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Chronic Pain Releases Parabrachial Activity from Central Amygdala Inhibition

Pia-Kelsey O'Neill[1] **and Jozsef Meszaros**[2]

[1]Mortimer B. Zuckerman Mind Brain Behavior Institute and Department of Neuroscience, Columbia University, New York, New York 10027, and [2]Department of Psychiatry, New York State Psychiatric Institute, New York, New York 10032

Review of Raver et al.

Chronic pain is a complex neurologic condition that involves both sensory and affective components. A crucial integrator of pain-related sensation and affect may be a set of neurons within the parabrachial nucleus. This area receives nociceptive inputs from spinal projection neurons and transmits signals to the amygdala, thalamus, and, ultimately, the cortex, regions that are associated with cognitive and emotional regulation. A simplified model describing the role of the parabrachial nucleus in chronic pain suggests that hyperactive parabrachial neurons contribute to pain hypersensitivity and that restoring normal activity provides relief. In support of this hypothesis, studies in rodents have demonstrated a correlation between enhanced parabrachial activity and hypersensitivity to innocuous stimuli, a phenomenon called allodynia (Matsumoto et al., 1996; Uddin et al., 2018). Activity within the parabrachial nucleus is believed to reflect an intricate balance between excitation and inhibition. The hyperactivity of parabrachial neurons could reflect increased activity from spinal inputs, inhibitory controls gone awry, or both. Indeed, there is evidence that spinal inputs become hyperactive in response to injury (Chapman et al., 1998; Prescott, 2015). This leads to the following question: what are the inhibitory controls that place a brake on the parabrachial nucleus under normal conditions, and what kind of injuries can cut that brake? Resolving this question is critical for understanding how allodynia develops.

In a recent article in *The Journal of Neuroscience*, Raver et al. (2020) investigated projections from the central amygdala to the parabrachial nucleus. Using a combination of behavioral, electrophysiological, and optogenetic approaches, the authors identified a direct, inhibitory pathway from the central amygdala to parabrachial nucleus that becomes weakened during chronic injury. The weakening of this connection leads to disinhibition of parabrachial activity and a corresponding increase in measures of pain.

To induce chronic pain in mice, Raver et al. (2020) used chronic constriction injury of the infraorbital nerve above the roof of the mouth (Uddin et al., 2018). Three weeks after surgery, subjects in the constriction injury group expressed more behaviors indicative of pain than sham-operated subjects: they exhibited a higher sensitivity to mechanical stimuli and a greater intensity of facial grimace. Moreover, extracellular recordings in the parabrachial nucleus of anesthetized animals revealed that in response to a facial pinch, parabrachial neurons in the constriction injury group were more strongly activated than those in the control group: neurons discharged for a longer time and were more likely to discharge even after the facial pinch had ended. Furthermore, spontaneous firing rates of parabrachial neurons in the injury group were higher than in controls. These results replicated the authors' previous findings that parabrachial nucleus is hyperactive after chronic injury in rats.

Raver et al. (2020) next explored the mechanism that causes parabrachial neurons to become more active after chronic constriction injury. They addressed the following two possibilities: alteration in presynaptic input (e.g., reduced inhibitory input) or alteration in intrinsic properties (e.g., altered ion channel function). To distinguish between these possibilities, the authors conducted whole-cell patch-clamp recordings from parabrachial neurons in brain slices from injured and control mice. Finding that input resistance and resting membrane potential were similar between the groups, they shifted away from a cell-autonomous mechanism. They then recorded miniature IPSCs, which reflect the size and number of spontaneous GABA release events onto the parabrachial cell. In slices from the injury group, there was a 50% reduction in the frequency of miniature IPSCs, indicating that a decrease in inhibitory input could be a source of hyperactivity in parabrachial neurons.

The authors went on to identify the population of GABAergic neurons that innervate parabrachial cells. They found that the parabrachial nucleus contained very

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Correspondence should be addressed to Pia-Kelsey O'Neill at pto2102@columbia.edu or Jozsef Meszaros at jm3648@columbia.edu.

