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1 **Diverse roles and modulations of I_A in spinal cord pain circuits**

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9 **Summary**

10 **This review highlights recent findings of different amplitude ranges, roles and**
11 **modulations of A-type K^+ currents (I_A) in excitatory (GAD67-GFP⁻) and inhibitory**
12 **(GAD67-GFP⁺) interneurons in mouse spinal cord pain pathways. Endogenous**
13 **neuropeptides, such as TFAFA4, oxytocin and dynorphin in particular, have been**
14 **reported to modulate I_A in these pain pathways, but only TFAFA4 has been shown to**
15 **reverse fully the opposing modulations that occur selectively in LIIo GAD67-GFP⁻ and**
16 **LIII GAD67-GFP⁺ interneurons following both neuropathic and inflammatory pain. If,**
17 **as hypothesized here, Kv4 subunits underlie I_A in both GAD67-GFP⁻ and GAD67-GFP⁺**
18 **interneurons, then I_A diversity in spinal cord pain pathways may depend on the**
19 **interneuron subtype-selective expression of Kv4 auxiliary subunits with functionally**
20 **different N-terminal variants. Thus, I_A emerges as a good candidate for explaining the**
21 **mechanisms underlying injury-induced mechanical hypersensitivity.**

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26 **Introduction**

27 A-type K^+ currents (I_A) were initially characterized in invertebrate neurons (Connor and
28 Stevens, 1971a, b). They take their name from their typical profile generated by the opposing
29 effects of fast activation and inactivation. Within the central nervous system and heterologous
30 expression systems, I_A can be generated by multiple Kv pore-forming α subunits: Kv1.4,
31 Kv3.3, Kv3.4, all members of the Kv4 subfamily and Erg3 (Strube et al., 2015; Zemel et al.,
32 2018). In addition, Kv1 subfamily α subunits yielding delayed rectifier currents are rapidly
33 inactivated when co-expressed with auxiliary $\beta 1$ subunits (Jerng et al., 2004; Pongs and
34 Schwarz, 2010). I_A can thus be generated by a wide variety of molecular entities with
35 different gating and pharmacological properties, as summarized by (Zemel et al., 2018) and
36 (Johnston, 2021). I_A is a low-voltage activating current when carried by Kv1.4 and Kv4s, but
37 a high-voltage activating current when carried by Kv3.4. Recovery from inactivation is slow
38 for I_A carried by Kv1.4 and Kv3.4, but fast for I_A carried by Kv3.3 and Kv4s. Remarkably, the
39 I_A carried by Kv4s has the most hyperpolarized voltage dependence for steady-state
40 inactivation.

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

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

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

56 Fewer data are available for the spinal cord dorsal horn, but it is becoming clear that I_A plays
57 diverse roles in this structure. The spinal cord dorsal horn is a central nervous system
58 structure divided into six Rexed laminae [with Lamina II (LII) subdivided into LII outer
59 (LIIo) and inner (LIIi)] that receive the central terminals of primary afferent neurons and
60 descending supraspinal projections (see (Moehring et al., 2018; Peirs and Seal, 2016; Todd,
61 2010). It is, therefore, the principal site for primary afferent input integration and descending
62 supraspinal modulation. Most C- and A δ - nociceptive primary afferents form synaptic
63 contacts in the superficial laminae (LI and LII), whereas low-threshold A δ - and A β -primary
64 afferents mostly project onto deeper laminae (LIII to LV). At the border between LII and LIII,
65 LIIi is the projection site of the low-threshold C- primary afferents, which are unusual in
66 being able to transmit pleasant touch and modulate unpleasant mechanical pain sensations
67 (Olausson et al., 2010; Seal et al., 2009; Yoo et al., 2021). The spinal cord dorsal horn
68 includes a large number of excitatory and inhibitory interneuron populations (Abraira et al.,
69 2017; Haring et al., 2018). LI and LII interneurons mostly respond to noxious peripheral
70 stimulation, whereas the interneurons of deeper layers are more sensitive to touch. Projection
71 neurons with cell bodies in LI and LIII transfer information to supraspinal sites, giving rise to
72 the qualitative and affective aspects of pain sensation.

