Sensitization of Meningeal Sensory Neurons and the Origin of Headaches

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This was an early study from 1996, aimed at exploring the potential pathophysiology of migraine pain. Several previous studies had shown the responses of central neurons, within the spinal trigeminal nucleus and thalamus, to electrical, mechanical, and chemical (bradykinin, capsaicin) stimulation of the dura. Notably, the concept of sensitization had not been previously studied, and the authors suggested that chemical sensitization of meningeal afferent neurons to mechanical stimuli might play a role in migraine pain pathophysiology. For this reason, this study explored this possibility by directly assessing primary afferent neurons in the rat trigeminal ganglion that innervate the dural venous sinuses.

Experimental Design: This study used single-unit electrophysiological recording techniques in rat trigeminal neurons, which supplied afferent innervation to the intracranial meninges. To isolate these neurons, male rats underwent craniotomy to expose the transverse sinus, superior sagittal sinus, and dura. Exposed dura was bathed in synthetic interstitial fluid (SIF), and microelectrodes were advanced into the trigeminal ganglion through the cortex. Single-shock electrical stimuli were delivered to stimulation electrodes on the dura overlying the transverse sinus as a microelectrode was advanced through the ganglion. This helped assess mechanosensitivity. To then assess for chemosensitivity, chemical agents were also delivered directly to the exposed dura or by infusion into the superior sagittal sinus, in a select group of neurons. Chemical agents tested included KCI, NaCl, low osmolarity SIF, high osmolarity SIF, capsaicin and an inflammatory "soup" (bradykinin, histamine, serotonin, prostaglandin E). Neuronal responses were quantified after exposure to mechanical and chemical stimuli, and the class of transmitting sensory fiber was identified. To investigate sensitization, a graded series of von Frey monofilaments was applied in order of increasing stiffness/force to measure the mechanical threshold or von Frey threshold,[1] of the tested neurons. This was measured at 10-30-minute intervals, before and after a conditioning chemical stimulus (acidic or inflammatory agents) was applied. Each data point was obtained by applying a single monofilament, 5-10 times to the neuron's mechanical receptive field on the transverse sinus. The percentage of times that the neuron responded was plotted against the force of the monofilament.

Results: Mechanosensitivity was found more frequently in neurons with C-fibers and slowly conducting A-delta fibers than in neurons with rapidly conducting A-delta fibers (**Fig 1**). A subset of the neurons that responded to dural shock (18/22 mechanosensitive and 1/5 mechanically-insensitive neurons) also responded to topical application of chemical agents to the dura **(Fig 2).** As above, sensitization was investigated by determining mechanical sensitivity before and after topical application of an acidic (n = 2) or inflammatory (n = 15) agent[2]; both agents were applied to the measured, dural receptive fields of isolated neurons. Sensitization was eventually demonstrated by a lowered von Frey threshold (to mechanical stimulation) in 10 of the 15 mechanosensitive neurons identified previously, and also in some neurons (1 of 2) that were initially found to be mechanically-insensitive **(Fig 3).** This chemically-

[1] Von Frey Filament testing: a pin-like von Frey filament is applied with increasing force to the subject being tested (here, isolated trigeminal neurons; in other studies, hindpaw of a rat). A positive response here was neuronal firing; in live animals, this would be hindpaw withdrawal. A lowered threshold is demonstrated by a positive response after a smaller, applied force.
[2] Both acidic and the inflammatory agents used were known to sensitize cutaneous nociceptive afferent neurons to subsequent mechanical stimuli

induced sensitization to mechanical stimuli (i.e., lowered von Frey threshold) was statistically significant ($p < 0.01$, Wilcoxon signed-rank test).

Conclusions: This study was among the first to demonstrate chemically-induced sensitization to mechanical stimulation in afferent neurons that directly supply the dura. Further, this study showed that central, dura-responsive neurons had nociceptive, facial receptive fields, with a distribution reminiscent of the pattern of pain referral evoked by dural stimulation. This suggested a role for these neurons in referred pain of intracranial origin, and additionally hypothesized that dural mechanosensitivity and sensitization likely contribute to the throbbing pain of migraine headaches. Specifically, the authors suggest that dural sinus afferents may first be chemically sensitized by the release of chemicals through the cortex (substance P, CGRP) then later "over-excited" by minor mechanical stimulation (head turn, head movement), leading to progressive pain during migraine (or other headaches). This was an important contribution to the field, as the throbbing pain in migraine had been previously attributed to pulsations of abnormally dilated blood vessels, a phenomenon that had not been supported by empirical evidence.

Additional reading, if interested:

1) Recober, A. (2021). Pathophysiology of migraine. *CONTINUUM: Lifelong Learning in Neurology*, *27*(3), 586–596. https://doi.org/10.1212/con.0000000000000983

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