Diverse roles and modulations of IA in spinal cord pain circuits

Nadine Clerc, Aziz Moqrich

Diverse roles and modulations of I_A in spinal cord pain circuits

Nadine Clerc[1,*] and Aziz Moqrich[1]

[1]Aix-Marseille-université, CNRS, Institut de Biologie du Développement de Marseille, UMR 7288, case 907, 13288 Marseille Cedex 09, France.

Correspondance : nadine.clerc@univ-amu.fr

Summary

This review highlights recent findings of different amplitude ranges, roles and modulations of A-type K^+ currents (**I_A**) **in excitatory (GAD67-GFP$^-$) and inhibitory (GAD67-GFP$^+$) interneurons in mouse spinal cord pain pathways. Endogenous neuropeptides, such as TAFA4, oxytocin and dynorphin in particular, have been reported to modulate I_A in these pain pathways, but only TAFA4 has been shown to reverse fully the opposing modulations that occur selectively in LIIo GAD67-GFP$^-$ and LIIi GAD67-GFP$^+$ interneurons following both neuropathic and inflammatory pain. If, as hypothesized here, Kv4 subunits underlie I_A in both GAD67-GFP$^-$ and GAD67-GFP$^+$ interneurons, then I_A diversity in spinal cord pain pathways may depend on the interneuron subtype-selective expression of Kv4 auxiliary subunits with functionally different N-terminal variants. Thus, I_A emerges as a good candidate for explaining the mechanisms underlying injury-induced mechanical hypersensitivity.**

Introduction

A-type K^+ currents (I_A) were initially characterized in invertebrate neurons (Connor and Stevens, 1971a, b). They take their name from their typical profile generated by the opposing effects of fast activation and inactivation. Within the central nervous system and heterologous expression systems, I_A can be generated by multiple Kv pore-forming α subunits: Kv1.4, Kv3.3, Kv3.4, all members of the Kv4 subfamily and Erg3 (Strube et al., 2015; Zemel et al., 2018). In addition, Kv1 subfamily α subunits yielding delayed rectifier currents are rapidly inactivated when co-expressed with auxiliary β1 subunits (Jerng et al., 2004; Pongs and Schwarz, 2010). I_A can thus be generated by a wide variety of molecular entities with different gating and pharmacological properties, as summarized by (Zemel et al., 2018) and (Johnston, 2021). I_A is a low-voltage activating current when carried by Kv1.4 and Kv4s, but a high-voltage activating current when carried by Kv3.4. Recovery from inactivation is slow for I_A carried by Kv1.4 and Kv3.4, but fast for I_A carried by Kv3.3 and Kv4s. Remarkably, the I_A carried by Kv4s has the most hyperpolarized voltage dependence for steady-state inactivation.

Native neuronal I_A ion channels function as macromolecular protein complexes including one or more Kv α subunits (from a single subfamily) in association with cytosolic and/or transmembrane accessory subunits and regulatory proteins influencing channel stability, localization and biophysical properties. For example, for the I_A carried by Kv4 α subunits, two types of auxiliary subunits are classically described: Kv channel-interacting proteins (KChIPs) and dipeptidyl peptidase-like proteins (DPLPs). KChIPs are cytoplasmic calcium-binding proteins that interact with the intracellular moieties of the Kv4 α subunits, and DPLPs are type II transmembrane proteins (with a large extracellular portion) that associate with the Kv4 channel core (see (Pongs and Schwarz, 2010); (Jerng and Pfaffinger, 2014a). The roles of

the Kv α subunits and I_A channel accessory subunits depend on cell type (see (Carrasquillo and Nerbonne, 2014).

The roles of these different functional entities in dorsal root ganglion (DRG) neurons has recently been reviewed by (Zemel et al., 2018), and our understanding of the changes in I_A channel expression, function and modulation in these neurons in pain models has progressed (Tsantoulas and McMahon, 2014; Zemel et al., 2018).

Fewer data are available for the spinal cord dorsal horn, but it is becoming clear that I_A plays diverse roles in this structure. The spinal cord dorsal horn is a central nervous system structure divided into six Rexed laminae [with Lamina II (LII) subdivided into LII outer (LIIo) and inner (LIIi)] that receive the central terminals of primary afferent neurons and descending supraspinal projections (see (Moehring et al., 2018; Peirs and Seal, 2016; Todd, 2010). It is, therefore, the principal site for primary afferent input integration and descending supraspinal modulation. Most C- and Aδ- nociceptive primary afferents form synaptic contacts in the superficial laminae (LI and LII), whereas low-threshold Aδ- and Aβ-primary afferents mostly project onto deeper laminae (LIII to LV). At the border between LII and LIII, LIIi is the projection site of the low-threshold C- primary afferents, which are unusual in being able to transmit pleasant touch and modulate unpleasant mechanical pain sensations (Olausson et al., 2010; Seal et al., 2009; Yoo et al., 2021). The spinal cord dorsal horn includes a large number of excitatory and inhibitory interneuron populations (Abraira et al., 2017; Haring et al., 2018). LI and LII interneurons mostly respond to noxious peripheral stimulation, whereas the interneurons of deeper layers are more sensitive to touch. Projection neurons with cell bodies in LI and LIII transfer information to supraspinal sites, giving rise to the qualitative and affective aspects of pain sensation.

The gate control theory of pain was first proposed in 1965 (Melzack and Wall, 1965), and has evolved considerably since (Cui et al., 2016; Duan et al., 2014; Zhang et al., 2018). There is

now a consensus that lamina II neuron circuits play a major role in pain gating processes. (Hu et al., 2006), who used cultured mouse neurons from the LI to LIII laminae and pain models in mice (inflammation after carrageenan injection into the paw and chronic constriction of the sciatic nerve), were the first to put highlight on the importance of I_A in pain processing in the spinal cord dorsal horn. They identified Kv4.2 α subunits as crucial components by showing that the genetic elimination of this α subunit decreased I_A and increased membrane excitability in cultured dorsal horn neurons. These features were correlated with enhanced sensitivity to tactile and thermal stimuli and were ERK-dependent.

Several studies based on patch clamp recordings on neurons in spinal cord slices have recently revealed an unexpected diversity of amplitude ranges, roles and modulations of I_A in LII pain pathways and the regulation of these modulations by endogenous neuropeptides with potential antinociceptive action. We provide here an overview of these recent advances and present some hypotheses that could be used as starting points for deciphering the cell and signaling pathways underlying these effects. Ionic current recording remains challenging in the spinal cord, and we, therefore, also consider ways in which it could be optimized.

Optimizing I_A resolution and isolation in the spinal cord in mouse models of pain

Extensive data on I_A are lacking for spinal cord slices, probably because the spinal cord presents many obstacles to the accurate measurement of ionic currents. In particular, it becomes heavily myelinated and less resistant to anoxia during the first few weeks after birth, even though pain processes are immature at this point in development (see (Mitra and Brownstone, 2012). Each experimental step must be optimized to overcome these problems to make it possible to obtain data for the mature spinal cord (see Table 1). Alternatively, once I_A pain-induced modulation has been observed in specific interneuron subtype using spinal cord slices, cultured neurons may be a more appropriate study model for deciphering cell signaling pathways, as they can bypass the obstacles specific to slices. Regardless of the study model

chosen, it is useful to begin by considering the situation from the viewpoint of the biophysicist, exploring the voltage dependence of the Kv channels at a large range of potentials. This generates useful information that can feed into physiological interpretations. The appropriateness of this strategy is illustrated by the fact that, for example, using a holding potential close to the resting membrane potential partly inactivates the I_A carried by Kv4 α subunits (see above), which may prevent its detection, particularly if it is a small I_A.

