Behavioral effects of cortico-striatal neuronal inhibition in an animal model of neuropathic pain

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Introduction

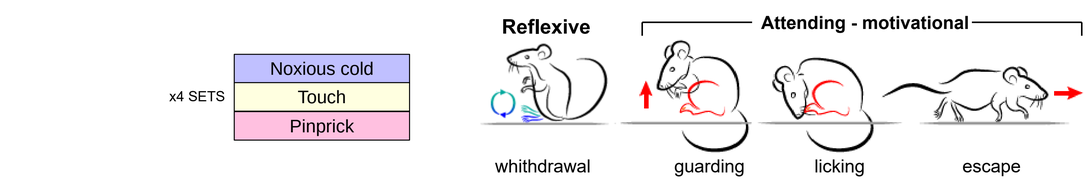
The perception of pain is a multidimensional experience arising from distributed brain activity. Maladaptive changes in the pain matrix are thought to play a role in this experience. However, how the brain encodes the perception of pain is elusive. Particularly, little is known about the neuronal mechanisms associated with the unpleasantness that characterizes pain.

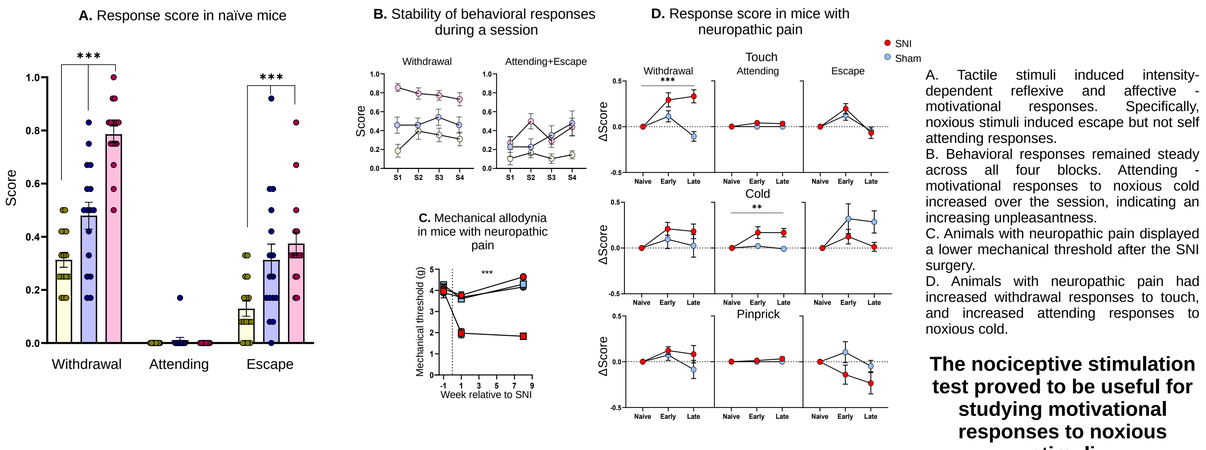
A key structure involved in the affective processing of pain is the Anterior Cingulate Cortex (ACC). The dense excitatory connections between the ACC and limbic system structures, such as the Dorso-medial Striatum (DMS), suggest that cortico-striatal (CS) neurons may converge nociceptive information to the mesolimbic system. To investigate the role of these neurons, we used a chemogenetic approach to interfere with their activity. For this, we injected a retro-cre virus in the DMS and a cre-dependent inhibitory dreadd in the ACC.

To study the behavioral effects of CS inhibition, we used the spared-nerve injury model of neuropathic pain. First, we characterized the reflexive and more complex behaviors that reflect the subject's motivation to alleviate aversive sensations. Then, we used a conditioned place avoidance paradigm, where we observed that while control mice avoided spending time in a compartment paired with a nociceptive stimulus, mice treated with inhibitory dreadd spent more time in that chamber, which suggests a role of ACC-CS neurons in the expression of pain related behaviors.

We also studied the neuronal activity of freely moving mice using a miniature microscope to obtain calcium imaging recordings in order to assess how this activity changes in response to a variety of stimuli.

