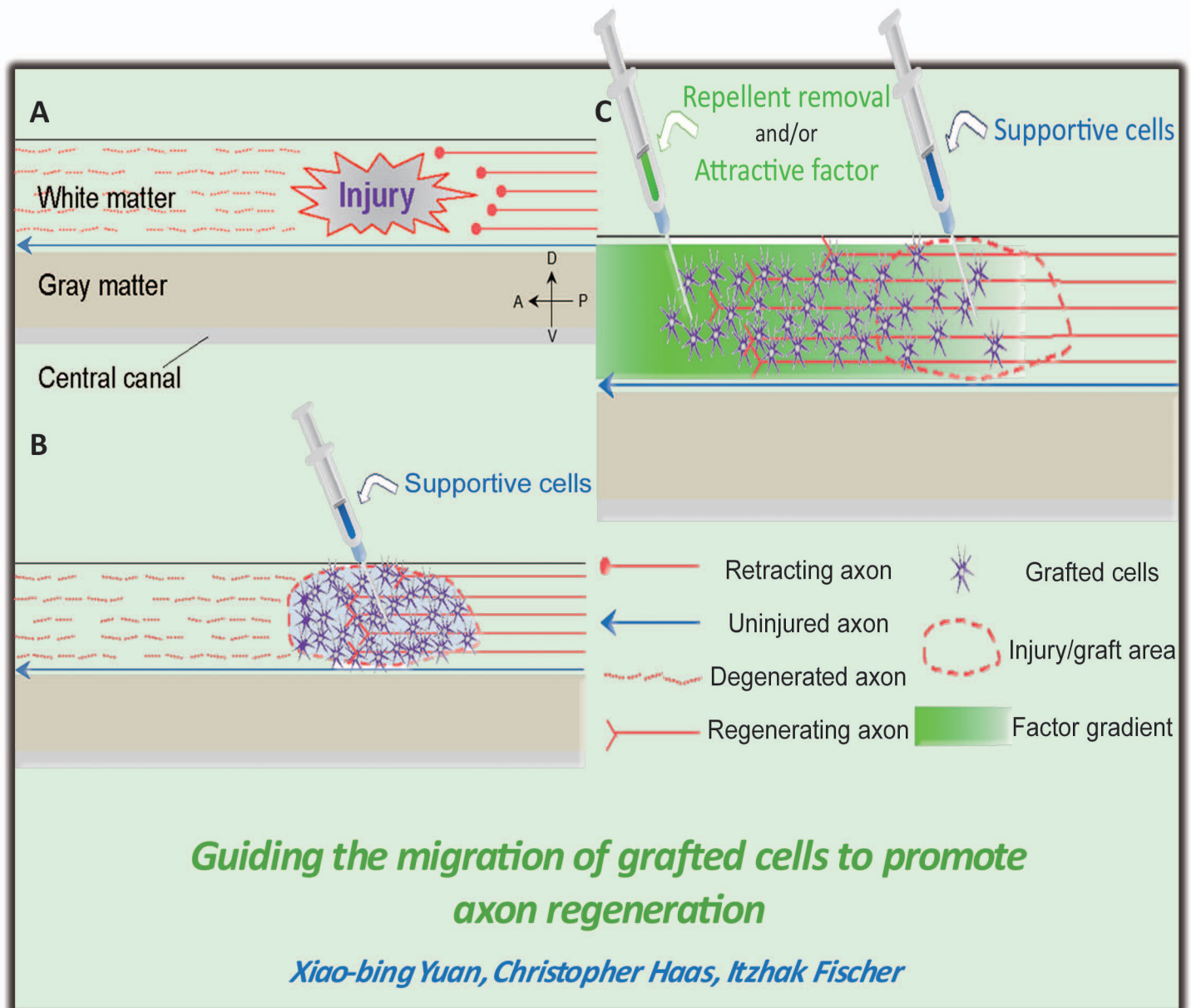




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● PERSPECTIVE

## Differing roles for parvalbumin neurons after nerve injury

Neuropathic pain is abnormal, persistent pain that is caused by injury to the somatosensory system. It commonly involves damage to the peripheral nerves as a result of physical trauma, metabolic diseases such as diabetes, infections such as HIV or shingles, or toxicity induced neuropathies as a result of cancer treatments. Neuropathic pain is common, with epidemiological studies suggesting that around 18–30% of American, European and Australian adults suffer from chronic pain. This leads to significant disabilities and suffering. People with chronic pain complain of spontaneous pains that may be electric-shock like or continuous. Often coexisting with these are abnormal, evoked sensations such as tactile allodynia (pain in response to innocuous stimuli *e.g.*, light brush or pressure) and cold allodynia (pain in response to innocuous cooling), or hyperalgesia (increase pain in response to an already noxious stimulus), as well as ataxia and proprioceptive deficits. The management of these conditions is often suboptimal for two main reasons: firstly, an incomplete knowledge of the mechanisms that are involved (and which of them are the most important). Secondly, patients often have compliance issues with pharmacological medications (Attal, 2012). Both peripheral and central neuronal mechanisms contribute to the pathophysiology and since the turn of the century particular emphasis has focused on the role of glial cells in pain (West et al., 2015).

Primary sensory afferents convey information about peripheral stimuli to the central nervous system (CNS). Their cell bodies are located in dorsal root ganglia (DRGs) and constitute a heterogeneous population that has been classified in several ways. Initially, they were split into the small dark and large light groups based on neurofilament (and myelination) content that correlated well with conduction velocities. With the advent of immunohistochemistry, and the discovery of different trophic factors, these