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TITLE

Differential Regenerative Ability of Sensory and Motor Neurons

AUTHORS

Menghon Cheah, James W. Fawcett*, Barbara Haenzi*

AFFILIATIONS

John van Geest Centre for Brain Repair, University of Cambridge, Cambridge CB2 0PY, United Kingdom.

*Co-correspondence: B.H. (bh432@cam.ac.uk), J.W.F. (jf108@cam.ac.uk)

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CONFLICT OF INTERESTS

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ABSTRACT

After injury, the adult mammalian central nervous system (CNS) lacks long-distance axon regeneration. This review discusses the similarities and differences of sensory and motor neurons, seeking to understand how to achieve functional sensory and motor regeneration. As these two types of neurons respond differently to axotomy, growth environment and treatment, the future challenge will be on how to achieve full recovery in a way that allows regeneration of both types of fibers simultaneously.

INTRODUCTION

After spinal cord injury (SCI), long-distance axon regeneration in the adult mammalian CNS is a challenging task. There is a vast diversity of axonal tracts in the spinal cord that need to grow for long distances and contact appropriate targets. This review compares the regenerative responses of sensory and motor neurons, focusing particularly on their differences and on what this teaches us about regeneration.

For sensory neurons, we focus on dorsal root ganglion (DRG) neurons which are the afferent neurons relaying sensory information from the periphery to the brain. With cell bodies in the peripheral nervous system (PNS) and axons in both the PNS and CNS, DRG neurons give us insights as to why axons regenerate differently in the PNS and CNS environments [1]. For motor neurons, we focus on upper motor neurons, particularly the corticospinal tract (CST) whose neurons are located in the deeper layers of the sensorimotor cortex with axons projecting down the spinal cord.

Sensory and motor neurons are different from each other in many aspects: anatomy, surrounding environment, response to injury, and growth requirements. In this review, we aim to decipher some of these differences, analyzing how these two types of neurons respond to injury, and therefore provide an insight into how they can be stimulated to promote regeneration (Figure 1A