Characterization of the behavioral repertoire in response to nociceptive stimuli in male mice using a protocol compatible with calcium imaging and neuronal inhibition

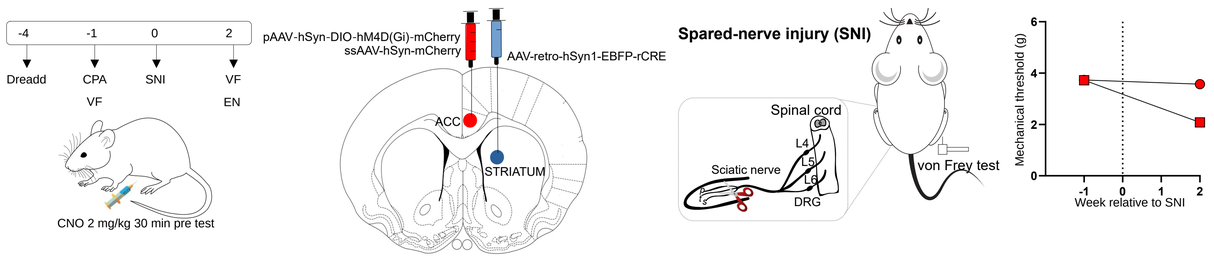


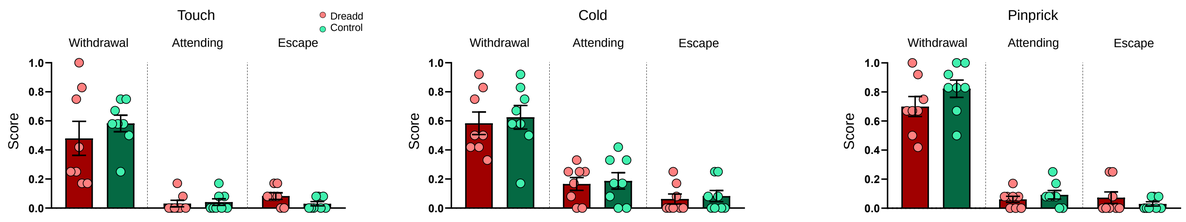


A. Tactile stimuli induced intensity-dependent reflexive and affective - motivational responses. Specifically, noxious stimuli induced escape but not self attending responses.
B. Behavioral responses remained steady across all four blocks. Attending - motivational responses to noxious cold increased over the session, indicating an increasing unpleasantness.
C. Animals with neuropathic pain displayed a lower mechanical threshold after the SNI surgery.
D. Animals with neuropathic pain had increased withdrawal responses to touch, and increased attending responses to noxious cold.

The nociceptive stimulation test proved to be useful for studying motivational responses to noxious stimuli

Inhibition of ACC-CS neurons did not alter the responses to nociceptive stimulation in a model of neuropathic pain

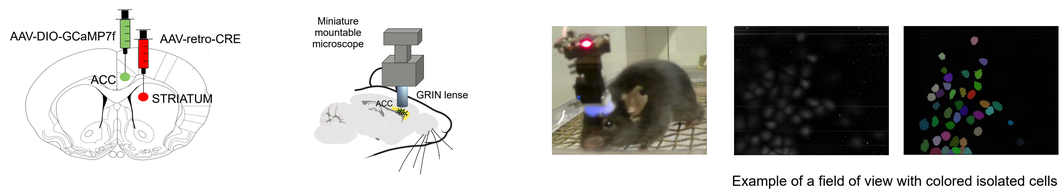




Inhibition of ACC-CS neurons did not have an effect on behavioral responses to nociceptive stimulation

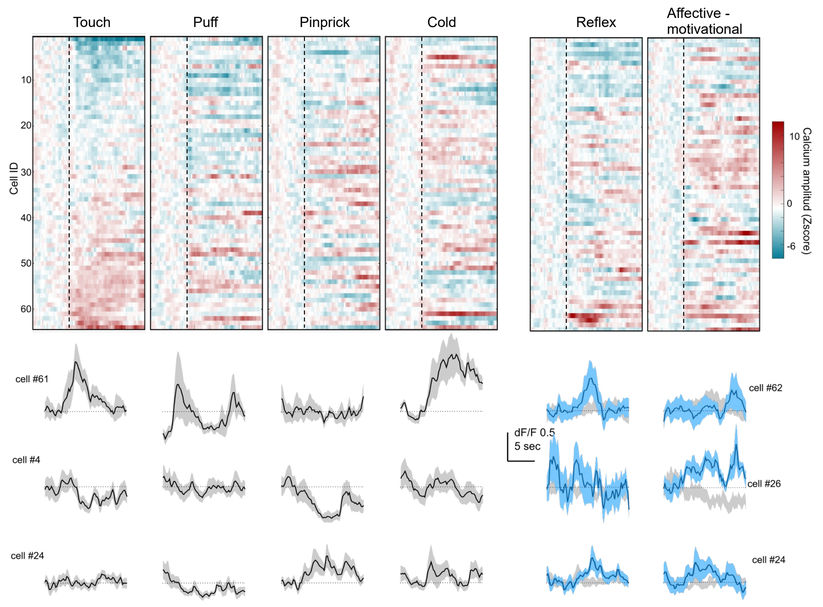
Representation of nocifensive behaviors by cortico-striatal neurons of the Anterior Cingulate Cortex