I_A is expressed in excitatory and inhibitory dorsal horn interneurons

Possible differences in I_A expression between the inhibitory and excitatory interneurons of the spinal cord dorsal horn have been investigated by patch-clamp recordings on interneurons from transgenic mice expressing enhanced GFP under the control of the promoter of the gene encoding the GABA-synthesizing enzyme GAD67 (GAD67-GFP$^+$ interneurons). I_A was recorded (with markedly different voltage protocols) with cultured neurons (Hu et al., 2006) or interneurons in spinal cord slices from young adult mice (Figure 1; (Yoo et al., 2021), the corresponding transgenic lines being generated by (Oliva et al., 2000) and (Tamamaki et al., 2003), respectively. In cultured neurons obtained from LI to LIII, I_A was induced with a +40 mV step after a -80 mV conditioning prepulse (Hu and Gereau, 2011). In these conditions, I_A densities were ~250 pA/pF in GAD67-GFP$^-$ neurons (excitatory neurons) and ~140 pA/pF in GAD67-GFP$^+$ neurons (inhibitory interneurons) which included a small percentage of neurons with no I_A. In interneurons recorded on slices, I_A was induced with a +70 mV step after a -120 mV conditioning prepulse (Yoo et al., 2021). With this protocol, I_A densities were determined for GAD67-GFP$^-$ and GAD67-GFP$^+$ interneurons selectively located in LIIi or in LIIo. The values obtained were ~ 260 pA/pF for LIIi GAD67-GFP$^-$ interneurons, ~295 pA/pF for LIIo GAD67-GFP$^-$ interneurons, ~40 pA/pF for LIIi GAD67-GFP$^+$ interneurons and finally ~95 pA/pF for LIIo GAD67-GFP$^+$ interneurons. The data obtained when using the two different models were remarkably consistent leading to the conclusion that both types of

interneuron express I_A in naïve mice, but with striking differences in amplitude. Indeed, in GAD67$^-$GFP$^-$ interneurons, which are excitatory, the I_A is large (see Figure 1); by contrast, in GAD67$^-$GFP$^+$ interneurons, which are inhibitory, it is small (see Figure 1), sometimes at the limit of detection. The higher values obtained for cultured neurons than for neurons in slices probably reflect the possibility of recording ionic currents with lower access resistances (Ra) than in slices (Ra= 6-15 MΩ for cultured neurons in Hu and Gereau's study; (Hu and Gereau, 2011) and ≤ 19 MΩ for neurons in slices in the study by (Yoo et al., 2021). See Table 1 for the consequences of high Ra.

The I_A in LII GAD67$^-$GFP$^-$ interneurons is generally thought to be carried by Kv4.2 α subunits (Hu et al., 2006; Hu and Gereau, 2011; Huang et al., 2005), but further investigations are required to identify unambiguously the ion channels carrying the I_A in LII GAD67-GFP$^+$ interneurons. After the off-line subtraction of IK_{DR} from IK_{total} on full current traces (see Table 1), the I_A in LII GAD67-GFP$^+$ interneurons typically displays fast inactivation (Figure 1; (Yoo et al., 2021). (Yoo et al., 2021) systematically performed their recordings in the presence of a high TEA concentration to ensure that any current potentially carried by Kv3.3 or Kv3.4 channels was blocked, due to the high TEA-sensitivity of these channels (see (Johnston, 2021; Strube et al., 2015). We can deduce that the I_A in LII GAD67-GFP$^+$ interneurons may be carried by Kv1.4 and/or Kv4s (probably Kv4.2 and Kv4.3) subunits. Kv1.4 immunoreactivity has been reported for neuronal soma from the rat dorsal horn (Edwards et al., 2002). If the I_A in GAD67-GFP$^+$ interneurons is carried largely by Kv1.4 subunits it should recover slowly from inactivation (see (Johnston, 2021; Zemel et al., 2018). Remarkably, (Hu et al., 2006) found that most cultured GAD67-GFP$^+$ neurons were immunoreactive for Kv4.2. Less striking immunohistological staining results were obtained for slices. In particular, (Nowak et al., 2011) detected no immunoreactivity for either Kv4.2 or Kv4.3 in the soma of GAD67-GFP$^+$ interneurons. These negative results may reflect

immunostaining for Kv4s being below the threshold of detection at the cell body membrane, but stronger in neuronal processes. Consistent with this hypothesis, (Huang et al., 2005) detected Kv4.2 immunoreactivity in "long fibrous structures extending rostro caudally" and reminiscent of the processes of islet cells (which are exclusively inhibitory) but never in neuronal cell bodies in rats. (Strube et al., 2015) were also unable to visualize Kv4-immunoreactive neuronal cell bodies in the nucleus of the tractus solitarius of the Rat, even though AmmTX3, a scorpion toxin of the α-KTX 15 family that blocks all Kv4s, inhibited I_A by 80% in this structure. To unambiguously demonstrate that, as suspected, the I_A in GAD67-GFP$^+$ LIIi interneurons is mainly carried by Kv4s, similar pharmacological experiments should be performed on these interneurons, possibly after SNI to get relatively high I_A control values.

In neuropathic and inflammatory pain, I_A is modulated differentially in excitatory and inhibitory dorsal horn interneurons

The induction of irreversible neuropathic pain by spared nerve injury (SNI) in gad1^{GFP} mice leads to an upregulation of I_A in LIIi GAD67-GFP$^+$ interneurons and a downregulation of this current in LIIo GAD67-GFP$^-$ interneurons (Table 2; (Tamamaki et al., 2003; Yoo et al., 2021). Interestingly, I_A is unaffected in LIIo GAD67-GFP$^+$ interneurons and in LIIi GAD67-GFP$^-$ interneurons. The increase in I_A reached 74% of control values for I_A evoked by depolarizing steps of +70 mV after a -120 mV conditioning prepulse, in LIIi GAD67-GFP$^+$ interneurons (Table 2) and the I_A in LIIo GAD67-GFP$^-$ interneurons was about 35% lower than control values (Table 2). A decrease in I_A has also been observed in LIIo excitatory interneurons (particularly LIIo SOM^{Cre}-negative interneurons) during inflammation induced by capsaicin injection into the hind paw (Zhang et al., 2018). (Harris et al., 2014) described a similar effect in the upper cervical superficial dorsal horn neurons following acute neck muscle inflammation due to carrageenan injection. These neurons were blindly patched and

were not, therefore, functionally identified, but it can be inferred that they were excitatory. Indeed, in these neurons, I_A was evoked by the application of a low depolarizing voltage (from -90 to -40 mV), which would have been much too low to produce a detectable I_A in LIIi GAD67-GFP$^+$ interneurons, or even LIIo GAD67-GFP$^+$ interneurons (Yoo et al., 2021).

Overall, these data suggest that, in mice, LII interneurons are sensitized, during both neuropathic and inflammatory pain, by opposing modulations of I_A in LIIi inhibitory and LIIo excitatory interneurons. The role of I_A therefore differs between these two interneuron subpopulations.