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

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

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

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

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

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

106 **I_A is expressed in excitatory and inhibitory dorsal horn interneurons**

107 Possible differences in I_A expression between the inhibitory and excitatory interneurons of the
108 spinal cord dorsal horn have been investigated by patch-clamp recordings on interneurons
109 from transgenic mice expressing enhanced GFP under the control of the promoter of the gene
110 encoding the GABA-synthesizing enzyme GAD67 (GAD67-GFP⁺ interneurons). I_A was
111 recorded (with markedly different voltage protocols) with cultured neurons (Hu et al., 2006)
112 or interneurons in spinal cord slices from young adult mice (Figure 1; (Yoo et al., 2021), the
113 corresponding transgenic lines being generated by (Oliva et al., 2000) and (Tamamaki et al.,
114 2003), respectively. In cultured neurons obtained from LI to LIII, I_A was induced with a +40
115 mV step after a -80 mV conditioning prepulse (Hu and Gereau, 2011). In these conditions, I_A
116 densities were ~250 pA/pF in GAD67-GFP⁻ neurons (excitatory neurons) and ~140 pA/pF in
117 GAD67-GFP⁺ neurons (inhibitory interneurons) which included a small percentage of
118 neurons with no I_A . In interneurons recorded on slices, I_A was induced with a +70 mV step
119 after a -120 mV conditioning prepulse (Yoo et al., 2021). With this protocol, I_A densities were
120 determined for GAD67-GFP⁻ and GAD67-GFP⁺ interneurons selectively located in LIIIi or in
121 LIIo. The values obtained were ~ 260 pA/pF for LIIIi GAD67-GFP⁻ interneurons, ~295 pA/pF
122 for LIIo GAD67-GFP⁻ interneurons, ~40 pA/pF for LIIIi GAD67-GFP⁺ interneurons and
123 finally ~95 pA/pF for LIIo GAD67-GFP⁺ interneurons. The data obtained when using the two
124 different models were remarkably consistent leading to the conclusion that both types of

125 interneuron express I_A in naïve mice, but with striking differences in amplitude. Indeed, in
126 GAD67-GFP⁻ interneurons, which are excitatory, the I_A is large (see Figure 1); by contrast, in
127 GAD67-GFP⁺ interneurons, which are inhibitory, it is small (see Figure 1), sometimes at the
128 limit of detection. The higher values obtained for cultured neurons than for neurons in slices
129 probably reflect the possibility of recording ionic currents with lower access resistances (R_a)
130 than in slices (R_a = 6-15 M Ω for cultured neurons in Hu and Gereau's study; (Hu and Gereau,
131 2011) and \leq 19 M Ω for neurons in slices in the study by (Yoo et al., 2021). See Table 1 for
132 the consequences of high R_a .

133 The I_A in LII GAD67-GFP⁻ interneurons is generally thought to be carried by Kv4.2 α
134 subunits (Hu et al., 2006; Hu and Gereau, 2011; Huang et al., 2005), but further investigations
135 are required to identify unambiguously the ion channels carrying the I_A in LII GAD67-GFP⁺
136 interneurons. After the off-line subtraction of $I_{K_{DR}}$ from $I_{K_{total}}$ on full current traces (see
137 Table 1), the I_A in LII GAD67-GFP⁺ interneurons typically displays fast inactivation (Figure
138 1; (Yoo et al., 2021). (Yoo et al., 2021) systematically performed their recordings in the
139 presence of a high TEA concentration to ensure that any current potentially carried by Kv3.3
140 or Kv3.4 channels was blocked, due to the high TEA-sensitivity of these channels (see
141 (Johnston, 2021; Strube et al., 2015). We can deduce that the I_A in LII GAD67-GFP⁺
142 interneurons may be carried by Kv1.4 and/or Kv4s (probably Kv4.2 and Kv4.3) subunits.
143 Kv1.4 immunoreactivity has been reported for neuronal soma from the rat dorsal horn
144 (Edwards et al., 2002). If the I_A in GAD67-GFP⁺ interneurons is carried largely by Kv1.4
145 subunits it should recover slowly from inactivation (see (Johnston, 2021; Zemel et al., 2018).
146 Remarkably, (Hu et al., 2006) found that most cultured GAD67-GFP⁺ neurons were
147 immunoreactive for Kv4.2. Less striking immunohistological staining results were obtained
148 for slices. In particular, (Nowak et al., 2011) detected no immunoreactivity for either Kv4.2 or
149 Kv4.3 in the soma of GAD67-GFP⁺ interneurons. These negative results may reflect

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

161 **In neuropathic and inflammatory pain, I_A is modulated differentially in excitatory and** 162 **inhibitory dorsal horn interneurons**