In vivo calcium imaging of cortico-striatal neuronal activity



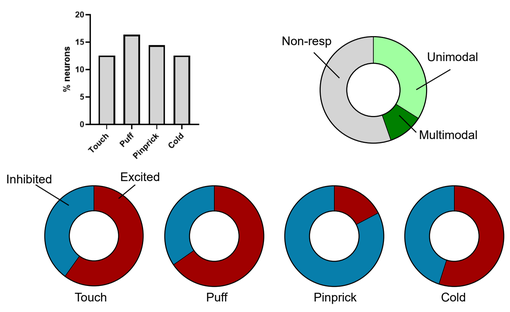
Example of a field of view with colored isolated cells

ACC cortico-striatal neurons responded to stimulus identity and / or to the manifestation of nocifensive behaviors

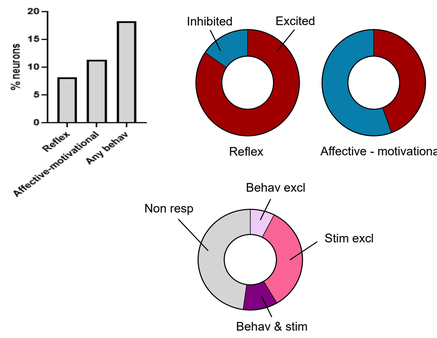


- Mean activity over time across all trials aligned to stimulus presentation (dashed line) from a single session of one filed of view
- Trials were sorted according to the presented stimulus (left) or to manifestation of nocifensive behaviors (right).
- Neurons were ordered according to their maximal response evoked by touch.
- Average evoked calcium transients (solid line: mean; shaded area: ± SEM) induced by stimulus presentation.
- Blue and light gray traces represent trials where the corresponding behavior was observed or not, respectively.

