

● PERSPECTIVES

“Bad regenerators” die after spinal cord injury: insights from lampreys

In mammalian species, including humans, spinal cord injury (SCI) leads to permanent disability. A major cause of disability after SCI is the failure of axotomized descending axons to regenerate across the trauma zone and to reconnect to their appropriate targets distal to the site of injury. Currently, major research efforts are devoted to find new ways of promoting the regrowth of damaged descending axons. However, activation of axonal regrowth will depend on the survival of the axotomized descending brain neurons. This will be especially important in chronically injured patients. Neuronal degeneration can be a slow and delayed process, so descending neurons of long-term injured patients would have more time to degenerate after the injury making it more difficult to activate regenerative processes. If descending neurons of the brain die after the damage to the axon in the spinal cord, regenerative therapies in long-term injured patients will fail. There is then a need to prevent the retrograde degeneration of axotomized brain neurons after SCI if we want to promote axonal regeneration. It may be necessary to protect from retrograde degeneration very early after the injury if we then want to enhance axonal regeneration later on.

It is generally accepted that several types of central nervous system (CNS) neurons die after axotomy. Indeed, most of the studies in mammalian models have reported the death of at least some brain descending neurons after SCI (Hains et al., 2003; Latini et al., 2014). The death of brain descending neurons after SCI appears to involve an apoptotic process, as indicated by the appearance of TUNEL staining, cytochrome c release or activated caspase-3 immunoreactivity in rubrospinal (Latini et al., 2014) or corticospinal (Hains et al., 2003) neurons. However, there is still some controversy whether corticospinal neurons die after axotomy due to a SCI. The recent work of Steward's group has suggested that corticospinal neurons of rats may not die after the SCI and that they may only suffer a process of atrophy (Nielson et al., 2011). This could indicate that several types of neurons could be more vulnerable to the axotomy at the spinal level than others. In addition, the type of injury, the distance between the point of injury and the neuronal soma and the time of analysis post-injury are factors that also influence the detection or not of neuronal death in spinal-projecting neuronal groups of the brain. In any case, most of the studies in mammals indicate that it is important to prevent the death or the degeneration (atrophy) of spinal-projecting neurons after SCI if we want to promote axonal regeneration.

Lampreys as an animal model for the study of spinal cord regeneration and death of spinal-projecting neurons: In contrast to mammals, lampreys spontaneously recover locomotion from a complete SCI (see Rodicio and Barreiro-Iglesias, 2012). This amazing recovery process involves the regeneration of descending spinal axons (Jacobs et al., 1997) and the formation of synaptic connections between the regenerated descending axons and neurons caudal to the lesion (Rodicio and Barreiro-Iglesias, 2012). Brain descending neurons of lampreys have a complex cytoarchitecture, with 36 identifiable giant reticulospinal neurons (Jacobs et al., 1997) (**Figure 1**). These include the Mauthner neurons and several pairs of Müller cells. Interestingly, these identifiable descending neurons vary greatly in their regenerative abilities (Jacobs et al., 1997). Some of these neurons are considered “good regenerators” (*i.e.*, they regenerate their axon through the site of injury more than 55% of the times) and others are considered “bad regenerators” (*i.e.*, they regenerate their axon less than 50% of the times) (**Figure 1**). Thus, in lampreys, there is an opportunity to follow up the fate of individually identifiable neurons of the brain after a traumatic SCI. An additional advantage of the lamprey model of SCI is that the identifiable spinal-projecting neurons and their descending axons can be visualised *in vivo* and in CNS whole-mounts due to the transparency of the lamprey brain. Lampreys are the only vertebrate model in which the issue of “good” vs. “poor” regenerators can be adequately addressed within the context of the CNS. In other models, such as in mammals, the neurons are too small and are unidentified, and so it is impossible to track the fate of such neurons across large populations of animals as is needed for molecular studies after SCI.

Recently, it has been reported, by us and others, that a complete SCI induces the delayed death of lamprey descending neurons that, at earlier times post-injury, had been identified as bad regenerators (Shifman et al., 2008) (**Figure 1**). The appearance of TUNEL staining (Shifman et al., 2008) and activated caspases (Barreiro-Iglesias and Shifman, 2012; Zhang et al., 2014) in the soma of axotomized neurons suggested that the death of the bad-regenerating neurons in lampreys is apoptotic. A recent report by Barreiro-Iglesias and Shifman (2015) has shown that caspase activation in the cell soma of bad regenerator descending neurons of lampreys is preceded by the initial activation of caspases in the descending axotomized axons at the lesion site within the first hours after the injury. This suggested that the degenerative process is initiated very early at spinal levels and lasts for a long period of time after the injury.

