priestley EV, McMahon SB, Aldskogius H (2002) The dorsal root transitional zone model of CNS axon regeneration: morphological findings. J Anat 200:214.
36. Neumann S, Woolf CJ (1999) Regeneration of dorsal column fibers into and beyond the lesion site following adult spinal cord injury. Neuron 23:83-91.
37. Cao Z, Gao Y, Bryson JB, Hou J, Chaudhry N, Siddiq M,

 http://dx.doi.org/10.5607/en.2012.21.3.83

Martinez J, Spencer T, Carmel J, Hart RB, Filbin MT (2006) The cytokine interleukin-6 is sufficient but not necessary to mimic the peripheral conditioning lesion effect on axonal growth. J Neurosci 26:5565-5573.

38. Chong MS, Woolf CJ, Haque NS, Anderson PN (1999) Axonal regeneration from injured dorsal roots into the spinal cord of adult rats. J Comp Neurol 410:42-54.
39. Zhang Y, Zhang X, Wu D, Verhaagen J, Richardson PM, Yeh J, Bo X (2007) Lentiviral-mediated expression of polysialic acid in spinal cord and conditioning lesion promote regeneration of sensory axons into spinal cord. Mol Ther 15:1796-1804.
40. Carlstedt T (1985) Regenerating axons form nerve terminals at astrocytes. Brain Res 347:188-191.
41. Dockery P, Fraher JP, Mobarak MS, O'Leary D, Ramer MS, Bishop T, Kozlova E, Aldskogius H, Priestley JV, McMahon SB (2002) 24: The dorsal root transitional zone model of CNS axon regeneration: morphometric findings. J Anat 200:207
42. Fraher JP (2000) The transitional zone and CNS regeneration. J Anat 196:137-158.
43. Sims TJ, Gilmore SA (1994) Regrowth of dorsal root axons into a radiation-induced glial-deficient environment in the spinal cord. Brain Res 634:113-126.
44. Carlstedt T, Dalsgaard CJ, Molander C (1987) Regrowth of lesioned dorsal root nerve fibers into the spinal cord of neonatal rats. Neurosci Lett 74:14-18.
45. Fawcett JW, Asher RA (1999) The glial scar and central nervous system repair. Brain Res Bull 49:377-391.
46. Grimpe B, Pressman Y, Lupa MD, Horn KP, Bunge MB, Silver J (2005) The role of proteoglycans in Schwann cell/astrocyte interactions and in regeneration failure at PNS/CNS interfaces. Mol Cell Neurosci 28:18-29.
47. Busch SA, Silver J (2007) The role of extracellular matrix in CNS regeneration. Curr Opin Neurobiol 17:120-127.
48. Pindzola RR, Doller C, Silver J (1993) Putative inhibitory extracellular matrix molecules at the dorsal root entry zone of the spinal cord during development and after root and sciatic nerve lesions. Dev Biol 156:34-48.
49. Fidler PS, Schuette K, Asher RA, Dobbertin A, Thornton SR, Calle-Patino Y, Muir E, Levine JM, Geller HM, Rogers JH, Faissner A, Fawcett JW (1999) Comparing astrocytic cell lines that are inhibitory or permissive for axon growth: the major axon-inhibitory proteoglycan is NG2. J Neurosci 19:8778-8788.
50. Donnelly EM, Strappe PM, McGinley LM, Madigan NN, Geurts E, Rooney GE, Windebank AJ, Fraher J, Dockery P, O'Brien T, McMahon SS (2010) Lentiviral vector-mediated knockdown of the NG2 [corrected] proteoglycan or expression of neurotrophin-3 promotes neurite outgrowth in a cell culture model of the glial scar. J Gene Med 12:863-872.
51. Shen Y, Tenney AP, Busch SA, Horn KP, Cuascut FX, Liu K, He Z, Silver J, Flanagan JG (2009) PTPsigma is a receptor for chondroitin sulfate proteoglycan, an inhibitor of neural regeneration. Science 326:592-596.
52. Fisher D, Xing B, Dill J, Li H, Hoang HH, Zhao Z, Yang XL, Bachoo R, Cannon S, Longo FM, Sheng M, Silver J, Li S (2011) Leukocyte common antigen-related phosphatase is a functional receptor for chondroitin sulfate proteoglycan axon growth inhibitors. J Neurosci 31:14051-14066.
53. Zhang Y, Tohyama K, Winterbottom JK, Haque NS, Schachner M, Lieberman AR, Anderson PN (2001) Correlation between putative inhibitory molecules at the dorsal root entry zone and failure of dorsal root axonal regeneration. Mol Cell Neurosci 17:444-459.
54. Bradbury EJ, Moon LD, Popat RJ, King VR, Bennett GS, Patel PN, Fawcett JW, McMahon SB (2002) Chondroitinase ABC promotes functional recovery after spinal cord injury. Nature 416:636-640.
55. McKeon RJ, Höke A, Silver J (1995) Injury-induced proteoglycans inhibit the potential for laminin-mediated axon growth on astrocytic scars. Exp Neurol 136:32-43.
56. Hunt D, Coffin RS, Anderson PN (2002) The Nogo receptor, its ligands and axonal regeneration in the spinal cord; a review. J Neurocytol 31:93-120.
57. Hunt D, Mason MR, Campbell G, Coffin R, Anderson PN (2002) Nogo receptor mRNA expression in intact and regenerating CNS neurons. Mol Cell Neurosci 20:537-552.
58. Venkatesh K, Chivatakarn O, Lee H, Joshi PS, Kantor DB, Newman BA, Mage R, Rader C, Giger RJ (2005) The Nogo-66 receptor homolog NgR2 is a sialic acid-dependent receptor selective for myelin-associated glycoprotein. J Neurosci 25:808-822.
59. Atwal JK, Pinkston-Gosse J, Syken J, Stawicki S, Wu Y, Shatz C, Tessier-Lavigne M (2008) PirB is a functional receptor for myelin inhibitors of axonal regeneration. Science 322:967-970.
60. Kerschensteiner M, Schwab ME, Lichtman JW, Misgeld T (2005) In vivo imaging of axonal degeneration and regeneration in the injured spinal cord. Nat Med 11:572-577.
61. Davies SJ, Goucher DR, Doller C, Silver J (1999) Robust regeneration of adult sensory axons in degenerating white