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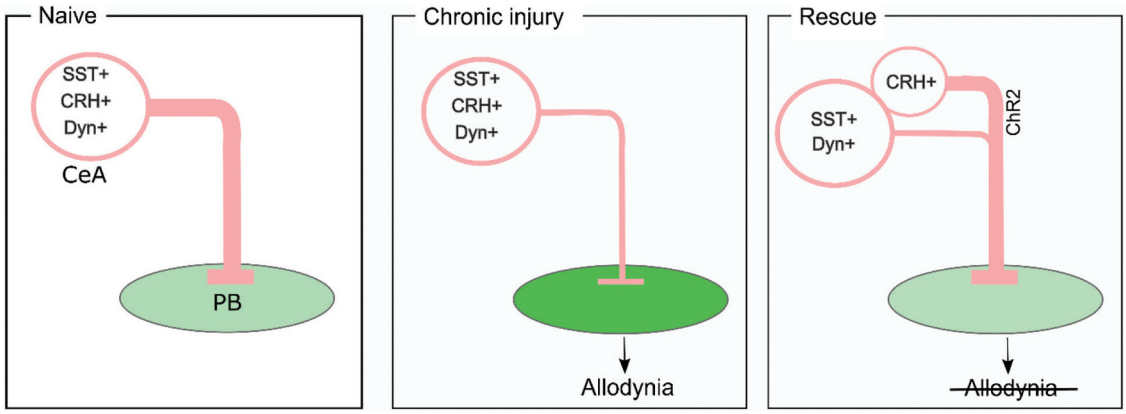

Figure 1. GABAergic projections from the central amygdala (CeA) to the parabrachial nucleus (PB) express somatostatin (SST^+), CRH^+, and/or dynorphin (Dyn^+; left). After chronic injury, central amygdala-mediated GABAergic inhibition of parabrachial nucleus is reduced while mechanical sensitivity is increased, producing allodynia (middle). Optogenetic activation of all central amygdala GABAergic axons in parabrachial nucleus or selectively of CRH^+ GABAergic neurons, leads to a reduction in allodynia, presumably by reducing parabrachial nucleus activity (right).

few GABAergc interneurons and thus reasoned that the inhibitory control must arise from a long-range projection. Connections between central amygdala and parabrachial nucleus have previously been described, making them a potential candidate for the inhibitory input (Jia et al., 2005). By selectively tracing GABAergic neurons from the central amygdala, the authors discovered a robust input from this population to parabrachial nucleus—a finding they confirmed in mice and rats of both sexes. Using *in situ* hybridization in rats, they further revealed that the population of GABAergic central amygdala neurons that project to parabrachial nucleus contained mRNA transcripts for dynorphin, somatostatin, and corticotropin-releasing hormone (CRH; Fig. 1, left). The largest fraction of the neurons (almost half) expressed dynorphin, an opioid peptide.

To confirm that central amygdala projections inhibit the parabrachial nucleus, the authors expressed channelrhodopsin selectively in central amygdala neurons that contain glutamic acid decarboxylase, an enzyme that mediates the production of GABA, and photostimulated axonal terminals of these neurons in the parabrachial nucleus while recording inhibitory postsynaptic currents in parabrachial neurons. Stimulation evoked strong currents in the parabrachial nucleus of controls, but the amplitude of evoked current was fivefold smaller in injured animals. In addition, paired-pulse ratio in the injured group was almost twice as large, suggesting the presence of weakened inhibitory synapses.

After confirming that the amygdalo-parabrachial pathway is weaker after chronic injury (Fig. 1, middle), Raver et al. (2020) proceeded to establish a causal role for the central amygdalo-parabrachial pathway in ongoing behavior. To do this, they paired optogenetic stimulation with mechanical stimulation of the hindpaw. Stimulating amygdalo-parabrachial terminals increased hindpaw withdrawal thresholds in injured animals. Importantly, the same effect was produced when CRH^+ central amygdala terminals were selectively activated in parabrachial nucleus (Fig. 1, right). This finding suggests that the CRH^+ subpopulation of central amygdala inputs to parabrachial nucleus may be sufficient for producing analgesia.

Having shown that activating the central amygdalo-parabrachial nucleus pathway could reverse allodynia in animals with chronic constriction injury, the authors investigated the potential for the pathway to provide relief after an acute inflammatory injury mediated by injection of formalin into the left hindpaw. As with chronic injury, optical stimulation increased hindpaw withdrawal thresholds.