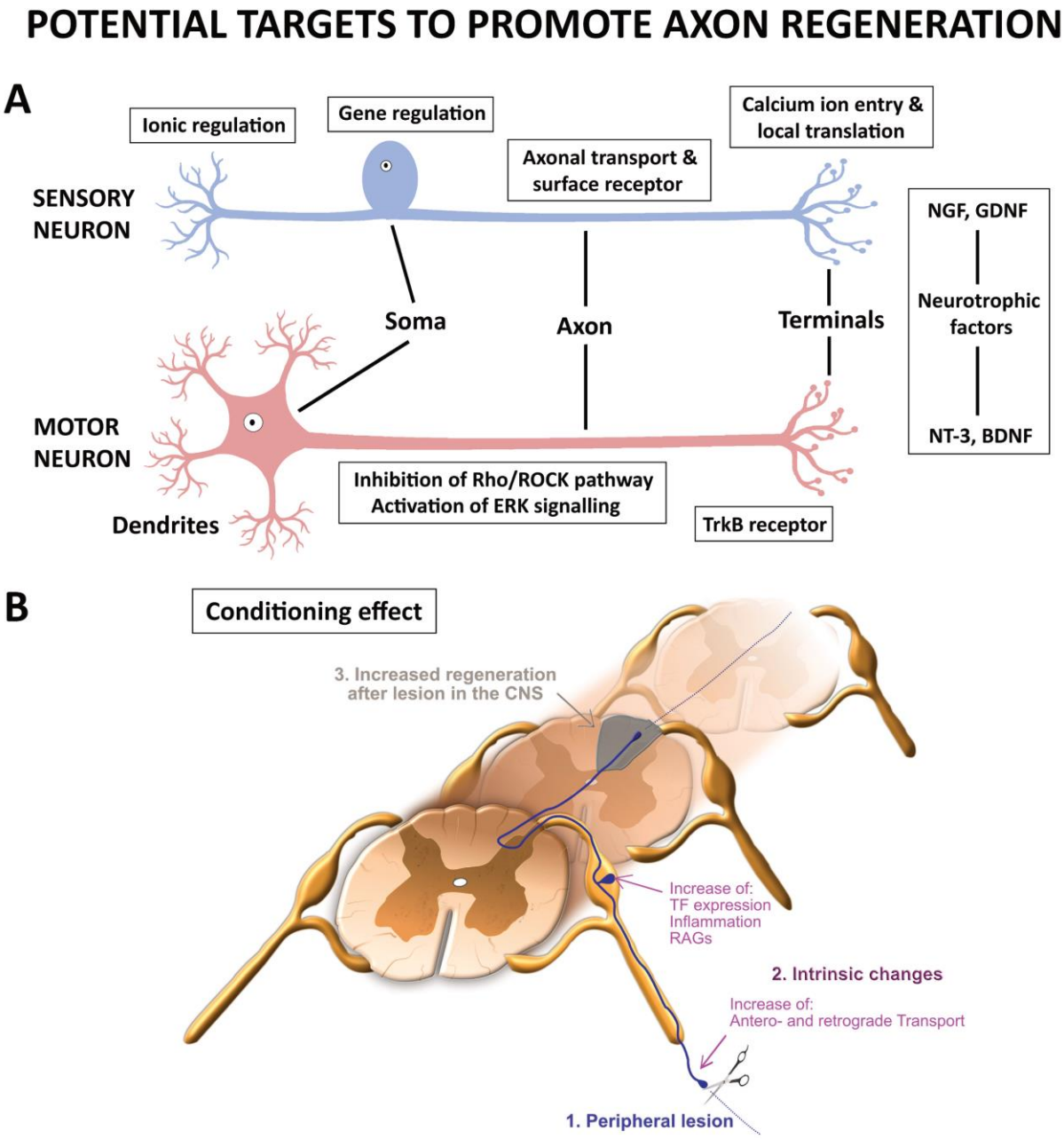

Figure 1. Potential targets to promote axon regeneration

(A) Sensory and motor neurons can be targeted differently for regeneration. **(B)** Conditioning effect facilitates sensory regeneration in the CNS due to intrinsic changes in the DRG neuron and axon following peripheral lesion.

INTRINSIC DIFFERENCES

Sensory and motor neurons have different developmental origins which arise during neurulation. The neural tube gives rise to components of the brain and spinal cord including motor neurons, while the neural plate border develops the neural crest to form components of the PNS including DRG neurons. During dorsal-ventral patterning of the neural tube, the roof plate is exposed to a concentration gradient of bone morphogenic proteins (BMPs) whereas the floor plate to an opposing gradient of Sonic Hedgehog (SHH) [2]. As both BMPs and SHH are morphogens with cell-fate-determining activity [3], they critically affect the development of sensory and motor tracts in the spinal cord, long before the presence of a functioning nervous system. Anatomically, motor neurons have a single axon and multiple dendrites, while sensory neurons lack dendrites but their axon splits into a central and peripheral branch destined to exist in different environments.

Early Events after Injury

Ionic changes

Axotomy disrupts the axonal membrane resulting in extracellular Ca^{2+} influx, which stimulates axonal degeneration and regeneration initiation. The Ca^{2+} rise is two-phasic, first a leak into the proximal axon, then a delayed entry through Ca^{2+} channels. This results in trains of action potentials in both sensory and motor neurons [4]. Ca^{2+} influx is crucial for resealing the impaired plasma membrane, intracellular ionic regulation and growth cone formation [5]. As demonstrated in DRG neurons, the lack of Ca^{2+} influx after axotomy significantly reduces their regenerative capacity [1] and local protein synthesis essential for growth cone initiation [6]. Physiological responses to injury can include changes to the resting membrane potential and membrane polarization [7]. These changes can be triggered by the Na^{+}-K^{+}-$2Cl^{-}$ type 1

cotransporter (NKCC1). As NKCC1 can regulate the concentration of intracellular Cl^-, it has a substantial effect in changing the resting membrane potential and modulating GABAergic activity [8]. This results in GABA having a depolarising effect on DRG and immature neurons, but a hyperpolarising effect on other adult neurons including motor neurons. As DRG neurons have a higher NKCC1 activity than motor neurons, they have a smaller variation of intracellular Cl^- during neuronal activity and are less prone to GABAergic presynaptic inhibition [9].

