

● HIGHLIGHTS

## Regulation of axonal remodeling following spinal cord injury

Central nervous system injuries, such as spinal cord injury (SCI), are a leading cause of disability in young adults. SCIs generally have severe clinical consequences and often lead to loss of motor or sensory input below the segment of injury. While complete injuries generally show very little improvement over time, functional recovery can be seen following partial injuries. A number of studies over the last decade have aimed to identify the anatomical basis of this recovery process. They showed that while long-distance axonal regeneration is restricted in the mammalian central nervous system (CNS), supraspinal and intraspinal CNS axons can remodel after injury (Weidner et al., 2001; Bareyre et al., 2004; Girgis et al., 2007; van den Brand et al., 2012; Zörner et al., 2014).

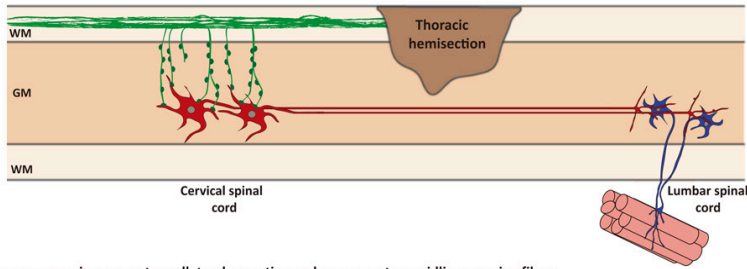
The potential of axons to remodel following CNS injury has been studied for several axonal tract systems including the corticospinal tract (CST), an important descending motor pathway. It could for example be shown that a complete transection of the main dorsal corticospinal pathway triggers sprouting of the minor CST components: for instance, sprouting of the ventral corticospinal tract that is associated with functional recovery (Weidner et al., 2001) as well as sprouting of the dorsolateral corticospinal tract (Bareyre et al., 2005). Fouad and collaborators (2001) further demonstrated that a thoracic hemisection that interrupts the main hindlimb dorsal and dorsolateral components of the corticospinal tract triggers sprouting of dorsal corticospinal collaterals into the cervical spinal cord, remotely from the level of injury. Subsequent studies showed that this newly sprouted collaterals were able to contact spinal relay neurons, such as propriospinal neurons (PSNs) located in the intermediate and ventral laminae of the cervical spinal cord (**Figure 1A**; Bareyre et al., 2004; Lang et al., 2012). Two populations of propriospinal relay neurons were examined more closely in these studies: Short propriospinal neurons (SPSNs), which are important for visually-guided target reaching and project from cervical to upper thoracic segments and long propriospinal neurons (LPSNs), which project in the ventral funiculus from cervical to lumbar segments and are important for the coupling of the hindlimb and forelimb movements. Importantly the long propriospinal pathway, due to its ventral localization is spared in classical thoracic hemisection models. While early following SCI, both populations of relay neurons are contacted by new hindlimb CST collaterals in the cervical cord, refinement of the contacts takes place over time and many contacts onto short propriospinal neurons are eliminated. In the meanwhile, contacts onto long propriospinal neurons are maintained and these neurons in turn increased their synaptic contacts onto lumbar motoneurons, the initial targets of the dorsal hindlimb CST. This remodeling that takes place at multiple spinal levels thus leads to the formation of an intraspinal detour circuit that allows bypassing the lesion site and contributes at least partially to the functional recovery that is seen after SCI in rodents (**Figure 1A**; Bareyre et al., 2004; Lang et al., 2012). In recent years,

additional examples of supraspinal and intraspinal remodeling processes involving different axonal track systems and different injury paradigms have been reported (Courtine et al., 2008; Filli et al., 2014) indicating that axonal remodeling is a more general hallmark of the adaptive response to CNS injuries. While the importance of such circuit remodeling for functional recovery is now well established (van den Brand et al., 2012), the molecular regulation of the different steps of the remodeling is much less understood.