163 The induction of irreversible neuropathic pain by spared nerve injury (SNI) in gad1^{GFP} mice
164 leads to an upregulation of I_A in LIIi GAD67-GFP⁺ interneurons and a downregulation of this
165 current in LIIo GAD67-GFP⁻ interneurons (Table 2; (Tamamaki et al., 2003; Yoo et al.,
166 2021). Interestingly, I_A is unaffected in LIIo GAD67-GFP⁺ interneurons and in LIIi GAD67-
167 GFP⁻ interneurons. The increase in I_A reached 74% of control values for I_A evoked by
168 depolarizing steps of +70 mV after a -120 mV conditioning prepulse, in LIIi GAD67-GFP⁺
169 interneurons (Table 2) and the I_A in LIIo GAD67-GFP⁻ interneurons was about 35% lower
170 than control values (Table 2). A decrease in I_A has also been observed in LIIo excitatory
171 interneurons (particularly LIIo SOM^{Cre}-negative interneurons) during inflammation induced
172 by capsaicin injection into the hind paw (Zhang et al., 2018). (Harris et al., 2014) described a
173 similar effect in the upper cervical superficial dorsal horn neurons following acute neck
174 muscle inflammation due to carrageenan injection. These neurons were blindly patched and

175 were not, therefore, functionally identified, but it can be inferred that they were excitatory.
176 Indeed, in these neurons, I_A was evoked by the application of a low depolarizing voltage
177 (from -90 to -40 mV), which would have been much too low to produce a detectable I_A in LIII
178 GAD67-GFP⁺ interneurons, or even LIIo GAD67-GFP⁺ interneurons (Yoo et al., 2021).
179 Overall, these data suggest that, in mice, LII interneurons are sensitized, during both
180 neuropathic and inflammatory pain, by opposing modulations of I_A in LIII inhibitory and LIIo
181 excitatory interneurons. The role of I_A therefore differs between these two interneuron
182 subpopulations.

183 **I_A has opposite roles in LIIo excitatory and LIII inhibitory interneurons, in both the**
184 **presence and absence of pain**

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

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

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

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

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

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

223 **Functional role of I_A modulations in pain gate control circuits**

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

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

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

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

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

269 **Endogenous peptides with potential antinociceptive action via I_A modulation in LII**

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

274 potential in the spinal lamina II interneurons. The targets on which these endogenous
275 analgesics act are mostly membrane proteins, such as receptors for neurotransmitters or the
276 potassium-chloride exporter KCC2 [see (Kumamoto, 2019) for review]. As mentioned above,
277 DYN could potentially constitutively upregulate I_A in LIIo SOM^{Cre} -negative interneurons, but
278 the only other modulator reported to act on I_A in LII interneurons is oxytocin, a peptide
279 released by axons originating from a neuron subpopulation of the paraventricular nucleus of
280 the hypothalamus (PVN) and terminating in superficial layers of the spinal cord (Breton et al.,
281 2009). Another recently discovered potential endogenous analgesic, TAF4, a neuropeptide
282 produced by C-LTMRs, has been shown to act through the differential modulation of I_A in
283 LIIo $GAD67-GFP^-$ and LIII $GAD67-GFP^+$ interneurons (Yoo et al., 2021).

284 Oxytocin -whether secreted in the spinal cord after PVN stimulation applied or exogenously
285 by intrathecal infusion- has been shown to have antinociceptive properties during the
286 development of inflammatory or neuropathic pain [see (Breton et al., 2009; Kumamoto,
287 2019). A study based on patch-clamp recordings of blindly patched neurons in rat spinal cord
288 slices showed that bath applications of the specific oxytocin receptor agonist TGOT decreased
289 I_A and IK_{DR} respectively by 45% and 42%, in LII interneurons. These down regulations of
290 potassium currents synergically decreased the firing ability of LII interneurons, specifically
291 those with bursting firing patterns (Breton et al., 2009). In mice, oxytocin receptor-expressing
292 interneurons are always immunoreactive for ChAT (and may therefore be considered
293 probably excitatory) and have been reported to be interspersed with protein kinase $C\gamma$
294 interneurons in LIII (Wrobel et al., 2011). Further studies are required to determine whether I_A
295 is modulated in these ChAT positive interneurons in conditions of neuropathic pain and
296 whether this modulation is reversed by oxytocin.