These studies in lampreys reveal some interesting features that provide important clues for future mammalian studies:

1) The studies in lampreys indicate that the bad regenerators are also poor survivors, which confirms the importance

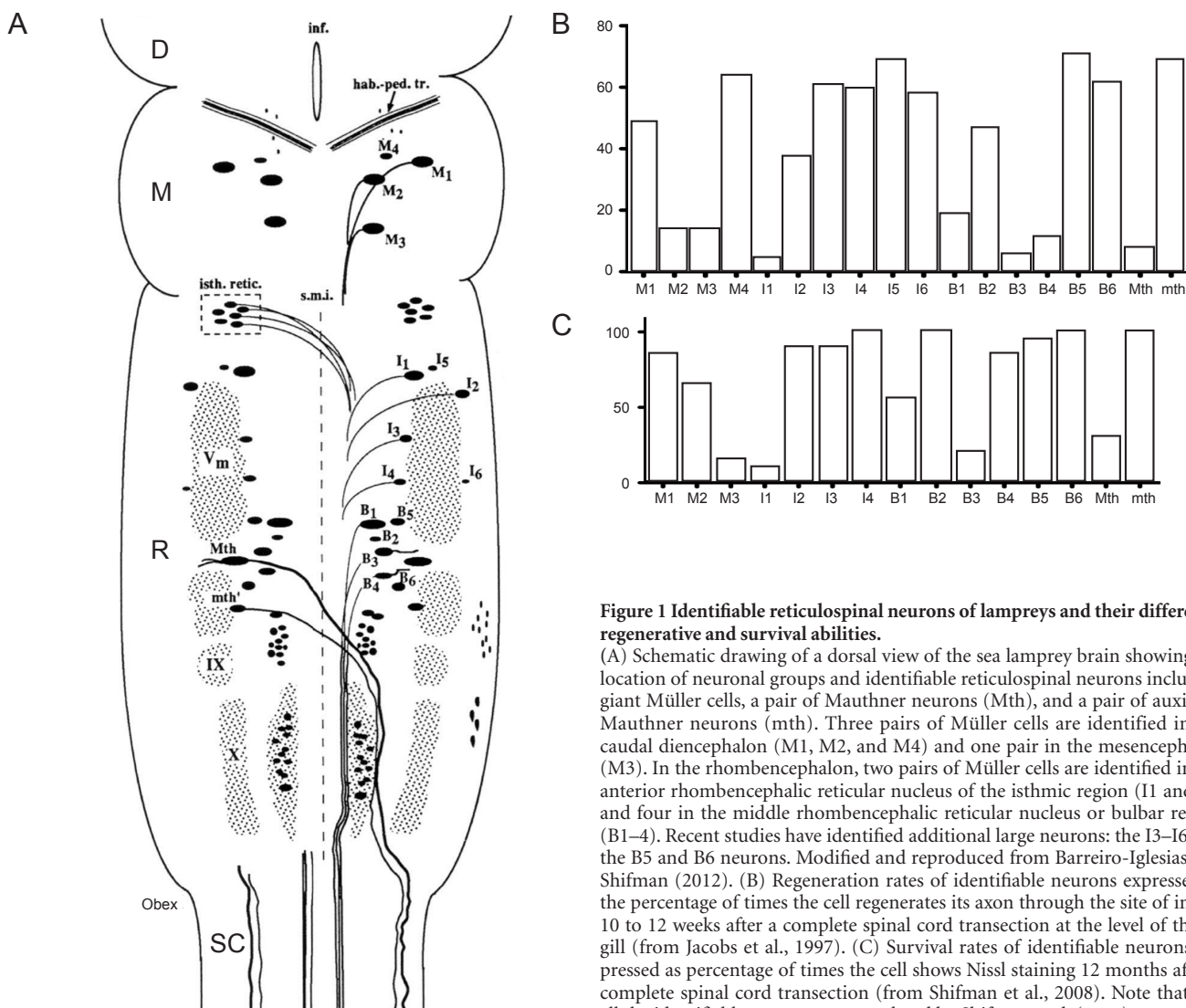


Figure 1 Identifiable reticulospinal neurons of lampreys and their different regenerative and survival abilities.

