PERSPECTIVE

When the left side knows something happened to the right – sensing injury in neurons contralateral and remote to injury

How sensory neurons encode axonal injury signals has been a longstanding area of neurological research. Insights into the cellular and transcriptional changes in the injured neurons have driven many new therapeutic strategies to improve repair. In contrast, less focus has been centered on the systemic or transneuronal changes that may arise from these injuries and how they may impact or factor into the alterations in gene expression, physiology and neuropathology, such as mirror image pain, arising in regions either directly contralateral or those remote to the injury site. Research in this area is clinically relevant. In patients with chronic unilateral pain due to multiple pathologies, many of which involve nerve trauma, there is a very high incidence of bilateral sensory abnormalities reported with 33–50% of patients with mechanical abnormalities reporting contralateral sensory abnormalities (Konopka et al., 2012). This has implications when evaluating sensory abnormalities or pain states in patients, as the contralateral side is often used as the non-affected control. Further, the bilateral nature of these sensory abnormalities, albeit usually less severe on the contralateral side, support that a systemic or central nervous system component is involved in this aspect of the pathology. It highlights the need to better understand the mechanisms underlying these bilateral responses as this will likely impact therapeutic treatments for these patients.

The secondary consequences of injury for sensory neurons are more extensive than initially posited, being detected in neurons not only contralateral to injury, but also remote and imposing either an increased pain state or heightened plasticity to the affected cells. In preclinical investigations, mirror pain (mechanical hypersensitivity) induced in rats by unilateral L5/6 spinal nerve ligation is associated with bilateral increases in nerve growth factor expression in the activated perineuronal satellite glial cells in dorsal root ganglia. Intrathecal delivery of either nerve growth factor antibodies or post-synaptic density-95 short hairpin RNA constructs, the latter to prevent the formation of synapse-like structures, attenuated the creation of the synapse-like structures around large sensory neurons by sympathetic and calcitonin-gene-related peptide sensory fibres and alleviated the mirror pain. The mirror pain was shown to involve nerve growth factor-dependent sprouting of sympathetic and calcitonin-gene-related peptide sensory fibres around large sensory neurons (Cheng et al., 2015).

Unilateral injury has also been shown to impact contralateral target innervation by motor and sensory neurons. Compelling early studies described increased sprouting of motor neuron terminals in the contralateral intact rat muscle, the detection of which after unilateral injury inversely correlated with the proximity of the lesion to the neuronal cell body (reviewed in Koltzenburg et al., 1999). The contralateral changes can persist long after injury. For example, injury to the rat saphenous nerve, the terminal cutaneous branch of the femoral nerve in the leg was found to suppress the ability of the contralateral nerve to evoke a plasma extravasation response. This response restricted itself to the contralateral saphenous, was not contingent on factors released from the injured nerve and was not an early response, being detected at 6 weeks post-injury and persisting until the last time point examined, 26 weeks post-injury (reviewed in Koltzenburg et al., 1999). Plastic changes invoked by unilateral injury can also lead to bilateral decreased innervation. Unilateral denervation involving tibial and common peroneal nerve branches of the rat sciatic nerve, while leaving the sural nerve branch intact, resulted in bilateral loss of tibial nerve target innervation within days of the unilateral nerve transection. The loss of PGP9.5^{+ve} innervation in the tibial territory of the contralateral paw epidermis reached 46% at 1 week post-injury. Notably, this contralateral loss of innervation remained up to 21 weeks post-injury, but did not result in an altered contralateral algesic state (Oaklander and Brown, 2004). Because this change appeared restricted to the tibial innervation and did not impact the sural innervation patterns they concluded that this response likely does not involve a humoral signal, but may involve rapid transcellular signals, as the changes were evident as early as one day after injury. Indeed, the conclusions drawn by Koltzenburg et al. (1999) with respect to contralateral effects after injury implicate a transneuronal mechanism (*versus* humoral), likely involving commissural interneurons in the studies described at that point.

We contend however, that the response to unilateral injury also involves a systemic component. We and others observe upregulation of regeneration- and stress-associated proteins, transcripts, and transcription factors in neurons both contralateral and remote to injury, supporting the involvement at least in part, of a systemic component in the response (Dubovy et al., 2018, 2019; Hasmatali et al., 2019).

Finely tuned regulation of transcription factor expression and nuclear translocation is essential for the activation and transitioning of a neuron to a regeneration-competent state. In studying the temporal responses of injured and uninjured neurons we discovered parallel multi-phasic alterations in Luman/CREB-3 protein levels and nuclear translocation in injured, and contralateral uninjured rat neurons (Hasmatali et al., 2019), that mirror the recently described distinct transcriptional phases in injured neurons in response to peripheral nerve injury. These injury-associated changes in the transcriptome include an initial rapid phase as neurons respond to the stress of the injury, a pre-regeneration phase and then a transition into the regeneration phase between 4–7 days post-injury (Li et al., 2015). We observed a distinct separation between the pre-regeneration and regeneration phases in sensory neurons with the initial peak in the transcription factor Luman/CREB3 cytoplasmic and nuclear levels observed at two days post-injury, declining to pre-injury levels or lower at 4 days post-injury and then increasing dramatically by 7 days post-injury. The parallel, albeit lower phasic Luman/CREB3 responses in contralateral uninjured neurons were also observed in C4 ganglia remote from injury, supporting the existence of a humoral component that likely drives the contralateral and remote responses in uninjured neurons, the nature of which remains to be identified (Hasmatali et al., 2019).

