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Novel insights into astrocyte-neuron interactions: Astrocytogenic synaptic depression as a central mechanism for modulating nociception

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Introduction

The modulation and processing of pain-related signals in the spinal cord is an enormously complex process, orchestrated by a diverse array of cell types. Among these, astrocytes, once primarily viewed as supportive cells for neurons, have emerged as important modulators of nociceptive signal processing at the spinal level. Their capacity to release a variety of neuromodulatory substances positions them as indispensable regulators of nociception, capable of signifcantly affecting the synaptic activity of spinal neurons. This ability suggests that astrocytes play a critical role in both the attenuation and amplifcation of pain signals.

Despite current understanding, the precise mechanisms governing neuron-astrocyte interactions remain largely elusive, necessitating comprehensive investigation. By leveraging G-protein coupled receptors, known as Designer Receptors Exclusively Activated by Designer Drugs (DREADDs), to selectively activate astrocytes, we aim to uncover the intricate pathways and interactions involved in astrocyte-to-neuron communication in the spinal cord dorsal horn. This approach seeks to provide deeper insights into the mechanisms of synaptic modulation and their broader implications for neurological function and therapeutic interventions.

Objectives

To elucidate the role of astrocytes in nociceptive processing in the spinal cord dorsal horn.
To evaluate potential sex-differences at the cellular and synaptic level.

Methods

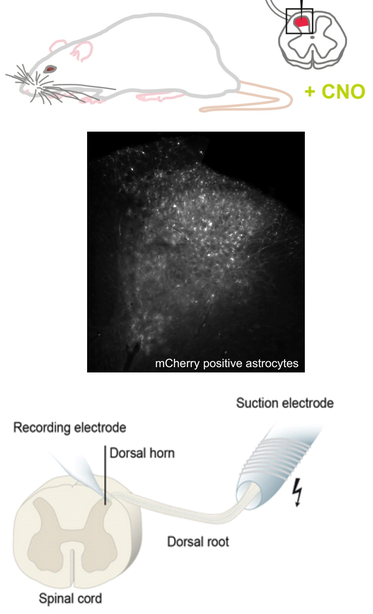
1. Sprague Dawley rats were used in all experiments. Astrocytes were targeted selectively using cell-specific Gq-DREADDs (Designer Receptors Exclusively Activated by Designer Drugs). The DREADDs were delivered via an AAV-vector that was injected unilaterally into the rat lumbar spinal cord. A non-DREADD virus was injected as a sham-control. DREADDs were activated using Clozapine-N-Oxide (CNO).

2. To assess DREADD specificity, we performed immunostainings in spinal cord tissue and assessed the colocalization of the DREADD marker mCherry with the neuronal marker NeuN, or the astrocytic marker GFAP, respectively.

3. To investigate the effects of astrocytic DREADD activation on astrocytic and neuronal calcium transients, we performed calcium imaging on acute spinal cord slices.

4. Patch-clamp recordings from dorsal horn neurons in acute spinal cord slices with long dorsal roots attached were performed to investigate the effect of spinal astrocyte activation on the cellular and synaptic levels.

5. Additionally, we recorded C-fiber-evoked field potentials in intact, deeply anesthetized rats to assess synaptic strength in vivo.