297 TAF4 is produced by C-LTMRs. In control conditions, TAF4-containing central terminals
298 project onto LIII, below the $PKC\gamma$ -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 LIIIi 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 LIIIi 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 LIIIi 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

324 production of ROS and RNS, consistent with the recently described anti-inflammatory
325 function of this molecule (Hoeffel et al., 2021). Following SNI, the elimination of TFAFA4
326 from the central terminals of the cut C-LTMRs probably contributes to the irreversibility of
327 pain, and only exogenous TFAFA4 is effective in this context. ROS increase I_A only when this
328 current is produced by Kv4 channels complexed with DPLP6a and/or DPLP10a (Jerng and
329 Pfaffinger, 2014a, b). It therefore seems possible that, after SNI, ROS increase I_A in LIII
330 GAD67-GFP⁺ interneurons but not in LIIo GAD67-GFP⁻ interneurons, based on the
331 assumption that DPLP6a and/or DPP10a are expressed in the former but not the latter.
332 Interestingly, in the dorsal horn of the spinal cord, the expression of DPPL6 and DPPL10 is
333 heterogeneous as most LI, LIII and LV dorsal horn projection neuron express DPPL10
334 whereas LII neurons express a strong DPPL6 immunoreactivity colocalized with Kv4.2 and
335 KChiP2 in rostrocaudal dendrites (Cheng et al., 2016). However, the DPLP6 paralog
336 subdistribution has not been determined and it remains unknown whether these rostrocaudal
337 dendrites emerge from islet cells (inhibitory neurons), as might be expected. A simple way of
338 testing the relevance of this hypothesis would be to check, in naive GAD67 mice, whether the
339 small I_A in LIII GAD67-GFP⁺ interneurons increases following addition of the highly
340 membrane-permeant H₂O₂ analog tert-butyl hydroperoxide (see (Jerng and Pfaffinger, 2014a).
341 If this is indeed the case, then another modulator would be expected to act on LIIo GAD67-
342 GFP⁻ interneurons to trigger a decrease in I_A . At least in the heart, I_A (I_{to} , carried by Kv4.3) is
343 downregulated by NO (Fernandez-Velasco et al., 2007; Gomez et al., 2008). For I_h in LIII
344 GAD67-GFP⁺ interneurons, the activity of HCN channels has also been shown to be
345 downregulated by NO, via a mechanism known as S-nitrosylation (see (Pires da Silva et al.,
346 2016).

347 (ii) Alternatively, a single cellular and molecular pathway (summarized in figure 2) may be
348 involved. In the SNI model, exogenous TFAFA4 may preferentially target LIII GAD67-GFP⁺

349 interneurons, the activity of which modulates I_A levels in LIIo GAD67-GFP⁻ interneurons. As
350 suggested above, the activity of LIII DYN⁺GLYT2⁺ interneurons and LIII PV⁺ interneurons
351 may decrease after SNI, subsequently being restored by TAF4, which would enable the LIIo
352 GAD67-GFP⁻ interneurons to recover their initial I_A amplitude.

353 This second scenario potentially works with any A-type channel α subunit. It implies that I_A
354 and I_h , in LIII GAD67-GFP⁺ and LIIo GAD67-GFP⁻ interneurons involved in a specific
355 pathway, are modulated by neurotransmitters acting in (or controlling) these pathways. The
356 first scenario requires the expression of specific Kv4 ternary complexes in LIII GAD67-GFP⁺
357 and LIIo GAD67-GFP⁻ interneurons. Whatever the LII pain pathways including these
358 interneurons and the identity of the involved neurotransmitters, I_A and I_h modulations are
359 conditioned by ROS or RNS acting on “adequat” Kv4 ternary complexes.

360 **Concluding remarks**

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

367 Endogenous neuropeptides, such as TAF4, oxytocin and DYN in particular, have been
368 reported to modulate I_A . In naïve conditions, oxytocin and DYN may have opposite effects,
369 probably on different excitatory interneuron subtypes. However, it remains unclear whether
370 these neuropeptides act in pain conditions. TAF4 is the only one of these neuropeptides for
371 which an effect has been shown in pain conditions. It has been shown to reverse pain-induced
372 alterations of I_A in both excitatory and inhibitory interneurons (specifically in LIIo GAD67-
373 GFP⁻ and LIII GAD-67-GFP⁺ interneurons).

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

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

395 If the pain-modulated I_A is carried by Kv4s in GAD67-GFP⁺ interneurons as in GAD67-GFP⁻
396 interneurons, then its functional diversity likely reflects that of the components of the full
397 ternary complex Kv4-KChIP-DPLP (coupled to T and L Ca_v channels; see (Anderson et al.,
398 2013; Jerng and Pfaffinger, 2014a)). These components should thus be identified in each

399 interneuron subtype exhibiting pain-modulated I_A because the expression of the different Kv4
400 auxiliary subunit paralogs is neuron specific; such knowledge should make it possible to
401 develop novel targeted therapeutics as stated by Jerng and Pfaffinger (2014a).
402 Concerning the mechanisms of I_A modulation, the ERK-signaling-dependent downregulation
403 of I_A, which was first described in superficial dorsal horn interneurons at the beginning of the
404 21st century (Hu et al., 2006; Hu and Gereau, 2003; Hu et al., 2003), is now known to occur in
405 some LIIo excitatory interneurons (Zhang et al., 2018). Different, as yet, undetermined
406 signaling processes probably occur in LIII GAD67-GFP⁺ interneurons to mediate the opposite
407 patterns of I_A modulation reported by Yoo et al (2021).
408 Thus, the diversity of I_A amplitude ranges, roles and modulations highlighted in this review
409 are probably dependent on interneuron subtype selectivity of expression of a large range of
410 molecular substrates (Cav-Kv4 complex) and multiple signaling pathways.