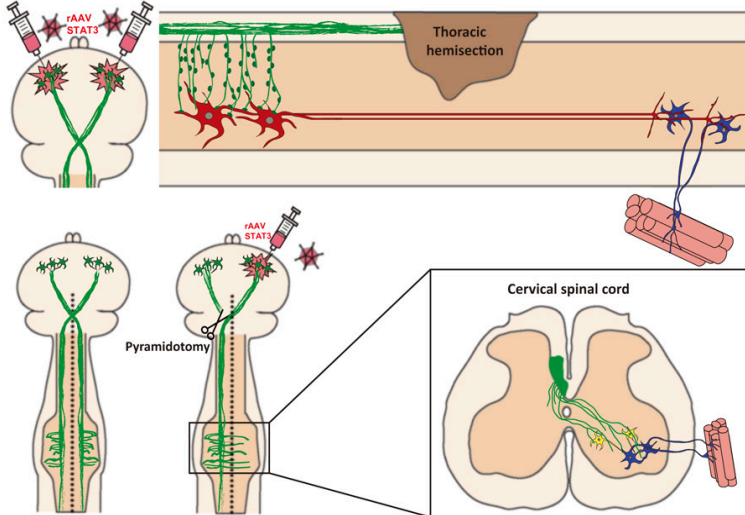
In general, the formation of detour circuits – as described above – can be divided into two steps, and both need to be accomplished to successfully bypass the injury and trigger recovery. First, the transected neurons need to induce the growth of new axon collaterals within the intraspinal architecture. Second these new collaterals need to form and maintain functional synapses onto appropriate interneuronal populations in order to ensure the transmission of neural signals from the upper motoneurons in the brain to the lower spinal motoneurons. Recent studies that shed light on the molecular regulation of these steps of the axonal remodeling process are summarized in the next paragraphs.

**Growth of axonal collaterals during injury-induced axonal remodeling: the role of STAT3:** In recent years, several molecules have been discovered that regulate the intrinsic growth program of neurons in the central or peripheral nervous system (Liu et al., 2011; Chen and Zheng, 2014). One key component of this intrinsic growth program is the transcription factor STAT3 (signal transducer and activator of transcription 3), which can be activated as part of the JAK-STAT transduction pathway. Several recent papers have demonstrated the importance of the JAK-STAT pathway in promoting axon growth after injury (Qiu et al., 2005; Smith et al., 2009; Sun et al., 2011). Using dorsal root ganglion (DRG) neurons that extend one branch to the central and another to the peripheral nervous system we could for example reveal the contribution of STAT3 to the differential injury response in the PNS and CNS (Bareyre et al., 2011). In this study, we showed that PNS but not CNS lesions lead to the persistent upregulation of STAT3 expression in DRG neurons. This differential expression is important as STAT3 deletion in DRG neurons impaired the regeneration of peripheral DRG branches after nerve cut, while the overexpression of STAT3 using gene therapy increased outgrowth and collateral sprouting of central DRG branches after a dorsal column lesion. Time-lapse *in vivo* imaging of PNS and CNS axons further revealed that STAT3 selectively regulates initiation but not later perpetuation of axonal growth. These findings raised the question whether STAT3 could also be a good candidate to modulate post-injury axonal remodeling. To test this, we overexpressed STAT3 in the cells of origin of the CST in the motor cortex using viral gene transfer and analyzed the effect of this overexpression on post-injury outgrowth of hindlimb CST collaterals in the cervical cord (Lang et al., 2013). The study shows that STAT3 overexpression increases the number of newly sprouted collaterals from the injured hindlimb CST after thoracic hemisection (**Figure 1B** upper panel). To our surprise, it also showed that even in absence of a lesion, STAT3 can induce the growth of *de novo* collaterals into the cervical spinal cord. This indicates that STAT3 can not only (moderately) promote the growth of injured fibers but also initiate the growth process in uninjured fibers. To

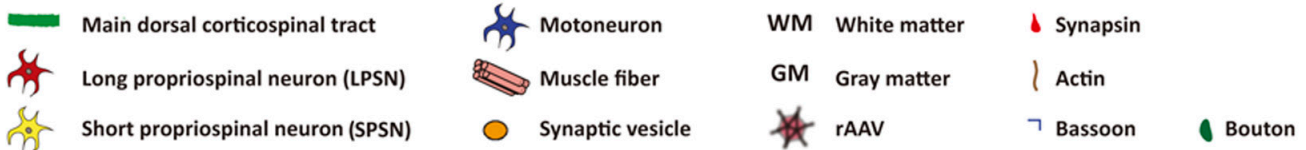
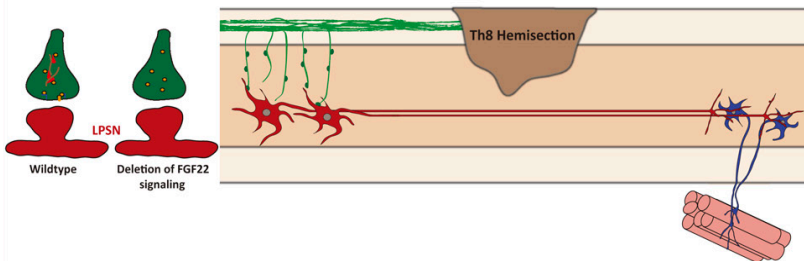
A. Intraspinal detour circuit formation



B. STAT3 overexpression promotes collateral sprouting and compensatory midline-crossing fibers



C. Deletion of FGF22 signaling decreases synapse formation and maturation



**Figure 1** Schematic representation of the formation and the regulation of intraspinal detour circuits following spinal cord injury.