I_A has opposite roles in LIIo excitatory and LIIi inhibitory interneurons, in both the presence and absence of pain

In LIIi inhibitory interneurons in naïve conditions, a small I_A should lead to high levels of neuron excitability, consistent with reports of mostly tonic firing in GAD67-GFP$^+$ or GAD65-GFP$^+$ interneurons (Cui et al., 2011; Daniele and MacDermott, 2009; Punnakkal et al., 2014; Zhang and Dougherty, 2011). A similar firing pattern has been reported for LIIi subpopulations of inhibitory neurons, such as glycinergic (GLY) interneurons expressing dynorphin (DYN$^+$; (Duan et al., 2014) or parvalbumin positive (PV$^+$) interneurons (Boyle et al., 2019). A much larger I_A after SNI probably results in lower rates of neuron firing. Consistent with this theoretical assumption, the excitability of PV$^+$ interneurons decreases after SNI (Boyle et al., 2019). More specifically, the amplitude of the current injection required to maintain tonic firing increased after SNI. PV$^+$ interneurons, with their islet cell-like morphology, are typified by a hyperpolarization-activated current (I_h; (Boyle et al., 2019; Hughes et al., 2012) carried by HCN channels and known to act in opposition to I_A (see (Biel et al., 2009). This is also a characteristic of the wider population of LIIi GAD67-GFP$^+$ interneurons, which present a large I_h in naïve conditions (Yoo et al., 2021). Yoo et al. (2021) reported a large decrease in this I_h after SNI, whereas (Boyle et al., 2019) reported no change.

This discrepancy can be explained by the restriction of the SNI-induced effect reported by (Yoo et al., 2021) to the interneurons located in the circumscribed area of SNI-induced microglial activation, rendering its detection unlikely when interneurons are sampled from a larger area ipsilateral to SNI (Boyle et al., 2019). In conclusion, through opposite effects on I_A and I_h, (increasing I_A and decreasing I_h), SNI generates synergistic modulations of I_A and I_h leading to a strong decrease in the excitability of LIIi inhibitory interneurons.

In LIIo excitatory interneurons, a large I_A is required to decrease excitability in naïve conditions. This is illustrated by LIIo SOM^{Cre}-negative interneurons, in which I_A strongly delays the action potential firing triggered by depolarizing steps. As expected, this delay is abolished when I_A is blocked with 4-AP (Zhang et al., 2018) and should be reduced after SNI decreasing I_A by one third in GAD67-GAD^- interneurons (Yoo et al., 2021).

Llli GAD67-GFP^+ interneurons display modulations of I_A (and I_h) that cannot be generalized to their LIIo counterpart. In naïve conditions, these LIIo interneurons have a large I_A (albeit much smaller than that in LIIo GAD67-GFP^- interneurons; see Figure 1) and a very small I_h. SNI does not significantly modify either of these currents in the total LIIo GAD-GFP^- interneuron population (Yoo et al., 2021).

I_A modulation is not a general feature of all GAD67-GFP^- interneurons. I_A and I_h have large amplitudes in LIIi and LIIo GAD67-GFP^- interneurons in control conditions (see Fig. 1), but SNI modulates I_A only in LIIo neurons after SNI, with I_h remaining unaffected in all GAD67-GFP^- interneuron subtypes.

The selectivity of I_A and I_h modulations between different subtypes of interneuron at strategic locations within LII probably reflects specific functions of I_A and I_h in pain gate control circuits.

Functional role of I_A modulations in pain gate control circuits

In the pain gate control circuits, I_A function differs between interneuron types (Figure 2). In LIIo excitatory interneurons, particularly those that transmit pain [the T neurons of the gate control theory; see (Duan et al., 2014) and (Zhang et al., 2018)], I_A establishes permissive conditions for feed-forward inhibition, whereas, in LIIi inhibitory interneurons, it modulates the feed-forward inhibition capacity.

As elegantly demonstrated by (Zhang et al., 2018), the I_A in LIIo acts as an electric filter operating in LIIo SOM^{Cre} negative interneurons, a subtype of excitatory interneurons acting as T neurons. In these interneurons, when I_A is large (in naïve conditions), most attempts to stimulate Aβ fibers fail to produce action potentials, reflecting the closed configuration of the gate (Figure 2; left panel). By contrast, when the I_A in these interneurons is blocked with intracellular 4-AP (in the absence of the sensitization of other gate-control circuit elements), most stimulations of Aβ fibers generate action potentials, indicating that the gate is open. A similar effect is observed when I_A in these interneurons is decreased due to the sensitization (expressed by mechanical hypersensitivity) following the injection of capsaicin into mouse hind paw (Zhang et al., 2018). It likely also occurs after I_A decrease in LIIo GAD67-GFP^- interneurons following SNI (Yoo et al. 2021; Figure 2).

The I_A in LIIo excitatory interneurons may be large due to a constitutive release of dynorphin (DYN). This hypothesis emerged from observations of a decrease in I_A in LIIo excitatory interneurons (in particular LIIo SOM^{Cre}-negative interneurons) in conditions in which DYN release is suppressed by the genetic ablation of DYN^+ interneurons (Zhang et al., 2018). According to this hypothesis, DYN release should decrease, I_A should increase and action potential firing should decrease in LIIo DYN^+ $GLYT2^-$ interneurons (the most abundant DYN-positive neurons in LIIo), following the lesioning of nerves or inflammation. However, as Zhang et al. (2018) did not selectively manipulate DYN signaling but ablated the entire DYN neuronal population, I_A expression might be regulated by other factors released from

DYN neurons or by indirect consequences of widespread cell death within the dorsal horn following the ablation. In addition, the fact that, in GAD67-GFP$^-$ interneurons, I_A is large in both slices and cultures (see above), suggests that this current is shaped by intrinsic factors in these interneurons.

Another set of DYN$^+$ interneurons, the LIIi DYN$^+$ GLYT2$^+$ interneurons located at the LII/LIII border and thus included in the LIIi GAD67-GFP$^+$ interneuron population, may also have an important function. Indeed, in this area of the dorsal horn, the number of interneurons generating Aβ-evoked action potentials increases considerably following genetic ablation of the entire DYN$^+$ interneuron population (Zhang et al., 2018). These LIIi DYN$^+$ GLYT2$^+$ interneurons may not display a change in I_h (this current being known to be substantial only in PV$^+$ interneurons), but they could be involved in the increase in I_A observed in LIIi GAD67-GFP$^+$ interneurons after SNI (Yoo et al., 2021). Finally, within the population of LIIi GAD67-GFP$^+$ interneuron population, the PV$^+$ interneurons are the most likely to display an increase in I_A accompanied by a decrease in I_h after SNI (Yoo et al., 2021). As indicated above, these patterns of I_A and I_h modulations in LIIi GAD67-GFP$^+$ interneurons should markedly reduce their electrical activity, and, thus, their classical inhibitory function via inhibitory postsynaptic potentials and currents. This inhibition probably has multiple consequences, as LIIi DYN$^+$ GLYT2$^+$ interneurons are subject to feed-forward inhibition (Duan et al., 2014), whereas PV$^+$ interneurons target several populations of LII interneurons (Boyle et al., 2019) and have been reported to inhibit Aβ terminals presynaptically (Boyle et al., 2019).