groups were further subdivided into peptide-rich and peptide-poor nociceptors, low threshold mechanoreceptor and proprioceptor subgroups (Scott, 1992). Moreover, it is now known that different DRG neurons can express in > 30 different neurotransmitters or receptors associated with a wide variety of stimuli and that they show considerable phenotypic plasticity associated with peripheral injury (Fukuoka and Noguchi, 2002; West et al., 2015). Most of the attention has focussed on the changes associated with small DRG cells that primarily innervate skin, since these are presumed to be responsible for evoked pain sensations after injury. However, less attention has been paid to the effects of injury on proprioceptors in general, and muscle afferents in particular. This is, in part, due to the paucity of histological markers associated with such fibres.

The most commonly used marker of proprioceptive (muscle) afferents is parvalbumin (PV) (Medici and Shortland, 2015). PV is a calcium binding protein that is found in subsets of sensory neurons, inhibitory interneurons and motoneurons. Studies that have explored the effects of nerve injury on muscle afferents reported no changes in its staining patterns after injury. However, those early studies used nerve injury models where injured and non-injured cell bodies coexist within the same DRG. Therefore, plasticity could occur without changing the absolute number of positive cells (downregulation in injured and upregulation in spared neurons). Now there are neuronal markers, such as activating transcription factor 3 (ATF3), which can identify damaged afferents and nerve injury models where the injured and non-injured cell bodies are spatially segregated in adjacent spinal ganglia (Shortland et al., 2006). This prompted us to reinvestigate whether or not PV positive cells show phenotypic plasticity (Medici and Shortland, 2015).

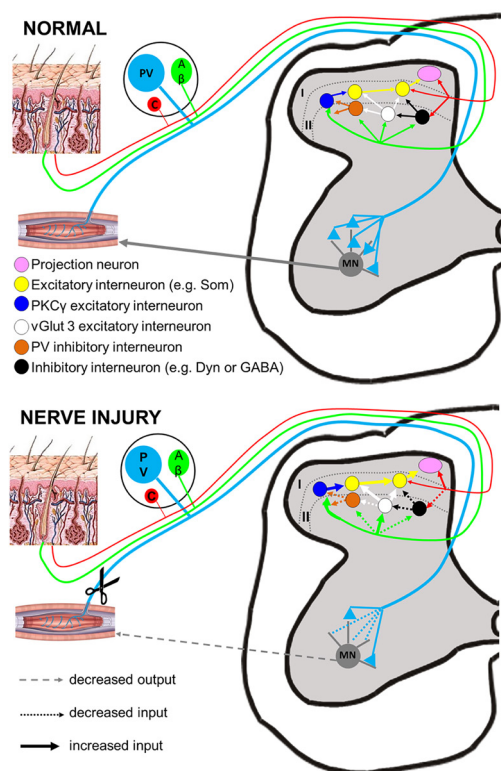
We first determined that around 25% of L<sub>4-5</sub> DRG cells innervating the sciatic nerve express PV. Following axotomy of the sciatic nerve, this number did not significantly change over 1–28 days,