(A) Intraspinal detour circuit formation: Following thoracic hemisection, the hindlimb corticospinal tract sprouts collaterals into the cervical spinal cord. Those collaterals form synapses with long propriospinal neurons which in turn increase their contacts onto lumbar motoneurons thereby forming a new detour pathway that reconnects upper corticospinal projection neurons to lumbar motoneurons.

(B) Effect of STAT3 overexpression on post-injury detour circuit formation (top panel) and post-injury compensatory midline sprouting fibers (bottom panel). Following thoracic hemisection and STAT3 overexpression using recombinant adeno-associated viruses (top panel), the hindlimb corticospinal tract sprouts more collaterals than in the control situation. Furthermore, following a unilateral lesion of the corticospinal tract at the level of the medulla oblongata, and STAT3 overexpression using recombinant adeno-associated viruses (bottom panel), midline crossing fibers emerge from the treated unlesioned corticospinal tract and re-innervate the denervated cervical spinal cord.

(C) Perturbation of FGF22 signalling, using genetically deficient animals, decreases synapse formation and maturation during post-injury detour circuit formation. In turn this impairment in synapse formation triggers a decreased formation of detour circuits and leads to impairment functional recovery following spinal cord injury. This indicates that intact FGF22 signaling is an important requirement for efficient intraspinal axonal remodeling.

better understand whether the growth of uninjured fibers could contribute to recovery, we unilaterally cut the CST at the pyramidal decussation (pyramidotomy) and overexpressed STAT3 in layer V projection neurons of the unlesioned CST. In this paradigm, STAT3 induces the sprouting of unlesioned forelimb CST fibers in the cervical spinal cord. These fibers cross the spinal midline and form contacts onto spinal interneurons and cervical motoneurons on the denervated side – a remodeling process that improves forelimb function as assessed both behaviorally and electrophysiologically (Figure 1B lower panel; Lang et al., 2013). STAT3 is thus a key modulator of the initial phase of the remodeling process that can “jump-start” axonal growth and thereby recruit both injured and uninjured fibers to the remodeling

process. Interestingly, Tsuda et al. (2011) also reported that the JAK-STAT3 signaling pathway critically regulates astrocyte proliferation and neuropathic pain following nerve injury which highlights that STAT3 might be an interesting therapeutic target not only to induce axonal growth but also reduce allodynia following spinal cord injury.

**Induction of synapse formation during injury-induced axonal remodeling: the role of FGF22:** The second important step during detour circuit formation is the establishment of new synaptic contacts between CST collaterals that enter the cervical grey matter and the appropriate target cell in the spinal grey matter, e.g., the long propriospinal neurons. A number of the molecules that induce and maintain synapse

formation have been elucidated in the developing nervous system. One of the more recently identified molecules is a member of the fibroblast growth factor (FGF) family, FGF22 and its receptors, FGFR1 and FGFR2 (Umemori et al., 2004). Several studies have shown its importance for the induction of excitatory presynaptic differentiation during development, *i.e.*, in the cerebellum or in the hippocampus and suggested its involvement in neurological diseases such as epilepsy. To investigate whether FGF22 signaling could also regulate synapse formation during post injury remodeling in the adult CNS, we genetically deleted FGF22 or its receptors (Jacobi et al., 2015). Mice deficient for FGF22 or lacking both of its receptors in corticospinal projection neurons showed a significant decrease in the number of synaptic boutons formed on the newly sprouted CST collaterals in the cervical spinal cord following SCI (**Figure 1C**). Moreover the maturation of those synapses that formed was significantly delayed. Together these changes in synapse formation and maturation led to impaired detour circuit formation and delayed functional recovery of the animals following SCI. Indeed motor performance after SCI, evaluated using behavioral tasks such as the ladder rung test – in which the animals have to walk over a ladder with bars regularly and irregularly spaced – or the catwalk system – in which the fine stepping movements of the animals are recorded and analyzed – was significantly worse in mice in which FGF22 signaling was genetically interrupted. These data identify FGF22 and its receptors as crucial endogenous regulators of the formation and maturation of new synapses in the injured central nervous system that are required for efficient post-injury remodeling and recovery.

Timely and accurate axonal remodeling is one of the keys to functional recovery following spinal cord injury. To therapeutically support such remodeling processes in the future we first have to unravel the molecular signals that govern their endogenous formation. Recent studies such as the ones described here identify first molecular clues but much more work is required to obtain a comprehensive molecular understanding of circuit remodeling in the injured spinal cord.

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