Endogenous peptides with potential antinociceptive action via I_A modulation in LII

Numerous endogenous pain modulators (opioids, nociceptin, cannabinoids, adenosine, adenosine 5'-triphosphate (ATP), bradykinin, norepinephrine, serotonin, dopamine, somatostatin, galanin, substance P, neuropeptide Y, oxytocin and acetylcholine) have been shown to induce changes in excitatory or inhibitory synaptic transmission and membrane

potential in the spinal lamina II interneurons. The targets on which these endogenous analgesics act are mostly membrane proteins, such as receptors for neurotransmitters or the potassium-chloride exporter KCC2 [see (Kumamoto, 2019) for review]. As mentioned above, DYN could potentially constitutively upregulate I_A in LIIo SOM^{Cre}-negative interneurons, but the only other modulator reported to act on I_A in LII interneurons is oxytocin, a peptide released by axons originating from a neuron subpopulation of the paraventricular nucleus of the hypothalamus (PVN) and terminating in superficial layers of the spinal cord (Breton et al., 2009). Another recently discovered potential endogenous analgesic, TAFA4, a neuropeptide produced by C-LTMRs, has been shown to act through the differential modulation of I_A in LIIo GAD67-GFP^- and LIIi GAD67-GFP^+ interneurons (Yoo et al., 2021).

Oxytocin -whether secreted in the spinal cord after PVN stimulation applied or exogenously by intrathecal infusion- has been shown to have antinociceptive properties during the development of inflammatory or neuropathic pain [see (Breton et al., 2009; Kumamoto, 2019). A study based on patch-clamp recordings of blindly patched neurons in rat spinal cord slices showed that bath applications of the specific oxytocin receptor agonist TGOT decreased I_A and IKDR respectively by 45% and 42%, in LII interneurons. These down regulations of potassium currents synergically decreased the firing ability of LII interneurons, specifically those with bursting firing patterns (Breton et al., 2009). In mice, oxytocin receptor-expressing interneurons are always immunoreactive for ChAT (and may therefore be considered probably excitatory) and have been reported to be intersperced with protein kinase Cγ interneurons in LIIi (Wrobel et al., 2011). Further studies are required to determine whether I_A is modulated in these ChAT positive interneurons in conditions of neuropathic pain and whether this modulation is reversed by oxytocin.

TAFA4 is produced by C-LTMRs. In control conditions, TAFA4-containing central terminals project onto LIIi, below the PKCγ-positive interneurons, with peripheral terminals innervating

299 the hair follicles in the dermis (Delfini et al., 2013; Hoeffel et al., 2021; Yoo et al., 2021). In
300 the skin sunburn model, skin damage induces an increase in TAFA4 production by DRGs
301 leading to an increase in TAFA4 levels in the skin. This increase triggers a dysregulation of
302 macrophage activity leading to an increase in the concentration of IL-10, which has anti-
303 inflammatory effects, ultimately favoring skin repair (Hoeffel et al., 2021). No data are
304 currently available concerning putative changes in TAFA4 production within the dorsal spinal
305 cord after peripheral inflammation or sciatic nerve injury. After SNI, TAFA4 persists in the
306 projection territory of the spared C-LTMRs but disappears from the spinal territory displaying
307 strong microglial activation. I_A (together with I_h) has been shown to be modulated in specific
308 populations of interneurons within this highly perturbed area, as described above (Yoo et al.
309 2021). Each SNI-induced modulation can be reversed by incubating the spinal cord slices in
310 an external solution containing TAFA4. In other words, the exogenous administration of
311 TAFA4 results in opposite modulations of I_A in LIIo GAD67-GFP$^-$ and LIIi GAD67-GFP$^+$
312 interneurons (increasing and decreasing I_A, respectively). These modulations can be prevented
313 by adding RAP (receptor-associated protein), an LDL-receptor antagonist, together with
314 TAFA4. In parallel, TAFA4 reversed inflammatory, postoperative and SNI-induced
315 mechanical hypersensitivity in both male and female mice, also in an LDL receptor-dependent
316 manner (Yoo et al., 2021).
317 How does the exogenous administration of TAFA4 lead to opposite changes in I_A in LIIo
318 GAD67-GFP$^-$ and LIIi GAD67-GFP$^+$ interneurons? Several scenarios are possible:
319 (i) TAFA4 may activate different cellular and/or molecular pathways independently targeting
320 I_A in LIIo GAD67-GFP$^-$ and in LIIi GAD67-GFP$^+$ interneurons. For example, both neuronal
321 and non-neuronal sources of reactive oxygen and nitrogen (nitroxidative) species (ROS and
322 RNS) implicated in pain processing (Grace et al., 2016; Jerng and Pfaffinger, 2014b;
323 Kuboyama et al., 2011) might be involved. In this scenario, TAFA4 may act by decreasing the

production of ROS and RNS, consistent with the recently described anti-inflammatory function of this molecule (Hoeffel et al., 2021). Following SNI, the elimination of TAFA4 from the central terminals of the cut C-LTMRs probably contributes to the irreversibility of pain, and only exogenous TAFA4 is effective in this context. ROS increase I_A only when this current is produced by Kv4 channels complexed with DPLP6a and/or DPLP10a (Jerng and Pfaffinger, 2014a, b). It therefore seems possible that, after SNI, ROS increase I_A in LIIi GAD67-GFP$^+$ interneurons but not in LIIo GAD67-GFP$^-$ interneurons, based on the assumption that DPLP6a and/or DPP10a are expressed in the former but not the latter. Interestingly, in the dorsal horn of the spinal cord, the expression of DPPL6 and DPPL10 is heterogeneous as most LI, LIII and LV dorsal horn projection neuron express DPPL10 whereas LII neurons express a strong DPPL6 immunoreactivity colocalized with Kv4.2 and KChiP2 in rostrocaudal dendrites (Cheng et al., 2016). However, the DPLP6 paralog subdistribution has not been determined and it remains unknown whether these rostrocaudal dendrites emerge from islet cells (inhibitory neurons), as might be expected. A simple way of testing the relevance of this hypothesis would be to check, in naive GAD67 mice, whether the small I_A in LIIi GAD67-GFP$^+$ interneurons increases following addition of the highly membrane-permeant H_2O_2 analog tert-butyl hydroperoxide (see (Jerng and Pfaffinger, 2014a). If this is indeed the case, then another modulator would be expected to act on LIIo GAD67-GFP$^-$ interneurons to trigger a decrease in I_A. At least in the heart, I_A (I_{to}, carried by Kv4.3) is downregulated by NO (Fernandez-Velasco et al., 2007; Gomez et al., 2008). For I_h in LIIi GAD67-GFP$^+$ interneurons, the activity of HCN channels has also been shown to be downregulated by NO, via a mechanism known as S-nitrosylation (see (Pires da Silva et al., 2016).

(ii) Alternatively, a single cellular and molecular pathway (summarized in figure 2) may be involved. In the SNI model, exogenous TAFA4 may preferentially target LIIi GAD67-GFP$^+$

interneurons, the activity of which modulates I_A levels in LIIo GAD67-GFP$^-$ interneurons. As suggested above, the activity of LIIi DYN$^+$GLYT2$^+$ interneurons and LIIi PV$^+$ interneurons may decrease after SNI, subsequently being restored by TAFA4, which would enable the LIIo GAD67-GFP$^-$ interneurons to recover their initial I_A amplitude.