matter of the adult rat spinal cord. J Neurosci 19:5810-5822.
62. Lee JK, Geoffroy CG, Chan AF, Tolentino KE, Crawford MJ, Leal MA, Kang B, Zheng B (2010) Assessing spinal axon regeneration and sprouting in Nogo-, MAG-, and OMgp-deficient mice. Neuron 66:663-670.
63. Lee JK, Chow R, Xie F, Chow SY, Tolentino KE, Zheng B. (2010) Combined genetic attenuation of myelin and semaphorin-mediated growth inhibition is insufficient to promote serotonergic axon regeneration. J Neurosci 30:10899-10904.
64. Snow DM, Watanabe M, Letourneau PC, Silver J (1991) A chondroitin sulfate proteoglycan may influence the direction of retinal ganglion cell outgrowth. Development 113:1473-1485.
65. Li M, Shibata A, Li C, Braun PE, McKerracher L, Roder J, Kater SB, David S (1996) Myelin-associated glycoprotein inhibits neurite/axon growth and causes growth cone collapse. J Neurosci Res 46:404-414.
66. Marsh L, Letourneau PC (1984) Growth of neurites without filopodial or lamellipodial activity in the presence of cytochalasin B. J Cell Biol 99:2041-2047.
67. Jones SL, Selzer ME, Gallo G (2006) Developmental regulation of sensory axon regeneration in the absence of growth cones. J Neurobiol 66:1630-1645.
68. Jin LQ, Zhang G, Jamison C Jr, Takano H, Haydon PG, Selzer ME (2009) Axon regeneration in the absence of growth cones: acceleration by cyclic AMP. J Comp Neurol 515:295-312.
69. Liuzzi FJ, Lasek RJ (1987) Astrocytes block axonal regeneration in mammals by activating the physiological stop pathway. Science 237:642-645.
70. Ertürk A, Hellal F, Enes J, Bradke F (2007) Disorganized microtubules underlie the formation of retraction bulbs and the failure of axonal regeneration. J Neurosci 27:9169-9180.
71. Allen NJ, Barres BA (2009) Neuroscience: Glia - more than just brain glue. Nature 457:675-677.
72. Ullian EM, Christopherson KS, Barres BA (2004) Role for glia in synaptogenesis. Glia 47:209-216.
73. Lichtman JW, Sanes JR (2003) Watching the neuromuscular junction. J Neurocytol 32:767-775.
74. Bishop DL, Misgeld T, Walsh MK, Gan WB, Lichtman JW (2004) Axon branch removal at developing synapses by axosome shedding. Neuron 44:651-661.
75. Balice-Gordon RJ, Lichtman JW (1990) In vivo visualization of the growth of pre- and postsynaptic elements of neuromuscular junctions in the mouse. J Neurosci 10:894-908.
76. Trachtenberg JT, Chen BE, Knott GW, Feng G, Sanes JR, Welker E, Svoboda K (2002) Long-term in vivo imaging of experience-dependent synaptic plasticity in adult cortex. Nature 420:788-794.
77. Pan F, Gan WB (2008) Two-photon imaging of dendritic spine development in the mouse cortex. Dev Neurobiol 68:771-778.
78. Grutzendler J, Gan WB (2006) Two-photon imaging of synaptic plasticity and pathology in the living mouse brain. NeuroRx 3:489-496.
79. Misgeld T, Nikic I, Kerschensteiner M (2007) In vivo imaging of single axons in the mouse spinal cord. Nat Protoc 2:263-268.
80. Davalos D, Lee JK, Smith WB, Brinkman B, Ellisman MH, Zheng B, Akassoglou K (2008) Stable in vivo imaging of densely populated glia, axons and blood vessels in the mouse spinal cord using two-photon microscopy. J Neurosci Methods 169:1-7.
81. Steward O, Zheng B, Tessier-Lavigne M (2003) False resurrections: distinguishing regenerated from spared axons in the injured central nervous system. J Comp Neurol 459:1-8.
82. Hanna AS, Son YJ, Dempsey R (2011) Live imaging of dorsal root regeneration and the resurgence of a forgotten idea. Neurosurgery 69:N18-N21.
83. Skuba A, Himes BT, Son YJ (2011) Live imaging of dorsal root axons after rhizotomy. J Vis Exp (55):e3126.
84. Ramon Y Cajal S (1928) Degeneration and regeneration of the nervous system (May RM, translator), Haffner, New York.
85. Bernstein JJ, Bernstein ME (1971) Axonal regeneration and formation of synapses proximal to the site of lesion following hemisection of the rat spinal cord. Exp Neurol 30:336-351.
86. Mangin JM, Gallo V (2011) The curious case of NG2 cells: transient trend or game changer? ASN Neuro 3:e00052.
87. Sakry D, Karram K, Trotter J (2011) Synapses between NG2 glia and neurons. J Anat 219:2-7.
88. Richardson WD, Young KM, Tripathi RB, McKenzie I (2011) NG2-glia as multipotent neural stem cells: fact or fantasy? Neuron 70:661-673.
89. Sakry D, Karram K, Trotter J (2011) Synapses between NG2 glia and neurons. J Anat 219:2-7.
90. Maldonado PP, Vélez-Fort M, Angulo MC (2011) Is neuronal communication with NG2 cells synaptic or extrasynaptic? J Anat 219:8-17.
91. Fröhlich N, Nagy B, Hovhannisyan A, Kukley M (2011)