Signalling and transcriptional pathways

Immediately after injury, there is an upregulation of immediate early genes, and followed by regeneration associated genes (RAGs). A particular consequence of gene expression changes after sensory axotomy is the conditioning effect which is a phenomenon where prior peripheral branch injury results in increased central branch regeneration (Figure 1B). The peripheral branch of DRG neurons regenerates vigorously, but regeneration of the central branch located entirely in the CNS is comparable to motor axon regeneration [10]. The conditional effect was discovered when a dorsal column injury was performed in conjunction with grafting of a 2 mm piece of autologous sciatic nerve into the spinal cord lesion [11]. Analysis of the retrograde-traced L4-L5 DRGs revealed the peripherally-lesioned side regenerated more axons into the graft. Conditional lesioning can result in regeneration beyond the central lesion site given that peripheral lesioning happened 7-14 days before central lesioning [12]. This suggests that conditional lesioning results in intrinsic changes that are permissive for functional regeneration in the CNS. Changes that lead to the conditioning effect in sensory neurons give insights into the molecular mechanisms involved in axon regeneration.

Since then, more has been learnt about these intrinsic changes and this has generated an extensive literature, most of which is outside the scope of this article, and there have been numerous excellent reviews [13-16]. We will mention a few relevant issues here. Axotomy of sensory neurons leads to upregulation of many RAGs such as actin, growth associated tubulin isotopes, GAP-43 [17, 18], and cAMP [19]; downregulation of neurofilament proteins [20]; increase in expression of transcription factors such as ATF-3 [21], c-jun, Sox11 [22] and STAT3 [23] and regulators of translation such as arginase-1 [24]; reduced expression of ion channels and proteins involved in neurotransmitter synthesis; upregulation of local translation [25-27] and inflammation [28, 29] after sensory axotomy. The question remains if upregulation of these features in motor neurons of the CNS would lead to enhanced regeneration. While the underlying mechanism of conditional lesioning is still unknown, it is apparent that intrinsic and extrinsic factors are involved. Further mechanistic insight will hopefully allow to identify key factors that promote both sensory and motor axon regeneration. Additionally, local translation is another important mechanism of the conditioning effect. Preventing local translation in injured sensory axons reduces the expression of conditioning-associated genes and also reduces axon regeneration [6, 13, 30]. There are large numbers of mRNAs transported into sensory axons through specific mechanisms, and their translation is protected until they reach the regions of growth [31].

Intracellular signaling regulation is essential for axon outgrowth initiation. After injury, neurons upregulate the expression of a stress marker, ATF-3, which has a long-term effect in gene regulation. The level of ATF-3 is sustained in motor neurons resulting in stunted regeneration but only transient in sensory neurons which is favorable for regeneration [32].

Additionally, the Rho/ROCK pathway has been of particular interest as it regulates the actin cytoskeleton for axon outgrowth and growth cone motility. In the presence of growth inhibitors such as myelin and chondroitin sulfate proteoglycans (CSPGs), inactivation of Rho-associated kinase and inhibition of ROCK have been shown to promote axon outgrowth [33, 34]. However, the inhibition of the Rho/ROCK pathway differentially affects motor and sensory axon regeneration due to the diverse activation levels of RhoA [35]. Motor neurons were shown to be more responsive and extended longer axons in response to ROCK inhibition in a CSPG-environment than sensory neurons.

