Modulation of Mechanical Pain by Dynorphin Neurons in the Developing Spinal Dorsal Horn

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Introduction: Pediatric pain affects up to 40% of children causing disruption in social, emotional, and cognitive development. Evidence suggests that neonatal injury causes a "priming" effect with increased pain sensitivity following repeat injury later in life. It is unclear which subtypes of inhibitory neurons within the spinal dorsal horn are essential for regulating pain sensitivity in neonates or how their maturation is influenced by early life injury. Genetic ablation of spinal dynorphin (DYN) interneurons in adult mice results in mechanical allodynia, but evidence also shows that spinal inhibitory networks in neonates are poorly tuned and undergo major reorganization between postnatal day (P)3 and adulthood.

Hypothesis: Spinal DYN interneurons suppress mechanical pain with increased functionality during adulthood, but neonatal injury disrupts the formation of their inhibitory synapses onto projection neurons (PNs).

Methods:
Behavior: Spinal DYN neurons were selectively excited in mice by the insertion of an excitatory DREADD receptor using an intersectional genetic strategy. The effects of the DREADD agonist clozapine (0.1 mg/kg) on mechanical pain sensitivity were characterized at P6-7 and P49-53 using Von Frey hairs and a paintbrush test.
Immunohistochemistry: The presynaptic marker synaptophysin-tdTomato was selectively expressed in DYN neurons by crossing Dyn^{Cre} mice with Rosa26 $^{LSL\text{-}Syp/Tomato}$ mice. PNs were labeled by injection of Cholera toxin subunit B (CTB) into the parabrachial nucleus at P3. Spinal cord sections from P49-70 mice receiving an incision at P2, and naïve mice, were labeled using antibodies for VGAT (to identify inhibitory synapses) and CTB. The number of inhibitory DYN synapses onto PNs was quantified using confocal microscopy.

Results: 1) Clozapine administration at P6-7 or P49-60 reduced mechanical sensitivity to a similar degree in mice with and without the DREADD; 2) The number of synaptic connections between inhibitory DYN interneurons and lamina I PNs was not altered by neonatal incision.

Conclusions: The "priming" effect is not a consequence of fewer inhibitory DYN connections onto PNs, but may instead reflect changes in the functional properties of these synapses. The genotype-independent effects of clozapine on mechanical pain sensitivity could reflect off-target actions of the drug or the occurrence of stress-induced analgesia during the testing procedure.

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