

## Journal Club

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## Dorsal Horn PKC $\gamma$ Interneurons Mediate Mechanical Allodynia through 5-HT<sub>2A</sub> R-Dependent Structural Reorganization

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Review of Alba-Delgado et al.

Nociceptors function to protect tissue from potential damage by thermal, mechanical, and chemical stimuli. The central terminals of primary nociceptive mechanical and thermal afferents (C/A $\delta$  fibers) converge in superficial laminae I and II in the dorsal horn of the spinal cord. Nociceptive information is processed by excitatory and inhibitory interneurons in the dorsal horn of the spinal cord before being relayed to projection neurons in lamina I that transmit the information to higher brain centers that mediate the experience of pain (Koch et al., 2018) (Fig. 1).

Light touch does not normally evoke pain, but after nerve injury, innocuous light touch can evoke a pain-like response called allodynia. Information about light touch is carried by low-threshold mechanical primary afferents (A $\beta$  fibers) that synapse in laminae II–IV in the dorsal horn (Fig. 1). In inner lamina II, A $\beta$  fibers synapse directly onto excitatory interneurons that express the  $\gamma$  isoform of protein

kinase C (PKC $\gamma$ ) (Neumann et al., 2008; Lu et al., 2013). Although PKC $\gamma$  interneurons do not receive direct input from mechanical nociceptors, they are strongly implicated in mediating mechanical allodynia (Lu et al., 2013; Petitjean et al., 2015). Allodynia is thought to stem from the loss of strong feedforward inhibition by inhibitory interneurons that prevent innocuous input from being transmitted as painful (Fig. 1). After nerve injury, these inhibitory synapses onto PKC $\gamma$  interneurons are lost, and normally innocuous mechanical input from deep ventral laminae is transmitted to superficial lamina I to evoke pain (Miracourt et al., 2007; Lu et al., 2013; Petitjean et al., 2015). Thus, the merging of the innocuous and noxious pathways promotes mechanical allodynia. Spinal nociceptive transmission is also modulated via descending supraspinal projections, which are responsible for the top-down processing of pain. Many of these descending projections contain the neuromodulator serotonin, which may play a role in mechanical allodynia as epidural 5HT<sub>2A</sub>R antagonists dose-dependently attenuate mechanical allodynia after nerve injury (Van Steenwinckel et al., 2008).

Mechanical allodynia is a hallmark of inflammatory as well as neuropathic pain, but the underlying circuitry remains in-

completely understood. In particular, whether inflammatory pain also involves disinhibition of PKC $\gamma$  interneurons and 5HT<sub>2A</sub>Rs has not been clearly shown. Nonetheless, intrathecal administration of a PKC $\gamma$  inhibitor attenuates capsaicin-induced inflammatory mechanical allodynia in mice, suggesting that PKC $\gamma$  interneurons contribute to inflammatory allodynia (Petitjean et al., 2015). Because both PKC $\gamma$  (Neumann et al., 2008) and 5HT<sub>2A</sub>Rs (Fay and Kubin, 2000) are found predominately in excitatory interneurons of inner lamina II of the dorsal horn, Alba-Delgado et al. (2018) hypothesized that PKC $\gamma$  and 5HT<sub>2A</sub>Rs interact in PKC $\gamma$  interneurons to facilitate inflammation-induced mechanical allodynia.

To test this hypothesis, the authors first tested the effects of 5HT<sub>2A</sub>R agonists and antagonists on mechanical withdrawal thresholds in rats treated with complete Freund's adjuvant (CFA) to induce inflammation. Pharmacological blockade of 5HT<sub>2A</sub>Rs prevented CFA-induced mechanical facial allodynia in rats, and activation of 5HT<sub>2A</sub>Rs was sufficient to induce facial mechanical allodynia in naive rats. In addition, the authors showed that PKC $\gamma$  interneurons coexpress 5HT<sub>2A</sub>Rs and that activation of 5HT<sub>2A</sub>Rs increased levels of phosphorylated extracellular signal-regulated ki-