Stimulus-responsive neurons

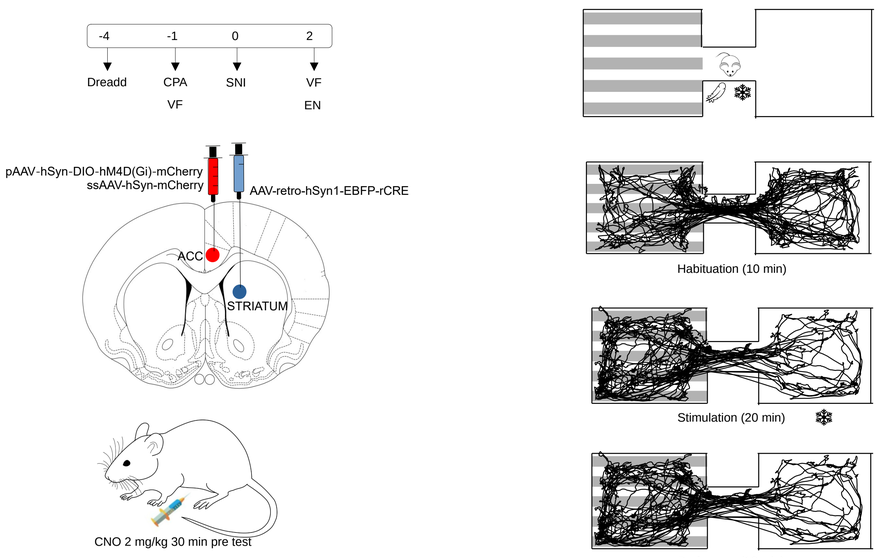


Nocifensive behaviors related neurons



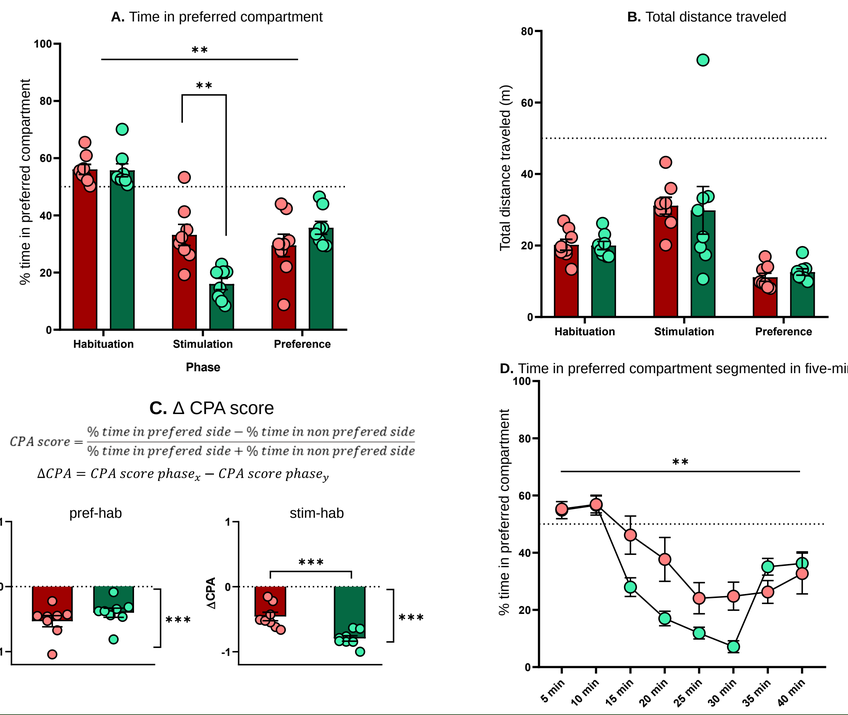
While some neurons responded specifically to sensory stimuli, others encoded preferentially the behavioral manifestation of pain, suggesting that ACC-CS neurons may encode a broad variety of information associated to noxious stimuli.

Chemogenetic inhibition of ACC-CS neurons impaired performance in a conditioned place avoidance paradigm



ACC cortico-striatal neurons were specifically targeted for inhibition injecting an AAV-retro-cre in the dorsomedial striatum and an AVV-hSyn-DIO-hM4D(Gi)-mCherry or AAV-hSyn-mCherry in the ACC. CNO (2 mg/kg) was administered i.p 30 minutes before the test.

Schematics of conditioned place avoidance chamber. A noxious stimulus was applied to the hind paw in the preferred compartment. Time spent in each compartment was measured as well as the preference shift.



A. DREADD animals spent more time in a compartment paired with a noxious stimulus than control animals during the stimulation phase of the test. Two-way ANOVA: interaction F (2, 28)=8.458, p=0.0013; Bonferroni post-hoc: significant differences in Stimulation phase (p=0.0053).

B. There were no differences in the total distance traveled.

C. Both groups shifted their baseline preference during the stimulation and preference phases, but a difference between groups was observed only in the first (unpaired t-test, t=4.380, df=14, p=0.0006).

D. The difference seen in A was stable throughout the entire stimulation phase. Two-way ANOVA: interaction F (7, 98)=3.393, p=0.0028

Inhibition of ACC cortico-striatal neurons decreased behavioral responses to noxious stimulation in a CPA paradigm, suggesting a role of this path in the expression of pain-related coping behaviors

Conclusion

The nociceptive stimulation test proved to be useful for studying attending – motivational responses to a variety of both nociceptive and neutral stimuli. The fact that responses to this test could not be modulated using a chemogenetic approach may be due to a floor-effect in the level of self-attenfding responses seen in animals with neuropathic pain. Even so, we found that ACC-CS neurons modulated their activity levels in response to stimulation by means of in vivo calcium imaging. While some of these neurons responded by increasing their activity, others were inhibited. Furthermore, some of these neurons responded specifically to stimulus identity, while others encoded the behavioral manifestation of pain. These results suggest a role of ACC-CS neurons in the manifestation of pain-related behaviors, which was later confirmed by further chemogenetic inhibition in a real time conditioned place avoidance paradigm, where animals treated with inhibitory dreadds showed less avoidance of a chamber paired with a noxious stimulus.
Taken together, these experiments suggest that the emotional processing of nociceptive information involves cortico-striatal transmission that plays a role both in identifying stimulus identity and in the behavioral manifestation of pain.

All experiments were conducted in accordance to the rules of the veterinary office of the canton of Bern, Switzerland and the Faculty of Medicine, University of Buenos Aires. This work was funded by IBRO (IBRO Return Home Fellowship), MINCyT – Conicet – Swiss National Science Found (Bilateral Cooperation Programme – Call 2016) and ANPCYT (PICT-2018-0835; PICT-2018-003).

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