(A) Schematic drawing of a dorsal view of the sea lamprey brain showing the location of neuronal groups and identifiable reticulospinal neurons including giant Müller cells, a pair of Mauthner neurons (Mth), and a pair of auxiliary Mauthner neurons (mth). Three pairs of Müller cells are identified in the caudal diencephalon (M1, M2, and M4) and one pair in the mesencephalon (M3). In the rhombencephalon, two pairs of Müller cells are identified in the anterior rhombencephalic reticular nucleus of the isthmic region (I1 and I2) and four in the middle rhombencephalic reticular nucleus or bulbar region (B1–4). Recent studies have identified additional large neurons: the I3–I6 and the B5 and B6 neurons. Modified and reproduced from Barreiro-Iglesias and Shifman (2012). (B) Regeneration rates of identifiable neurons expressed as the percentage of times the cell regenerates its axon through the site of injury 10 to 12 weeks after a complete spinal cord transection at the level of the 5th gill (from Jacobs et al., 1997). (C) Survival rates of identifiable neurons expressed as percentage of times the cell shows Nissl staining 12 months after a complete spinal cord transection (from Shifman et al., 2008). Note that not all the identifiable neurons were analysed by Shifman et al. (2008).

Abbreviations: B1–B6: Müller cells of the bulbar region (middle rhombencephalic reticular nucleus), D: diencephalon, hab.-ped. tr.: habenulopeduncular tract, I1–I6: Müller cells of the isthmic region, IX: glossopharyngeal motor nucleus, inf.: infundibulum, M: mesencephalon, M1–M4: Müller cells 1 to 4, isth. retic.: isthmic reticular formation, MRRN: middle rhombencephalic reticular nucleus, Mth: Mauthner cell, mth': auxiliary Mauthner cell, R: rhombencephalon, SC: spinal cord, s.m.i.: sulcus medianus inferior, Vm: trigeminal motor nucleus, X: vagal motor nucleus.

of protecting descending neurons after SCI if we want to promote axonal regeneration.

2) The appearance of TUNEL labelling in the soma of some of the spinal-projecting neurons several months (up to 1 year) after the injury shows that the retrograde degeneration process after SCI is a very slow process that progresses during a long period of time after the injury. This shows the importance of timing when looking at neuronal death in the brain after SCI in mammalian studies.

3) The lamprey studies also provide an answer to the following question: “Do the bad regenerators die because they fail to regenerate or do they do not regenerate their axon because they are dying?” The demonstration of the acute activation of caspases in the axotomized axons shows that the retrograde degenerative process is initiated very early,

even before the neurons would attempt to regrow their axon. Thus, some bad regenerator lamprey spinal-projecting neurons enter the slow degenerative process very early, which could act against the spontaneous activation of the regenerative processes and indicates that neuroprotective therapies targeting the descending neurons might need to be initiated very early.

Molecular pathways that could promote or decrease neuronal survival and subsequently affect regeneration:

Lampreys are living representatives of the jawless vertebrates, which are the oldest extant vertebrates. Although, there are important differences with the nervous system of jawed vertebrates, like the lack of a myelinated CNS, the nervous system of lampreys shows a similar organization and structure



to that of jawed vertebrates (Villar-Cerviño et al., 2011). In addition, the recent sequencing of the sea lamprey genome has revealed a high degree of similarity with mammals at the genetic and molecular level. This will clearly facilitate to translate the findings in lamprey studies to research in mammalian models. The lamprey model to study the retrograde degeneration of descending pathways after a complete SCI is already leading to the finding of the molecular pathways implicated in this process.

A study by Busch and Morgan (2012) has reported the occurrence of synuclein accumulation in the form of aggregated intracellular inclusions only in the “poor survivor/bad regenerator” reticulospinal neurons of the sea lamprey and a strong correlation between this and the subsequent death of the neuron.

The Selzer group has recently reported the expression of protein tyrosine phosphatase σ (PTP σ) and leukocyte common antigen-related phosphatase (LAR), which are specific chondroitin sulphate proteoglycans receptors, in bad regenerator/poor survivor neurons of lampreys after a complete SCI (Zhang et al., 2014). Expression of these receptors was observed in neurons with activated caspases and the presence of the receptors correlated with a poor axonal regenerative ability of the neurons. These results reveal a possible role for PTP σ and LAR in retrograde neuronal death.

This year, we have reported the surprising accumulation of GABA around some of the descending axons of identifiable neurons after a high and complete SCI (Fernández-López et al., 2014). In this study, we observed the massive release of glutamate and GABA from spinal cord neurons after a complete SCI in lampreys as it occurs in mammals. In mammals, there is a massive glutamate release after SCI, which causes glutamate excitotoxicity and neuronal death. On the other hand, GABA could exert neuroprotective roles (Fernández-López et al., 2014). Accordingly, we observed a statistical correlation between the presence of GABA accumulation in form of “halos” around the axotomized descending axons and a higher survival ability of the corresponding identifiable neurons (Fernández-López et al., 2014).

Lampreys are a convenient vertebrate model for the *in vivo* study of the mechanisms underlying the death/survival of descending neurons and how it relates to the activation of axonal regeneration after SCI. We need to find ways of protecting descending neurons after the injury if we want to promote regeneration. Lampreys can tell us how to do it.

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