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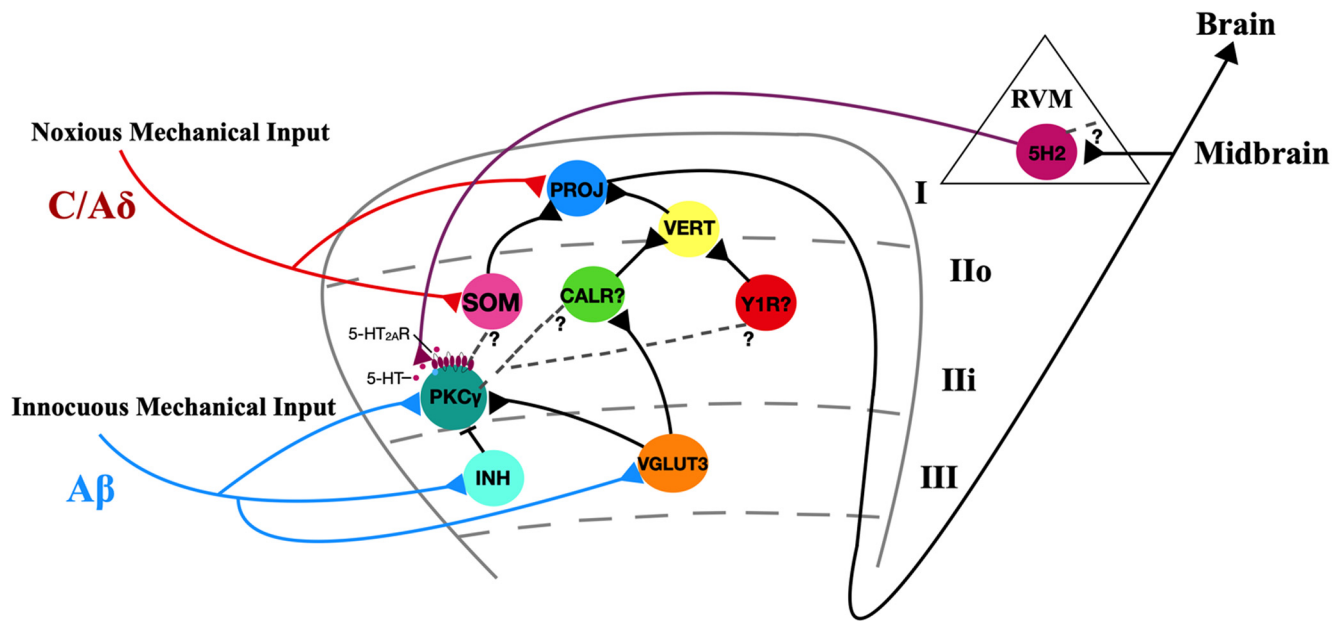
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**Figure 1.** A dorsal horn model for circuits mediating mechanical allodynia. 5-HT, serotonergic descending projections; PROJ, lamina I pain projection neurons; VERT, vertical cells; SOM, somatostatin; CALR, calretinin; Y1R, neuropeptide Y1-receptor expressing; PKC $\gamma$ , protein kinase C  $\gamma$ ; INH, inhibitory interneurons; VGLUT3, vesicular glutamate transporter 3; RVM, rostral ventromedial medulla.

nases 1/2 (pERK1/2), a marker of neuronal activation, in PKC $\gamma$ -expressing neurons. These results suggest that activation of dorsal horn 5HT $_{2A}$ R on PKC $\gamma$  interneurons leads to mechanical facial allodynia.

Alba-Delgado et al. (2018) also probed the electrophysiological and morphological effects of CFA-induced inflammation and 5HT $_{2A}$ R blockade on lamina II interneurons. Intrinsic electrophysiological properties (resting membrane potential and slope in current–voltage plots) of lamina II excitatory interneurons differed between CFA-treated and sham animals, but these changes occurred independently of 5-HT $_{2A}$ R activation. In contrast morphological changes (reduction in the number of tertiary branches of the dendritic arbor, increase in spine density) induced by CFA occurred selectively in lamina II PKC $\gamma$ -expressing interneurons, and these changes were partially prevented by blocking 5-HT $_{2A}$ Rs. Finally, specific activation of 5-HT $_{2A}$ Rs in naive rats replicated CFA-induced morphological changes in PKC $\gamma$  interneurons. Together, these results indicate that activation of 5-HT $_{2A}$ Rs on medullary dorsal horn PKC $\gamma$  interneurons induces rapid morphological remodeling of the dendritic arbor, which may lead to the development of facial mechanical allodynia.