confirming earlier studies. Co-expression with the neuronal injury marker ATF3 showed that approximately half the PV cells were axotomised suggesting that both injured and uninjured neurons express PV after sciatic nerve injury. However, around 50–60% of the L<sub>4</sub> & L<sub>5</sub> DRG neurons contribute axons to the sciatic nerve, so by itself this result did not really answer whether or not phenotypic plasticity was happening, since for switching to occur, one might predict that little or no colocalisation with ATF3 should occur. Therefore, we switched to the spinal nerve ligation model and selectively lesioned the L<sub>5</sub> spinal nerve, whilst being careful not to injure the adjacent L<sub>4</sub> spinal nerve. In this model, essentially all DRG neurons were injured and expressed ATF3 (Shortland et al., 2006).

The percentage of PV staining in the axotomized L<sub>5</sub> DRG was unchanged compared to the adjacent L<sub>4</sub> DRG, the contralateral L<sub>5</sub> DRG and naïve L<sub>5</sub> DRG. On the other hand, calcitonin gene related peptide (CGRP) staining, which is an accepted marker of peptidergic nociceptor afferents, including muscle afferents, was completely abolished in the ipsilateral L<sub>5</sub> DRG. Thus, these results unequivocally showed that nerve injury did not produce a phenotypic change in PV positive afferents. They also highlighted a clear difference between phenotypic plasticity in proprioceptors *versus* nociceptors in response to nerve injury.

There is now increasing knowledge of the molecular and biochemical repertoire of proprioceptors. Proprioceptors contain several calcium binding proteins such as calcineurin, calretinin and calbindin. They also contain metabolic markers such as carbonic anhydrase and cytochrome oxidase and the enzyme  $\alpha 3\text{Na}^+/\text{K}^+$  ATPase. Several ion channels are associated with proprioceptors such as Piezo 2, acid sensing ion channel-3 (ASIC3), transient receptor potential V2 (TRPV2), vesicular glutamate transporter-1 (vglut1) and other markers such as the PDZ scaffold protein whirlin. Piezo2,  $\alpha 3\text{Na}^+/\text{K}^+$  ATPase and whirlin are specific markers for muscle spindles. Following nerve injury, muscle afferents become spontaneously active and can change their neurotransmitter content. They can upregulate galanin, neuropeptide tyrosine (NPY) and brain derived neurotrophic factor (BDNF), but many of the markers do not change *e.g.*, TRPV2, ASIC3, carbonic anhydrase, whilst the response to others such as Piezo 2 or whirlin are unknown. A key player in contributing to chronic pain appears to be the *de novo* expression of BDNF in muscle afferents (Michael et al., 1999; Zhou et al., 2010). Recent evidence also implicates changes in the sodium channel Nav1.7 in proprioceptors as an important contributor to chronic pain (Fukuoka et al., 2015), as this is increased in muscle afferents after nerve injury. Yet the afferents retain their normal levels of PV. PV is important for regulating calcium homeostasis in a neuron. After nerve injury, muscle afferents have a reduced and redistributed presynaptic input onto homonymous motoneurons. Before injury, synapses were on the proximal dendrites, whilst after injury they were fewer in number and located further away from the soma. This explained the observations of weak excitatory post synaptic potentials to motoneurons and disappearance of stretch reflexes (Vincent et al., 2015). PV is found in the cell body and peripheral endings, but not in the central terminals of afferents in the spinal cord (Petitjean et al., 2015), suggesting PV is more important in physiological process such as speed of muscle contraction rather than neurotransmitter release. Nerve injury causes a selective loss of small, but not large-sized, DRG neurons and so PV may have a role in cell survival. Support for this idea comes from studies in motoneuron disease models, where PV is expressed in motoneuron groups that are resistant to amyotrophic lateral sclerosis (ALS) and over-expression studies where PV prevents excitotoxic cell death (Medici and Shortland, 2015).