To verify the link between the temporal stress response in uninjured neurons and the distinct transcriptional phases of injury, we recently investigated another stress-associated transcription factor expressed by sensory neurons, forkhead class box O3a. We noted that while its expression and nuclear localization in injured neurons is rapidly decreased following spinal nerve transection, temporal phasic alterations in nuclear localization in neurons contralateral and remote to injury parallels that which we observed for Luman/CREB3. This supports that with respect to uninjured neurons there appears to be shifts in systemic factors that have the same temporal consequences on nuclear translocation and expression of at minimum two stress-associated transcription factors. Further, the fact that forkhead class box O3a responses in injured neurons do not parallel those in the contralateral and remote uninjured neurons, reveals that the systemic component does not drive responses in both injured and uninjured neurons in an identical manner (JCDH and VMKV, unpublished observations).

The bilateral injury response with respect to Luman/CREB-3, a molecule that we have previously linked to the ability of injured sensory neurons to regenerate an axon through its regulation of the endoplasmic reticulum stress-associated unfolded protein response (Ying et al., 2014, 2015), implies that a heightened plasticity may be conferred on the uninjured sensory neurons in association with the changes that result in increased Luman expression. While no differences in neurite outgrowth capacity relative to naïve control sensory neurons were observed for contralateral sensory neurons from 2 day or 4 day injury-conditioned animals, significantly enhanced neurite outgrowth was observed when neurons contralateral to a 7-day spinal nerve lesion were assayed (Hasmatali et al., 2019). Notably, this increased ability to grow an axon manifested itself in the less branched mode of growth normally observed when injured neurons are assayed.

Insights into how a prior contralateral injury conditions contralateral sensory neurons with respect to increased regenerative capacity have been limited (Senger et al., 2018), with little information on what might underlie the changes observed in remote ganglia. Dubovy et al. (2018, 2019) have made some valuable recent contributions on this front. Specifically, they observe bilateral activation and nuclear translocation of the cytokine signaling-associated transcription factor STAT3 in sensory neurons and also observe the same response in remote (cervical segments) in response to 7-day sciatic nerve transection in the rat. The increases in phosphorylated STAT3 correlated with an increase in cere-

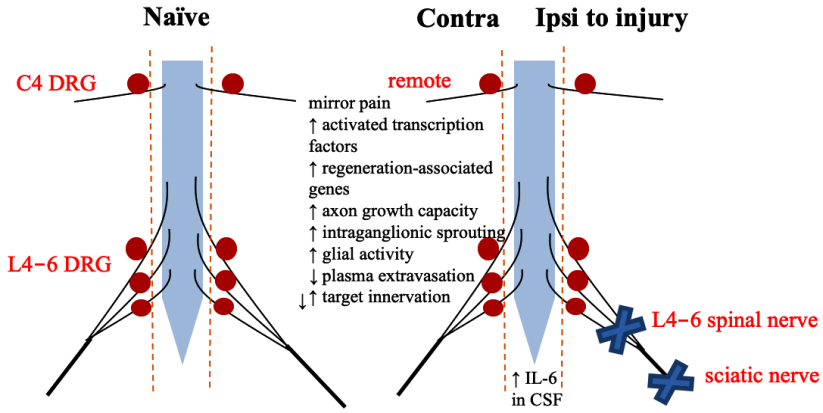

Figure 1 Schematic summary of cellular and functional responses to ipsilateral (Ipsi) nerve injury to sciatic nerve or associated spinal nerves detected in target tissue or sensory neurons in DRG contralateral (Contra L4–6 DRG) or remote (C4 DRG) to injury, illustrating the importance of including naïve controls in the experimental design. CSF: Cerebrospinal fluid; DRG; dorsal root ganglia; IL-6: interleukin-6.

bral spinal fluid interleukin-6 (IL-6) expression and could be mimicked by bolus intrathecal delivery of IL-6, suggesting a general neuroinflammatory reaction along the neural axis. In further studies, they demonstrated a heightened plasticity/pro-regenerative state in uninjured dorsal root ganglion (DRG) neurons remote to injury in response to a one week conditioning lesion that involved the IL-6 signaling pathway (Dubovy et al., 2019). These provide compelling data in support of systemic responses to nerve injury.

Finally, the creation of a novel transgenic fluorescent reporter mouse that assesses the secondary impacts of peripheral nerve injury, has facilitated examination of how broad reaching the injury-associated effects might be. The mouse was engineered to express red fluorescent protein under the control of the highly evolutionarily conserved stress-sensing heat shock protein-70 promoter region containing heat shock factor 1 binding sites in the heat shock-response elements. Using these reporter mice, an extensive network of stress-associated responses throughout both the peripheral nervous system and central nervous system have been described (Hashimoto-Torii et al., 2018). As heat shock protein-70 is ubiquitously expressed throughout the body, this system has the potential to report both primary and secondary perturbations resulting from a unilateral nerve lesion model that also involves neuroma formation. It revealed both the expected ipsilateral responses in sensory and motor neurons retrogradely identified from the site of lesion and also revealed lower responses in contralateral neurons and bilateral responses in microglia and rubrospinal neurons. In addition to these changes, the red fluorescent protein labelled wide dynamic range neurons ipsilateral to injury exhibited abnormal physiological responses with an increased hyperexcitability 4 weeks post-injury believed to contribute to associated neuropathic pain states.

Collectively these studies extend a cautionary note, beyond that of the clinical implications, namely, the invalidity of using the contralateral DRG in injury models as an internal normative control (**Figure 1**). While the responses of contralateral neurons can yield important insights into the responses of the injured neuron that are not due to systemic influences, it behooves researchers to include naïve animals as additional controls in the experimental design. This is the only way to ensure a true assessment of what is happening and to elucidate the broad pathological consequences for both injured and non-injured neurons, be they ipsilateral, contralateral, adjacent or remote to injury.

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