In another set of experiments, the authors showed that many of the features of the hypersensitization phenotype could be recapitulated by blocking GABA receptors in parabrachial nucleus and that normal sensitivity could be restored by exogenous application of muscimol, a GABA receptor agonist, in the parabrachial nucleus. Thus, the authors concluded that GABAergic central amygdala input to parabrachial nucleus, specifically from a subset of CRH^+ neurons, is sufficient to relieve allodynia.

With their study, Raver et al. (2020) have discovered that projections from the central amygdala to the parabrachial nucleus suppress nociceptive responses and that these projections are weakened after an injury that induces chronic pain (Fig. 1). Specifically, nerve constriction led to a reduction in miniature IPSC frequency in parabrachial neurons, a decrease in the amplitude of IPSCs evoked by stimulating GABAergic input from the central amygdala, and an increase in paired-pulse ratio at these synapses. These findings indicate a presynaptic mechanism. Whether this weakened synapse is matched by a decrease in the firing activity of parabrachial-projecting central amygdala neurons is not addressed, given that the authors did not directly measure activity in central amygdala. In a previous study, Wilson et al. (2019) recorded from $somatostatin^+$ central amygdala neurons and showed that this subpopulation decreased their activity after chronic injury (Wilson et al., 2019). This result is congruent with the finding in the study by Raver et al. (2020) that this subtype of central amygdala neuron may lose its inhibitory control over the parabrachial nucleus. In contrast to the $somatostatin^+$ neurons, another subpopulation of central amygdala neurons that express protein kinase C δ, exhibited higher activity following chronic injury (Wilson et al., 2019). These neurons are largely distinct from both $somatostatin^+$ and CRH^+ central amygdala neurons (Kim et al., 2017). Thus, it is possible that the central amygdala contains neurons that increase their activity following chronic injury, but that these neurons do not project to the parabrachial nucleus. Recording from parabrachial-projecting CRH^+ and dynorphin subtypes—let alone the large percentage of the parabrachial-projecting neurons that were unidentified may provide further insight.

Several recent studies have identified other brain areas that, like the central amygdala, can put a brake on parabrachial nucleus activity. For example, Alhadeff et al. (2018) describe a pathway from hypothalamus to parabrachial nucleus that suppresses chronic pain when an animal is hungry. Agouti-related protein (AgRP) neurons in the hypothalamus, active

during hunger, inhibit parabrachial nucleus through the release of neuropeptide Y to produce analgesia. The AgRP neurons also corelease GABA, which is necessary for inhibiting parabrachial nucleus neurons (Wu et al., 2009). Thus, AgRP neurons, like central amygdala neurons, may provide another brake on parabrachial nucleus activity and thereby produce pain relief.

Given that the parabrachial nucleus plays a role in a variety of functions, such as hunger, sleep, arousal, and respiration (Chamberlin, 2004; Qiu et al., 2016; Alhadeff et al., 2018), future work could hone in on the consequences of chronic pain for cells involved in these diverse functions. For example, CGRP (calcitonin gene-related peptide)-expressing parabrachial neurons are responsible for relaying a variety of aversive stimuli (Palmiter, 2018). Does activity in this cell type become excessive following chronic injury? Alterations in these complementary functions might exacerbate the experience of chronic pain, and restoring normal function at this synapse might therefore have far-reaching consequences for affected individuals.

The gradual development of allodynia belies an astonishing level of neurologic and biochemical complexity. Alterations in synaptic plasticity regularly occur as neurons are driven into prolonged states of decreased or increased activity (Turrigiano, 2007). If we suppose that acute pain leads to strong activation of central amygdala projections to the parabrachial nucleus, elevated inhibition in the parabrachial nucleus might trigger retrograde signaling back to central amygdala terminals that depresses the inhibitory influence over the parabrachial nucleus. Indeed, such a homeostatic mechanism has been shown to operate within the basolateral amygdala to depress GABAergic inhibitory currents (Marsicano et al., 2002). The CRH^{+} central amygdala neurons examined by Raver et al. (2020) express endocannabinoid receptors, which are known to be involved in retrograde signaling that reduces neurotransmitter release.

Chronic pain is a devastating condition. Raver et al. (2020) highlight a novel pathway from the central amygdala to the parabrachial nucleus that is critical for pain abatement. These findings could lead to the development of innovative new therapies in the treatment of pain.

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