This second scenario potentially works with any A-type channel α subunit. It implies that I_A and $I_{h,}$, in LIIi GAD67-GFP$^+$ and LIIo GAD67-GFP$^-$ interneurons involved in a specific pathway, are modulated by neurotransmitters acting in (or controlling) these pathways. The first scenario requires the expression of specific Kv4 ternary complexes in LIIi GAD67-GFP$^+$ and LIIo GAD67-GFP$^-$ interneurons. Whatever the LII pain pathways including these interneurons and the identity of the involved neurotransmitters, I_A and I_h modulations are conditionned by ROS or RNS acting on "adequat" Kv4 ternary complexes.

Concluding remarks

In the spinal cord dorsal horn, the amplitude range and role of I_A and its modulation after sensitization due to inflammation or nerve lesions differ considerably between interneuron subpopulations (as shown for LIIo GAD67-GFP$^-$ and LIIi GAD-67GFP$^+$ interneurons). The pathological consequences of perturbations of the intrinsic properties of spinal cord dorsal horn interneurons remain underestimated, but they could, as described here, play a major role in pain plasticity.

Endogenous neuropeptides, such as TAFA4, oxytocin and DYN in particular, have been reported to modulate I_A. In naïve conditions, oxytocin and DYN may have opposite effects, probably on different excitatory interneuron subtypes. However, it remains unclear whether these neuropeptides act in pain conditions. TAFA4 is the only one of these neuropeptides for which an effect has been shown in pain conditions. It has been shown to reverse pain-induced alterations of I_A in both excitatory and inhibitory interneurons (specifically in LIIo GAD67-GFP$^-$ and LIIi GAD-67-GFP$^+$ interneurons).

Further studies will be required to determine the biophysical properties, molecular determinants, modulation mechanisms and functional roles of I_A in spinal cord pain circuits. For this, it will be probably useful to refine the identification of the LII GAD67-GFP$^+$ and GAD67-GFP$^-$ interneurons that display pain-induced I_A modulations, and to focus on the interneuron subtypes for which deletions affect gating processes in the spinal cord dorsal horn. In the case of the GAD67-GFP$^+$ interneuron population, PV$^+$ and DYN$^+$ interneurons fulfil this criterion (see (Cui et al., 2016; Duan et al., 2014; Petitjean et al., 2015). PV$^+$ interneurons, which characteristically have a large I_h, probably account for a large proportion of the LIIi GAD67-GFP$^+$ interneurons displaying alterations of both I_A and I_h after SNI (Yoo et al., 2021). It remains unclear whether pain-induced modulations of I_A also occur in DYN$^+$ neurons. In addition, deep layer (LIII-V) inhibitory neurons that express the receptor tyrosine kinase Ret neonatally might be targeted as they mediate crosstalk between touch and pain pathways (Cui et al., 2016).

The LII GAD67-GFP$^-$ interneuron population contains numerous subpopulations of cells with a uniformly large I_A (Hu and Gereau, 2011; Yoo et al., 2021). The relatively large SOM^{Cre}-derived neuron population is essential for the expression of mechanical hypersensitivity (Cheng et al., 2017; Zhang et al., 2018), and might thus include some neuron subpopulations with pain-induced modulations of I_A. However, studies to date have focused on LIIo SOM^{Cre}-negative interneurons (not yet identified) because, unlike SOM^{Cre}-derived neurons, these cells receive frequent Aβ evoked action potentials after capsaicin treatment, due to I_A decrease in these neurons (Zhang et al., 2018).

If the pain-modulated I_A is carried by Kv4s in GAD67-GFP$^+$ interneurons as in GAD67-GFP$^-$ interneurons, then its functional diversity likely reflects that of the components of the full ternary complex Kv4-KChIP-DPLP (coupled to T and L Ca_v channels; see (Anderson et al., 2013; Jerng and Pfaffinger, 2014a)). These components should thus be identified in each

interneuron subtype exhibiting pain-modulated I_A because the expression of the different Kv4 auxiliary subunit paralogs is neuron specific; such knowledge should make it possible to develop novel targeted therapeutics as stated by Jerng and Pfaffinger (2014a).

Concerning the mechanisms of I_A modulation, the ERK-signaling-dependent downregulation of I_A, which was first described in superficial dorsal horn interneurons at the beginning of the 21st century (Hu et al., 2006; Hu and Gereau, 2003; Hu et al., 2003), is now known to occur in some LIIo excitatory interneurons (Zhang et al., 2018). Different, as yet, undetermined signaling processes probably occur in LIIi GAD67-GFP$^+$ interneurons to mediate the opposite patterns of I_A modulation reported by Yoo et al (2021).

Thus, the diversity of I_A amplitude ranges, roles and modulations highlighted in this review are probably dependent on interneuron subtype selectivity of expression of a large range of molecular substrates (Cav-Kv4 complex) and multiple signaling pathways.

ACKNOWLEDGMENTS

This work was funded by the French National Research Agency (ANR-CE16-Myochronic grant) and by the "Fondation pour la Recherche Médicale (FRM)" Equipe FRM-EQP202003010192, grants awarded to AM. This work was also supported by institutional funding from the CNRS and Aix-Marseille-Université awarded to IBDM.

AUTHOR CONTRIBUTIONS

N.C was responsible for performing the literature review and writing the article. A.M made substantial contributions to discussions concerning the content of the review article.

DECLARATION OF INTEREST

The authors have no competing interest to declare.

FIGURE LEGENDS

Figure 1: I_A in laminae IIo and IIi GAD67-GFP-positive and -negative interneurons in spinal slices

I_A (blue or purple traces) was evoked by a +70 mV step (as shown in the central panel) and isolated by off-line subtraction [**(Total IK at +70 mV from -120 mV) - (IK_{DR} at +70mV from -30 mV)**]. Note that $GAD67^-GFP^+$ LIIi interneurons had a large IK_{DR}, as previously described by Hu & Gereau (2011) in cultured neurons. The arrowhead before the red trace on the right indicates that, in LIIi $GAD67^-GFP^+$ interneurons in naïve conditions, I_A was preceded by a hyperpolarizing activated inward current (I_h) evoked by the -120 mV prepulse (see Yoo et al. 2021; Fig 4B and 5B). The same scaling was used for all current traces.

Figure 2: I_A and I_h modulations in LII interneurons after SNI or inflammation

In control conditions (left panel), a large I_A in LIIo excitatory interneuron (probably vertical cells and possibly SOM^{Cre}-negative interneurons; white with black plus) allows these neurons to gate Aβ-LTMR inputs (Zhang et al. 2018) and to prevent the activation of the mechanical pain pathway (doted vertical arrow). Such a large I_A in LIIo excitatory interneurons might depend (oblique dote arrow) on the LIIi inhibitory interneuron intense firing favored by a small I_A and a large I_h. These LIIi inhibitory interneurons (black with white minus) might be $PARV^+$ interneurons possibly also generating feed-forward inhibition onto LIIi DYN^+ $GLYT2^+$ interneurons and synaptically connected to vertical cells (Boyle et al. 2019). After nerve lesion such as SNI (right panel), LIIi inhibitory neurons lose their braking activity as I_A decreases and Ih increases with, as a final consequence, a decrease in I_A in LIIo excitatory interneurons that thus lose their gating ability as shown by Zhang et al. (2018). In these conditions, the mechanical pain pathway is activated (black vertical arrow).