Although PV is not present in the central terminals of afferents in the spinal cord, PV staining is clearly present in the dorsal horn. This is associated with the cell bodies and dendrites of a subset of inhibitory interneurons located in lamina III-III. Recent pharmacogenetic and selective ablation studies have shown that these PV neurons play a key gate keeping role in mechanical allodynia associated with nerve injury (Petitjean et al., 2015). These neurons receive input from low threshold cutaneous afferents and in turn provide presynaptic inhibition of A-fibres as well as postsynaptic



**Figure 1** Overview of the different roles of PV neurons in pain circuits of the spinal cord.

PV identifies sensory proprioceptive afferents that make monosynaptic connections with lower motoneurons (MN). After injury, PV levels in DRG neurons remains unchanged but there is a loss of presynaptic terminals from MNs resulting in reduced motor function. In the spinal cord, PV identifies a subset of inhibitory dorsal horn interneurons that gate information from A $\beta$  afferents by presynaptically inhibiting A-fibres and postsynaptically inhibiting PKC $\gamma$  excitatory interneurons. Additionally, A $\beta$  afferents contact other inhibitory (e.g., Dyn and gamma-amino-butyric acid (GABA)) and excitatory (e.g., SOM) interneurons forming a circuit that regulates nociceptive transmission. Nerve injury alters the synaptic efficacy and results in decreased activation of inhibitory PV cells and increased activation of excitatory neurons (e.g., vGlut3, PKC $\gamma$  and SOM) and so results in changes in the neuronal circuitry such that innocuous stimuli can now produce behavioural hypersensitivities such as mechanical and cold allodynia. DRGs: Dorsal root ganglia; A $\beta$ : low threshold mechanoreceptor afferents; SOM: somatostatin; vGlut3: vesicular glutamate transport 3; PKC $\gamma$ : protein kinase C- $\gamma$ .

inhibition to PKC $\gamma$  excitatory interneurons. Nerve injury induces a 50% loss of synaptic appositions between PV interneurons and PKC $\gamma$  interneurons resulting in disinhibition of pain circuits, without causing any death of PV neurons. Moreover, selective pharmacological blockade of PKC $\gamma$  neurons attenuated the injury-induced mechanical allodynia (Petitjean et al., 2015). Two other circuits have also been proposed for regulating nociceptive transmission: one involves the inhibition of somatostatin (SOM)-positive excitatory interneurons by dynorphin (Dyn)-positive inhibitory interneurons. The second involves vesicular glutamate transport 3 (vGlut3)-positive excitatory interneurons which regulate activity in PKC $\gamma$  or calretinin-positive excitatory interneurons (Gangadharan and Kuner, 2015). Under normal conditions, the vGlut3 interneurons are under powerful inhibitory control but after nerve injury they can drive mechanical allodynia (Arcourt and Lechner, 2015; Gangadharan and Kuner, 2015). Future experiments should determine whether PV interneurons also gate connections with these two neuronal subpopulations.

A final layer of complexity is added by the presence of another DRG subpopulation that can cause allodynia after nerve injury. C-low threshold mechanoreceptors (CLTMs) are attributed to conveying sensations of pleasant touch in normal circumstances but produce

allodynia after injury (Nagi et al., 2011; Samour et al., 2015). These fibres have a unique molecular repertoire and terminate in lamina II of the cord onto central, stalked and vertical cells (Arcourt and Lechner, 2015). Since PV cells have these morphologies (Hughes et al., 2012) they are ideally placed to receive input from such afferents, although this speculation requires confirmation.

Taken together, the results of our and other studies suggest that PV is an important protein related to sensory pathways and the plasticity that occurs after injury (Figure 1). We demonstrated that in primary afferents, PV did not change and so can still be used as an anatomical marker of proprioceptors to explore changes in other transmitters, ion channels or transcription factors after peripheral nerve injury. In the CNS, PV may confer preferential survival to excitotoxic insults and importantly gates abnormal sensory information after injury.

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