http://dx.doi.org/10.5607/en.2012.21.3.83

Fate of neuron-glia synapses during proliferation and differentiation of NG2 cells. J Anat 219:18-32.
92. Trotter J, Karram K, Nishiyama A (2010) NG2 cells: Properties, progeny and origin. Brain Res Rev 63:72-82.
93. Bergles DE, Jabs R, Steinhäuser C (2010) Neuron-glia synapses in the brain. Brain Res Rev 63:130-137.
94. Kukley M, Capetillo-Zarate E, Dietrich D (2007) Vesicular glutamate release from axons in white matter. Nat Neurosci 10:311-320.
95. Ziskin JL, Nishiyama A, Rubio M, Fukaya M, Bergles DE (2007) Vesicular release of glutamate from unmyelinated axons in white matter. Nat Neurosci 10:321-330.
96. GrandPré T, Nakamura F, Vartanian T, Strittmatter SM (2000) Identification of the Nogo inhibitor of axon regeneration as a Reticulon protein. Nature 403:439-444.
97. Barres BA (2008) The mystery and magic of glia: a perspective on their roles in health and disease. Neuron 60:430-440.
98. Morgenstern DA, Asher RA, Naidu M, Carlstedt T, Levine JM, Fawcett JW (2003) Expression and glycanation of the NG2 proteoglycan in developing, adult, and damaged peripheral nerve. Mol Cell Neurosci 24:787-802.
99. Beggah AT, Dours-Zimmermann MT, Barras FM, Brosius A, Zimmermann DR, Zurn AD (2005) Lesion-induced differential expression and cell association of Neurocan, Brevican, Versican V1 and V2 in the mouse dorsal root entry zone. Neuroscience 133:749-762.
100. Bergles DE, Roberts JD, Somogyi P, Jahr CE (2000) Glutamatergic synapses on oligodendrocyte precursor cells in the hippocampus. Nature 405:187-191.
101. Lin SC, Bergles DE (2004) Synaptic signaling between GABAergic interneurons and oligodendrocyte precursor cells in the hippocampus. Nat Neurosci 7:24-32.
102. Woolf CJ, Shortland P, Coggeshall RE (1992) Peripheral nerve injury triggers central sprouting of myelinated afferents. Nature 355:75-78.