The stylized traces are based on data from Zhang et al. (2018) and Yoo et al. (2021) for LIIo excitatory interneurons and exclusively from Yoo et al. (2021) for LIIi inhibitory interneurons.

The one * means that the data were exclusively obtained by Yoo et al. (2021). The two ** means that the data obtained by Yoo et al. (2021) were the same as those obtained by Zhang et al. (2018).

References

Abraira, V.E., Kuehn, E.D., Chirila, A.M., Springel, M.W., Toliver, A.A., Zimmerman, A.L., Orefice, L.L., Boyle, K.A., Bai, L., Song, B.J., *et al.* (2017). The Cellular and Synaptic Architecture of the Mechanosensory Dorsal Horn. Cell *168*, 295-310 e219.

Anderson, D., Engbers, J.D., Heath, N.C., Bartoletti, T.M., Mehaffey, W.H., Zamponi, G.W., and Turner, R.W. (2013). The Cav3-Kv4 complex acts as a calcium sensor to maintain inhibitory charge transfer during extracellular calcium fluctuations. J Neurosci *33*, 7811-7824.

Biel, M., Wahl-Schott, C., Michalakis, S., and Zong, X. (2009). Hyperpolarization-activated cation channels: from genes to function. Physiol Rev *89*, 847-885.

Boyle, K.A., Gradwell, M.A., Yasaka, T., Dickie, A.C., Polgar, E., Ganley, R.P., Orr, D.P.H., Watanabe, M., Abraira, V.E., Kuehn, E.D., *et al.* (2019). Defining a Spinal Microcircuit that Gates Myelinated Afferent Input: Implications for Tactile Allodynia. Cell reports *28*, 526-540 e526.

Breton, J.D., Poisbeau, P., and Darbon, P. (2009). Antinociceptive action of oxytocin involves inhibition of potassium channel currents in lamina II neurons of the rat spinal cord. Mol Pain *5*, 63.

Carrasquillo, Y., and Nerbonne, J.M. (2014). IA channels: diverse regulatory mechanisms. Neuroscientist *20*, 104-111.

Cheng, C.F., Wang, W.C., Huang, C.Y., Du, P.H., Yang, J.H., and Tsaur, M.L. (2016). Coexpression of auxiliary subunits KChIP and DPPL in potassium channel Kv4-positive nociceptors and pain-modulating spinal interneurons. J Comp Neurol *524*, 846-873.

Cheng, L., Duan, B., Huang, T., Zhang, Y., Chen, Y., Britz, O., Garcia-Campmany, L., Ren, X., Vong, L., Lowell, B.B., *et al.* (2017). Identification of spinal circuits involved in touch-evoked dynamic mechanical pain. Nat Neurosci *20*, 804-814.

Connor, J.A., and Stevens, C.F. (1971a). Inward and delayed outward membrane currents in isolated neural somata under voltage clamp. J Physiol *213*, 1-19.

Connor, J.A., and Stevens, C.F. (1971b). Voltage clamp studies of a transient outward membrane current in gastropod neural somata. J Physiol *213*, 21-30.

Cui, L., Kim, Y.R., Kim, H.Y., Lee, S.C., Shin, H.S., Szabo, G., Erdelyi, F., Kim, J., and Kim, S.J. (2011). Modulation of synaptic transmission from primary afferents to spinal substantia gelatinosa neurons by group III mGluRs in GAD65-EGFP transgenic mice. J Neurophysiol *105*, 1102-1111.

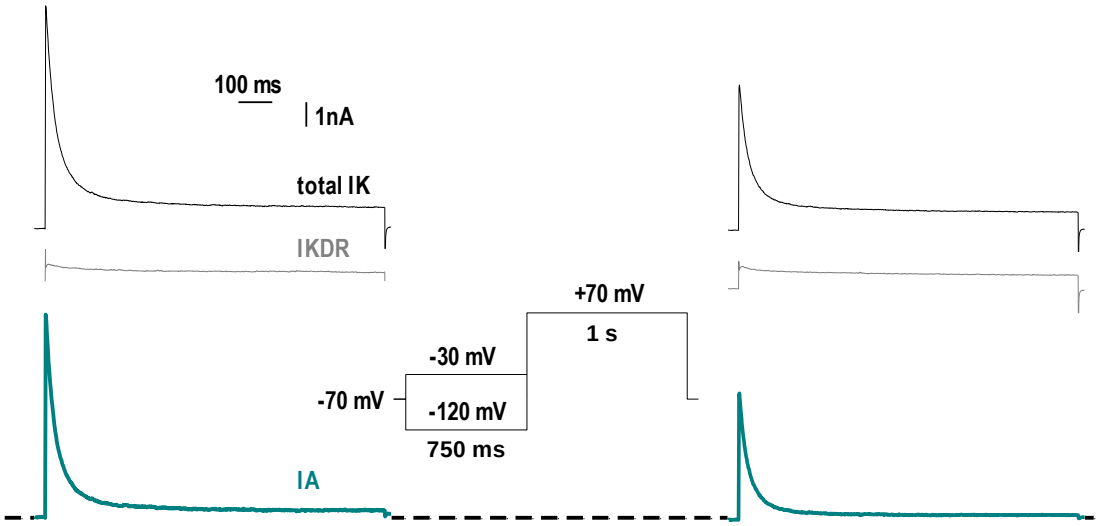
Cui, L., Miao, X., Liang, L., Abdus-Saboor, I., Olson, W., Fleming, M.S., Ma, M., Tao, Y.X., and Luo, W. (2016). Identification of Early RET+ Deep Dorsal Spinal Cord Interneurons in Gating Pain. Neuron *91*, 1413.

Daniele, C.A., and MacDermott, A.B. (2009). Low-threshold primary afferent drive onto GABAergic interneurons in the superficial dorsal horn of the mouse. J Neurosci *29*, 686-695.

Delfini, M.C., Mantilleri, A., Gaillard, S., Hao, J., Reynders, A., Malapert, P., Alonso, S., Francois, A., Barrere, C., Seal, R., *et al.* (2013). TAFA4, a chemokine-like protein, modulates injury-induced mechanical and chemical pain hypersensitivity in mice. Cell reports *5*, 378-388.