The 5-HT $_{2A}$ R-dependent morphological reorganization of PKC $\gamma$  interneuron dendrites is a key finding that expands our understanding of the circuit underlying

mechanical allodynia (Fig. 1). PKC $\gamma$  interneurons lose inhibitory connections after neuropathic injury (Lu et al., 2013; Petitjean et al., 2015), and this loss might result from apoptosis of inhibitory interneurons or simply a loss of inhibitory contacts onto the PKC $\gamma$  soma (Petitjean et al., 2015; Inquimbert et al., 2018). The results of Alba-Delgado et al. (2018) suggest that 5-HT $_{2A}$ R-mediated morphological reorganization reduces the dendritic arbor of PKC $\gamma$  interneurons during inflammation. This reduced dendritic arbor might lead to decreases in the number of inhibitory synapses onto these neurons, causing a loss of the feedforward inhibition that normally prevents innocuous touch stimuli from exciting PKC $\gamma$  interneurons. PKC $\gamma$  interneurons would then be able to excite yet unknown postsynaptic neurons, allowing innocuous stimuli to reach pain projection neurons in the superficial lamina I of the dorsal horn (Fig. 1).

Alba-Delgado et al. (2018) raise an important question: what are the postsynaptic targets of the PKC $\gamma$  interneurons that transmit innocuous mechanical input to superficial pain projection neurons? PKC $\gamma$  interneurons synapse directly onto excitatory transient central cells in lamina II, and these synapse onto vertical cells, which then target lamina I pain projection neurons (Lu and Perl, 2005; Todd, 2017). The identity of transient central cells remains unknown, and researchers are trying to uncover these neural populations

(Peirs and Seal, 2016). The most likely candidate is a subset of somatostatin-expressing excitatory interneurons in outer lamina II, as these neurons are necessary for mechanical pain (Duan et al., 2014) (Fig. 1). Calretinin interneurons are another probable candidate, as they are implicated in the development of mechanical allodynia (Peirs et al., 2015) (Fig. 1). The excitatory interneurons expressing neuropeptide Y1 receptors (Y1R), which are involved in both mechanical and thermal allodynia that arises after inflammatory and neuropathic injury, may also be a target of PKC $\gamma$  interneurons as Y1Rs do not colocalize with PKC $\gamma$  and are found superficial to PKC $\gamma$  interneurons (Diaz-delCastillo et al., 2018; Nelson et al., 2019) (Fig. 1).

While Alba-Delgado et al. (2018) focus on PKC $\gamma$  interneurons in mechanical allodynia, this is only a piece of the circuit. In deeper lamina III, interneurons that transiently express vesicular glutamate transporter 3 (VGLUT3) are upstream of PKC $\gamma$  interneurons and also receive information about innocuous touch from A $\beta$  fibers (Peirs and Seal, 2016) (Fig. 1). Most importantly, VGLUT3 interneurons in the spinal dorsal horn are both necessary and sufficient for mechanical allodynia (Peirs et al., 2015). Perhaps 5-HT $_{2A}$ R activation in PKC $\gamma$  interneurons and the subsequent reorganization of the dendritic arbor permits them to be excited by VGLUT3 interneurons to induce mechanical allodynia.

The final questions Alba-Delgado et al. (2018) raise concerning the inflammation-induced mechanical allodynia circuitry pertain to the origin and cause of 5-HT release into the dorsal horn to act on PKC $\gamma$  interneurons. Existing anatomical evidence indicates that 5-HT in the dorsal horn originates almost entirely from descending projections from the rostral ventromedial medulla (RVM) and is not released from local dorsal horn interneurons (Bannister and Dickenson, 2016). Inflammation may drive ascending pain signals from projection neurons that monosynaptically activate neurons in the RVM. The RVM's descending nociceptive projections, which likely include 5-HT fibers, release 5-HT to act on 5-HT<sub>2A</sub>R-expressing PKC $\gamma$  interneurons and drive morphological reorganization and subsequent mechanical allodynia. Another possible circuit involves ascending pain projection neurons that activate neurons in the periaqueductal gray, which in turn can activate RVM descending nociceptive 5-HT fibers to the dorsal horn (Ossipov et al., 2014). In summary, the mechanical allodynia circuit includes not only projections from local dorsal horn excitatory and inhibitory interneurons but also descending 5-HT fibers from the RVM (Fig. 1).

Alba-Delgado et al. (2018) implicate descending 5-HT projections as mediators of morphological rearrangement in PKC $\gamma$  interneurons that are both necessary and sufficient for inflammation-induced facial mechanical allodynia. This work is an important addition to the mechanical allodynia circuit summarized in

Figure 1. In future studies, it will be important to uncover the cause of 5-HT release into the dorsal horn and the postsynaptic targets of the PKC $\gamma$  interneurons that lead to the development of mechanical allodynia.

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