Axonal Transport

The morphology and behaviour of an axon depends on the molecules it contains. For highly polarised neurons such as motor neurons, selective axonal transport is required to maintain polarity [36]. Regeneration is inhibited if molecules required for growth are selectively excluded. We have studied the integrin transmembrane receptors because of their role in promoting long-distance functional axon regeneration in the CNS [37, 38]. Upon ligand binding and activation, integrin signalling has widespread effects ranging from short-term effects such as cell adhesion and mobility, to long-term effects such as proliferation and differentiation which may include changes in gene expression [39]. In sensory neurons, integrin can promote extensive axon regeneration: α9 integrin in conjunction with an integrin activator kindlin-1 promotes long-distance (25 mm) functional sensory axon regeneration in a growth-inhibitory CSPG and tenascin-environment [38]. However as adult CST motor neurons mature, integrins are selectively excluded from axons [40, 41], along with two other potentially regeneration-promoting receptors trkB and IGFR [42, 43]. This demonstrates a key difference between sensory and motor axons: in sensory neurons most expressed

molecules enter the axons, while in motor axons many growth-promoting molecules are excluded. Apart from transport differences, sensory and motor neurons express different integrins leading to different integrin-binding substrate preferences at early postnatal stages [44], further demonstrating the intrinsic differences between these neurons. Having said that, an important and unresolved question is the extent to which the local translation of axonal mRNAs, which is so important for sensory axon regeneration and the conditioning effect, also occurs in motor axons. There is evidence for some RNAs in CNS axons, but nothing is known about ribosomes in mature axons.

It is worth noting that currently there is only a limited number of studies that directly address the intrinsic molecular differences between sensory and motor neurons for regeneration. In the future, cell-specific analyses such as RNA-sequencing to study their individual profiles may be valuable to shed more light on this topic.

EXTRINSIC DIFFERENCES

In addition to intrinsic differences, the surrounding environment of the neurons also influence regeneration.

Neurotrophic Factors (NFs)

NFs are important for the survival and functioning of neurons. Neurotrophins have been shown to be a potential therapeutic tool to promote axon regeneration after injury as they serve as growth-promoting and guidance molecules [45, 46]. In a NF-embedded collagen matrix, sensory neurons showed a higher growth capacity than motor neurons [47]. The same

study also revealed that NGF has specific effects on sensory outgrowth, while BDNF on motor outgrowth, and GDNF enhances regeneration of both neurons. Having said that, experimental issues such as different quantification approaches, the age and type of neurons used across different studies can affect the results greatly, leading to inconsistencies in the literature. For instance, in one study it was shown that lentivirus-mediated overexpression of NGF and GDNF did not have an additional effect on increasing the number of regenerated sensory axons, and GDNF resulted in the trapping of motor axons and impairment of long-distance outgrowth [48]. These inconsistent results definitely highlight the need for the correct use of NFs at a specific time and dosage for the proper regeneration of each class of neurons [45].

Glial Cells

Glial cells provide neurons with trophic support and myelination. Schwann cells have an active role in supporting regeneration of peripheral neurons by clearing myelin debris, providing axonal guidance and remyelination. Due to this regeneration-supportive role, Schwann cell transplantation has long been an attractive treatment strategy for spinal cord injury [49]. A consistent observation has been that Schwann cell grafts attract many sensory axons, but less CNS axons [50]. The regenerative response of sensory axons in the PNS does not depend on the distance of axotomy from the cell body, but regeneration of motor axons into peripheral nerve grafts is more plentiful when the grafts are closer to the cell bodies [51]. In addition to the myelinating and non-myelinating phenotypes, Schwann cells also express sensory and motor phenotypes in response to cell type-specific promotion of regeneration [52]. Cutaneous Schwann cells preferentially express growth factors (such as NGF, BDNF, VEGF) that support sensory axon regeneration while ventral root Schwann cells

preferentially support motor axon regeneration with GDNF and pleiotrophin [52]. Additionally, Schwann cell remyelination alone can result in differential sensory and motor behavioural recovery despite having the same amount of axon regeneration [53]. By having a better understanding of axon-Schwann cell interactions, the outcome of cell type-specific axon regeneration can certainly be improved.