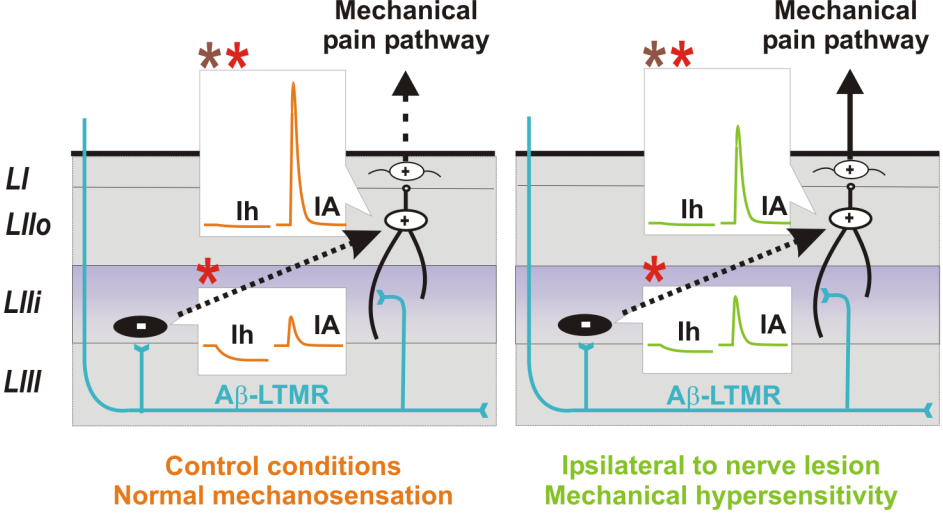
493 Duan, B., Cheng, L., Bourane, S., Britz, O., Padilla, C., Garcia-Campmany, L., Krashes, M., Knowlton,
494 W., Velasquez, T., Ren, X*., et al.* (2014). Identification of spinal circuits transmitting and gating
495 mechanical pain. Cell *159*, 1417-1432.
496 Edwards, L., Nashmi, R., Jones, O., Backx, P., Ackerley, C., Becker, L., and Fehlings, M.G. (2002).
497 Upregulation of Kv 1.4 protein and gene expression after chronic spinal cord injury. J Comp Neurol
498 *443*, 154-167.
499 Fernandez-Velasco, M., Ruiz-Hurtado, G., Hurtado, O., Moro, M.A., and Delgado, C. (2007). TNF-alpha
500 downregulates transient outward potassium current in rat ventricular myocytes through iNOS
501 overexpression and oxidant species generation. Am J Physiol Heart Circ Physiol *293*, H238-245.
502 Gomez, R., Nunez, L., Vaquero, M., Amoros, I., Barana, A., de Prada, T., Macaya, C., Maroto, L.,
503 Rodriguez, E., Caballero, R*., et al.* (2008). Nitric oxide inhibits Kv4.3 and human cardiac transient
504 outward potassium current (Ito1). Cardiovasc Res *80*, 375-384.
505 Grace, P.M., Gaudet, A.D., Staikopoulos, V., Maier, S.F., Hutchinson, M.R., Salvemini, D., and Watkins,
506 L.R. (2016). Nitroxidative Signaling Mechanisms in Pathological Pain. Trends Neurosci *39*, 862-879.
507 Haring, M., Zeisel, A., Hochgerner, H., Rinwa, P., Jakobsson, J.E.T., Lonnerberg, P., La Manno, G.,
508 Sharma, N., Borgius, L., Kiehn, O*., et al.* (2018). Neuronal atlas of the dorsal horn defines its
509 architecture and links sensory input to transcriptional cell types. Nat Neurosci *21*, 869-880.
510 Harris, B.M., Hughes, D.I., Bolton, P.S., Tadros, M.A., Callister, R.J., and Graham, B.A. (2014).
511 Contrasting alterations to synaptic and intrinsic properties in upper-cervical superficial dorsal horn
512 neurons following acute neck muscle inflammation. Mol Pain *10*, 25.
513 Hoeffel, G., Debroas, G., Roger, A., Rossignol, R., Gouilly, J., Laprie, C., Chasson, L., Barbon, P.V.,
514 Balsamo, A., Reynders, A*., et al.* (2021). Sensory neuron-derived TAFA4 promotes macrophage tissue
515 repair functions. Nature *594*, 94-99.
516 Hu, H.J., Carrasquillo, Y., Karim, F., Jung, W.E., Nerbonne, J.M., Schwarz, T.L., and Gereau, R.W.t.
517 (2006). The kv4.2 potassium channel subunit is required for pain plasticity. Neuron *50*, 89-100.
518 Hu, H.J., and Gereau, R.W.t. (2003). ERK integrates PKA and PKC signaling in superficial dorsal horn
519 neurons. II. Modulation of neuronal excitability. J Neurophysiol *90*, 1680-1688.
520 Hu, H.J., and Gereau, R.W.t. (2011). Metabotropic glutamate receptor 5 regulates excitability and
521 Kv4.2-containing K(+) channels primarily in excitatory neurons of the spinal dorsal horn. J
522 Neurophysiol *105*, 3010-3021.
523 Hu, H.J., Glauner, K.S., and Gereau, R.W.t. (2003). ERK integrates PKA and PKC signaling in superficial
524 dorsal horn neurons. I. Modulation of A-type K+ currents. J Neurophysiol *90*, 1671-1679.
525 Huang, H.Y., Cheng, J.K., Shih, Y.H., Chen, P.H., Wang, C.L., and Tsaur, M.L. (2005). Expression of A-
526 type K channel alpha subunits Kv 4.2 and Kv 4.3 in rat spinal lamina II excitatory interneurons and
527 colocalization with pain-modulating molecules. Eur J Neurosci *22*, 1149-1157.
528 Hughes, D.I., Sikander, S., Kinnon, C.M., Boyle, K.A., Watanabe, M., Callister, R.J., and Graham, B.A.
529 (2012). Morphological, neurochemical and electrophysiological features of parvalbumin-expressing
530 cells: a likely source of axo-axonic inputs in the mouse spinal dorsal horn. J Physiol *590*, 3927-3951.
531 Jerng, H.H., and Pfaffinger, P.J. (2014a). Modulatory mechanisms and multiple functions of
532 somatodendritic A-type K (+) channel auxiliary subunits. Front Cell Neurosci *8*, 82.
533 Jerng, H.H., and Pfaffinger, P.J. (2014b). S-glutathionylation of an auxiliary subunit confers redox
534 sensitivity to Kv4 channel inactivation. PLoS One *9*, e93315.
535 Jerng, H.H., Pfaffinger, P.J., and Covarrubias, M. (2004). Molecular physiology and modulation of
536 somatodendritic A-type potassium channels. Mol Cell Neurosci *27*, 343-369.
537 Johnston, J. (2021). Pharmacology of A-Type K(+) Channels. Handb Exp Pharmacol *267*, 167-183.
538 Kuboyama, K., Tsuda, M., Tsutsui, M., Toyohara, Y., Tozaki-Saitoh, H., Shimokawa, H., Yanagihara, N.,
539 and Inoue, K. (2011). Reduced spinal microglial activation and neuropathic pain after nerve injury in
540 mice lacking all three nitric oxide synthases. Mol Pain *7*, 50.
541 Kumamoto, E. (2019). Cellular Mechanisms for Antinociception Produced by Oxytocin and Orexins in
542 the Rat Spinal Lamina II-Comparison with Those of Other Endogenous Pain Modulators.
543 Pharmaceuticals (Basel) *12, 136*.
544 Melzack, R., and Wall, P.D. (1965). Pain mechanisms: a new theory. Science *150*, 971-979.