411

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418 **AUTHOR CONTRIBUTIONS**

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

421 **DECLARATION OF INTEREST**

422 The authors have no competing interest to declare.

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427 **FIGURE LEGENDS**

428 **Figure 1: I_A in laminae IIo and Iii GAD67-GFP-positive and -negative interneurons in** 429 **spinal slices**

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

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

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

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

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

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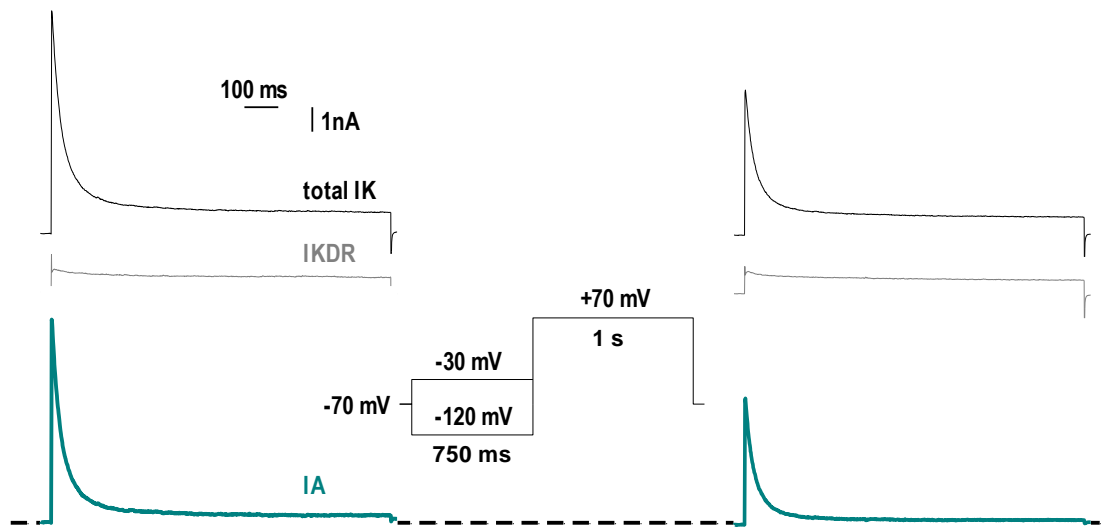
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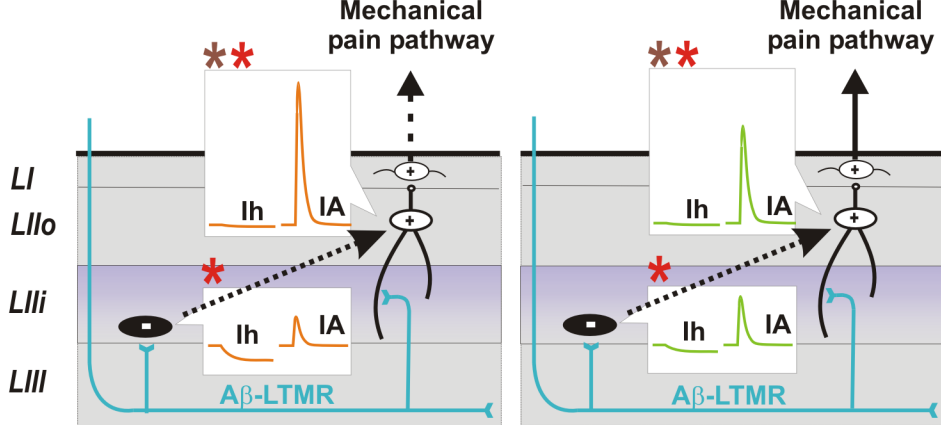
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GAD67-GFP- (excitatory) interneuron



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 (R_a ; between the pipette and the cell) as low as possible	Reducing R_a to obtain values $< 20 \text{ M}\Omega$ 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 $V_{1/2}$ 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 LII 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	<i>n</i>	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