Extracellular Matrix (ECM) Molecules

ECM molecules can be growth permissive or inhibitory to different types of neurons depending on their developmental stage and the type of receptors they express. Specific targeting of ECM molecules can promote axon regeneration. For instance, digestion of CSPGs by chondroitinase ABC promotes regeneration of both sensory and motor neurons, although the main effect on CST axons is sprouting rather than regeneration [54, 55]. Additionally, nerve injury also induces the expression of another family of ECM molecules, heparan sulfate proteoglycans (HSPGs) which are crucial for neuronal survival and sensory and motor regeneration [56, 57]. However, sensory and motor neurons can also respond differently to ECM molecules. For example, the glycoprotein osteopontin induced outgrowth of motor but not sensory neurons, while clusterin induced sensory but not motor axon outgrowth [58]. In another study, postnatal DRG neurons were shown to have a substrate preference for laminin while lower motor neurons prefer fibronectin [44]. Due to the difference in substrate preference, these environmental interactions can affect neuronal regeneration directly.

GRAFTS

Various types of grafts have been used for spinal cord repair, including Schwann cell (discussed above) and embryonic tissue grafts. Here, we discuss NF-secreting grafts (Table 1).

Injury	Graft	NF	Growth into graft/lesion	Growth beyond graft/lesion	CST	RST	5HT	TH	ChAT	Sensory	Ref
Thoracic dorsal hemisection	Secreting fibroblasts	NGF	Yes	No	No	-	No	Yes	Yes	CGRP	[59]
Chronic mid-thoracic dorsal hemisection	Secreting fibroblasts	NGF	Yes	-	No	-	-	Yes	Yes	CGRP	[60]
T7 complete transection or dorsal hemisection	Secreting fibroblasts	BDNF	Yes	No	-	-	Yes	-	Yes	-	[61]
Complete C3/4 lateral funicular	Secreting fibroblasts	GDNF	Yes	-	No	Yes	Yes	No	No	CGRP	[62]
Thoracic dorsal hemisection	None	NT-3 Injection	No	Yes	sprouting	-	-	-	-	-	[63]
T7 dorsal hemisection	Secreting fibroblasts	NT-3	Yes	No	Yes	-	-	-	-	-	[67]
T7 hemisection	Secreting fibroblasts	LIF	around	Yes	Yes	-	No	No	No	-	[68]
T9 dorsal column transection + conditional lesion	Pre-degenerated nerve	NGF, BDNF, NT-3 via pump	Yes	Yes	-	-	-	-	-	Yes	[70]
T6 dorsal column crush	None	BDNF, GDNF, NT-3 via pump	No No Yes	No No Yes	- - -	- - -	- - -	- - -	- - -	No No Yes	[71]

Table 1. Summary of selected studies using NF-secreting grafts to promote axon regeneration

NF: neurotrophic factor; NGF: nerve growth factor; BDNF: brain-derived neurotrophic factor; GDNF: glial-derived neurotrophic factor; NT-3: neurotrophic factor-3; LIF: leukemia inhibitory factor; CST: corticospinal tract; RST: rubrospinal tract; 5HT: serotonergic fibres; TH: tyrosine hydroxylase-positive coerulospinal fibres; ChAT: acetylcholine transferase-positive lower motor neurons.

In an early study where a NGF-secreting fibroblast graft was transplanted into the lesion cavity, ingrowth of diverse sensory fibres was observed three months after injury [59]. In

contrast, a lesser amount of ingrowth was observed in grafted uninjured animals, indicating that the injury upregulates responsiveness. In another closely-related study, NGF-graft transplantation was performed in a chronic model and similar results were reported [60]. These studies illustrate that sensory and motor neurons can be stimulated to re-grow and that the injury itself changes the responsiveness of neurons to NFs. Others have used GDNF- [61] or BDNF-secreting fibroblast grafts [62] and found a variety of motor and sensory fibers growing into and beyond these grafts. These studies show that cell grafts combined with NFs stimulate growth of different sensory and motor fibres and that responsiveness is determined by receptor expression of fibre subtypes.