Mitra, P., and Brownstone, R.M. (2012). An in vitro spinal cord slice preparation for recording from lumbar motoneurons of the adult mouse. J Neurophysiol *107*, 728-741.
Moehring, F., Halder, P., Seal, R.P., and Stucky, C.L. (2018). Uncovering the Cells and Circuits of Touch in Normal and Pathological Settings. Neuron *100*, 349-360.
Nowak, A., Mathieson, H.R., Chapman, R.J., Janzso, G., Yanagawa, Y., Obata, K., Szabo, G., and King, A.E. (2011). Kv3.1b and Kv3.3 channel subunit expression in murine spinal dorsal horn GABAergic interneurones. J Chem Neuroanat *42*, 30-38.
Olausson, H., Wessberg, J., Morrison, I., McGlone, F., and Vallbo, A. (2010). The neurophysiology of unmyelinated tactile afferents. Neurosci Biobehav Rev *34*, 185-191.
Oliva, A.A., Jr., Jiang, M., Lam, T., Smith, K.L., and Swann, J.W. (2000). Novel hippocampal interneuronal subtypes identified using transgenic mice that express green fluorescent protein in GABAergic interneurons. J Neurosci *20*, 3354-3368.
Peirs, C., and Seal, R.P. (2016). Neural circuits for pain: Recent advances and current views. Science *354*, 578-584.
Petitjean, H., Pawlowski, S.A., Fraine, S.L., Sharif, B., Hamad, D., Fatima, T., Berg, J., Brown, C.M., Jan, L.Y., Ribeiro-da-Silva, A*., et al.* (2015). Dorsal Horn Parvalbumin Neurons Are Gate-Keepers of Touch-Evoked Pain after Nerve Injury. Cell Rep *13*, 1246-1257.
Pires da Silva, M., de Almeida Moraes, D.J., Mecawi, A.S., Rodrigues, J.A., and Varanda, W.A. (2016). Nitric Oxide Modulates HCN Channels in Magnocellular Neurons of the Supraoptic Nucleus of Rats by an S-Nitrosylation-Dependent Mechanism. J Neurosci *36*, 11320-11330.
Pongs, O., and Schwarz, J.R. (2010). Ancillary subunits associated with voltage-dependent K+ channels. Physiol Rev *90*, 755-796.
Punnakkal, P., von Schoultz, C., Haenraets, K., Wildner, H., and Zeilhofer, H.U. (2014). Morphological, biophysical and synaptic properties of glutamatergic neurons of the mouse spinal dorsal horn. J Physiol *592*, 759-776.
Seal, R.P., Wang, X., Guan, Y., Raja, S.N., Woodbury, C.J., Basbaum, A.I., and Edwards, R.H. (2009). Injury-induced mechanical hypersensitivity requires C-low threshold mechanoreceptors. Nature *462*, 651-655.
Strube, C., Saliba, L., Moubarak, E., Penalba, V., Martin-Eauclaire, M.F., Tell, F., and Clerc, N. (2015). Kv4 channels underlie A-currents with highly variable inactivation time courses but homogeneous other gating properties in the nucleus tractus solitarii. Pflugers Arch *467*, 789-803.
Tamamaki, N., Yanagawa, Y., Tomioka, R., Miyazaki, J., Obata, K., and Kaneko, T. (2003). Green fluorescent protein expression and colocalization with calretinin, parvalbumin, and somatostatin in the GAD67-GFP knock-in mouse. J Comp Neurol *467*, 60-79.
Todd, A.J. (2010). Neuronal circuitry for pain processing in the dorsal horn. Nat Rev Neurosci *11*, 823-836.
Tsantoulas, C., and McMahon, S.B. (2014). Opening paths to novel analgesics: the role of potassium channels in chronic pain. Trends Neurosci *37*, 146-158.
Wrobel, L., Schorscher-Petcu, A., Dupre, A., Yoshida, M., Nishimori, K., and Tribollet, E. (2011). Distribution and identity of neurons expressing the oxytocin receptor in the mouse spinal cord. Neurosci Lett *495*, 49-54.
Yoo, S., Santos, C., Reynders, A., Marics, I., Malapert, P., Gaillard, S., Charron, A., Ugolini, S., Rossignol, R., El Khallouqi, A*., et al.* (2021). TAFA4 relieves injury-induced mechanical hypersensitivity through LDL receptors and modulation of spinal A-type K(+) current. Cell reports *37*, 109884.
Zemel, B.M., Ritter, D.M., Covarrubias, M., and Muqeem, T. (2018). A-Type KV Channels in Dorsal Root Ganglion Neurons: Diversity, Function, and Dysfunction. Front Mol Neurosci *11*, 253.
Zhang, H., and Dougherty, P.M. (2011). Acute inhibition of signalling phenotype of spinal GABAergic neurons by tumour necrosis factor-alpha. J Physiol *589*, 4511-4526.
Zhang, Y., Liu, S., Zhang, Y.Q., Goulding, M., Wang, Y.Q., and Ma, Q. (2018). Timing Mechanisms Underlying Gate Control by Feedforward Inhibition. Neuron *99*, 941-955 e944.



GAD67-GFP- (excitatory) interneuron

GAD67-GFP+ (inhibitory) interneuron



Control conditions
Normal mechanosensation

Ipsilateral to nerve lesion
Mechanical hypersensitivity

Table 1: Toolbox for recording IA in spinal cord slices

Goal	Methodological trick	References
Good slices	Rapid removal of the spinal cord to prevent anoxia; fast intracardiac perfusion with adequately oxygenated slicing solution	Mitra & Browstone 2012 Yoo et al. 2021 (methods)
Access resistance (Ra; between the pipette and the cell) as low as possible	Reducing Ra to obtain values < 20 MΩ by using recording pipettes with tips as large as possible; fire-polishing helps	Strube et al. 2015 (Fig. 4)
IA resolution & isolation (1)	Pharmacological identification of the Kv family carrying the IA	Johnston 2021 Strube et al. 2015
IA resolution & isolation (2)	Determination of at least V1/2 inactivation to identify the most appropriate hyperpolarizing potential for the conditioning prepulse	
IA resolution & isolation (3)	If not wishing to study the IA carried by Kv3.3 or Kv3.4, decrease the persistent IK (IK_{DR}*) with high [TEA] in the recording solution.	Strube et al. 2015 (Fig. 4) Yao et al. 2013 (methods)
IA resolution & isolation (4)	P/N subtraction method to eliminate the effects of leak current on whole-cell responses (but requires a longer recording time)	Melnick 2011 (methods)
IA resolution & isolation (5)	Obtain the difference current (IK_{total} – IK_{DR}) by subtracting the full current traces. Subtracting the maximum IK_{DR} from the total instantaneous IK would lead to: 1- Underestimation of IA amplitude (because IK_{DR} is activated more slowly than IA) 2- An impossibility of inactivation time course evaluation	Hess & El Manira 2001 (Fig.1) Luther & Tasker 2000 (Fig. 2A) Strube et al. 2015 (discussion)
Targeting the spinal area affected by a peripheral lesion	The SNI model, for example, can be used to target the site at which LIIi and LIIo interneurons should be patched with precision, as this injury circumscribes the activation of microglia	Yoo et al. 2021 (methods)

- * IK_{DR}: delayed rectifier K^+ current

Table 2: Comparison of IA densities in LII excitatory and inhibitory interneurons in slices from mice either naïve or subjected to SNI (data from Yoo et al. 2021)

Location	Neuron type	*n*	Mouse model	IA density (+70 mV; pA/pF)	Ih density (-120 mV; pA/pF)
LII outer	ExIN	46	NAIVE	**295**	-2
		26	SNI	**192 (35% decrease)**	-2
	InhIN	25	NAIVE	95	-2
		14	SNI	84	-1.5
LII inner	ExIN	28	NAIVE	258	-2
		21	SNI	245	-22
	InhIN	52	NAIVE	**42**	**-112**
		32	SNI	**73 (74% increase)**	**-6**