The CST is the most challenging tract for regeneration probably because CST axons are long and branched and show little response to axotomy. Nevertheless, NT-3 has shown to be promising. When a single injection of NT-3 rostral to the lesion site was given, CST sprouting but not growth was observed [63], similar results have been shown by others [64-66]. In contrast, CST axonal growth in the grey matter of up to 8 mm distal to the lesion site was observed when NT-3-secreting fibroblasts were grafted [67]. Other than NT-3, significant growth was also achieved using a leukaemia inhibitor factor (LIF)-secreting fibroblast graft [68]. Interestingly, since LIF secretion resulted in increased NT-3 expression it was inconclusive if the reported effect was due to a direct effect of LIF or via NT-3, or both. As compared to other axons, regenerating CST axons did not penetrate the graft but grew through the grey matter, indicating that the inhibitory environment of the scar might be more averse to CST axons than to other fibres and it could make CST growth more challenging to detect. Furthermore, these studies illustrate that it is crucial how NFs are delivered; while a single injection did not result in growth of CST axons, grafting of NT3-secreting fibroblasts did.

NT-3 also promotes regeneration of sensory fibres such as the trkC-expressing proprioceptive axons [69]. In a conditional lesioning study, the sciatic nerve was transected one week before injury with a piece of the distal stump collected and pre-degenerated before grafting [70]. At the time of injury, an osmotic minipump containing β-NGF, BDNF, NT-3, or a mixture of these was implanted and infused for two weeks. Only the NT3-treated animals showed sensory fibres of up to 3 mm into the distal tissue originating from the injured sciatic nerve. Infusion from osmotic minipumps presumably sets up a gradient of neurotrophins enabling sensory fibres to grow beyond the graft. This suggests that it is not just the graft/host barrier that prevents growth. In another study delivering BDNF, GDNF or NT-3 for four weeks, the lesion appeared more extensive in GDNF-treated animals with fibres growing around the lesion, but not into or beyond [71]. In NT-3-treated animals, an abundance of fibres sprouted at the lesion site with many fine fibres growing into and beyond (4 mm) the lesion. However, the fibres did not grow in a directed or aligned manner. In BDNF-treated animals, the fibres ascended in the gracile fasciculus and stopped at the lesion site. This is an unexpected result since it has been shown by others that BDNF-secreting fibroblast grafts result in ingrowth of sensory fibres into the graft [62], illustrating again how different studies will lead to different conclusions mainly due to experimental setup rather than true differences in regenerative potential.

In summary, sensory and motor fibres respond to grafts containing NFs. The differences observed could be partly due to differential expression of receptors or experimental setups. However, there is no bias towards sensory or motor neuron regeneration.

CONCLUSION

Intrinsic and extrinsic differences contribute to the differential regenerative abilities of sensory and motor neurons which are of different developmental origins and prefer different environments for growth and functioning. Both fibres respond to growth-promoting treatments to different degrees after injury. Upper motor neurons, such as the CST, are clearly the most challenging tracts for regeneration. The future challenge will be on how to achieve SCI recovery in a way that allows regeneration of both types of fibers simultaneously. Deeper understanding of the conditioning effect might allow us to understand successful regeneration and give us tools to manipulate upper motor neuron tracts for better regeneration. Local translation and expression of RAGs in injured CNS axons are promising approaches. Even though specific mechanisms, such as conditioning lesioning, axonal transport and local translation are better understood in sensory neurons, neither sensory nor motor neuron regeneration is to date in a satisfactory functional way. It is very likely that a combinatorial strategy is required to promote a diversity of injured axons to regenerate after SCI.

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