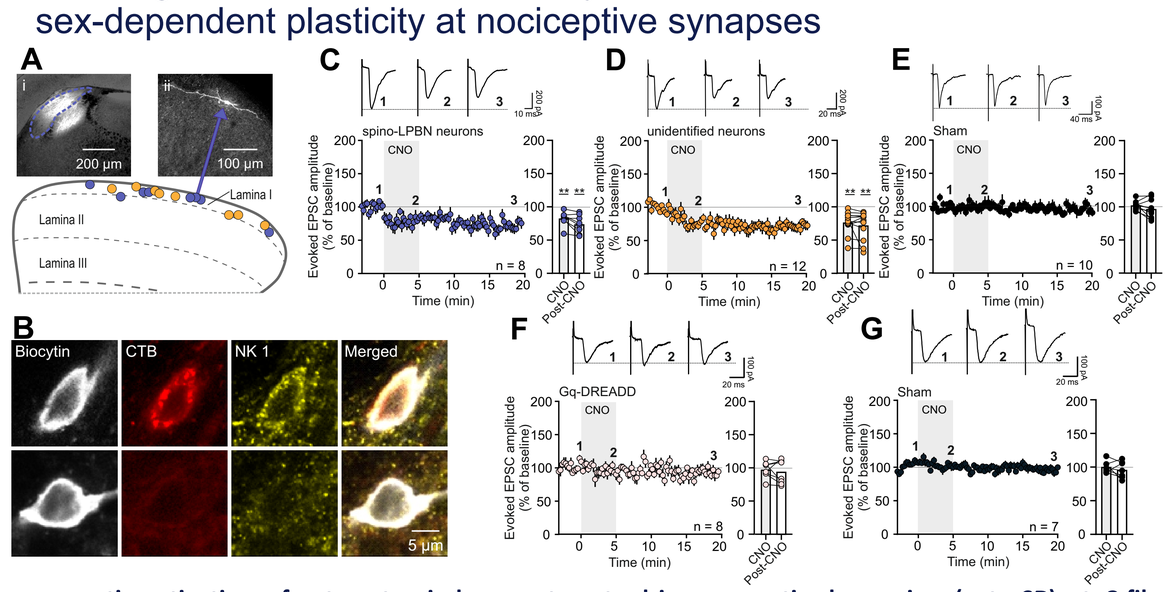


Results

1 Astrocyte-specific DREADD expression induces CNO-evoked calcium signaling selectively in astrocytes.

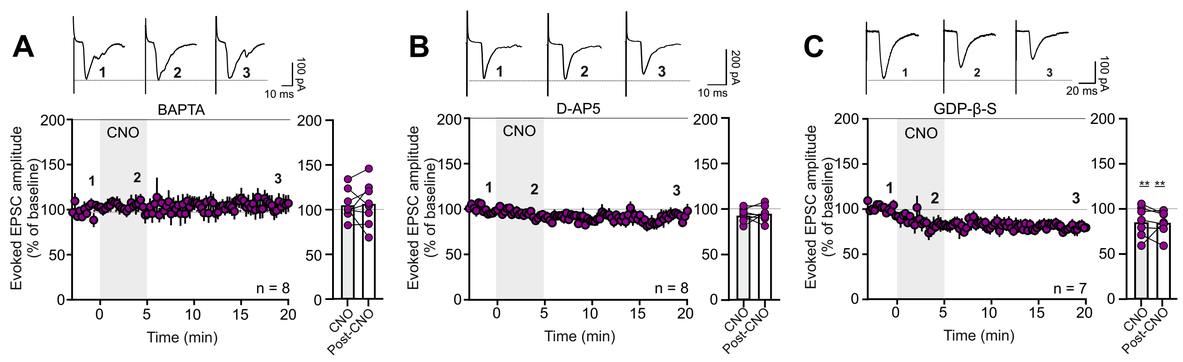
DREADD expression is astrocyte-specific and induces distinct calcium responses upon CNO activation. (A) Representative triple immunostaining shows mCherry (yellow) expression in GFAP+ astrocytes (cyan), but not in NeuN+ neurons (magenta), confirming astrocyte-specific DREADD expression. Scale bar: 50 µm. (B) Quantification of mCherry+ cells co- or not co-localized with NeuN. Calcium imaging in acute spinal cord slices shows that CNO application (10 µM) increases calcium levels in astrocytes in male (C) and female (G) rats, but not in neurons. No calcium response to CNO was observed in astrocytes of male (E) and female (I) Sham animals. (D, F, H, J) Application of a positive control consisting of phenylephrine (10 µM), DHPG (50 µM), and Mg-ATP (50 µM) reliably induced Ca^{2+} signaling in all tested cells across groups.

2 Chemogenetic activation of astrocytes via Gq-DREADDs induces sex-dependent plasticity at nociceptive synapses



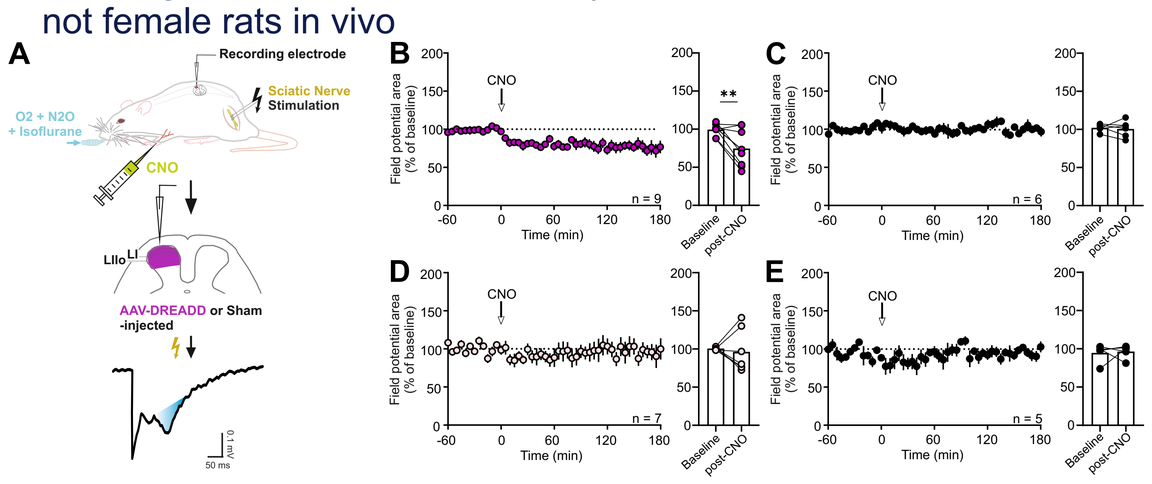
Chemogenetic activation of astrocytes induces astrocyte-driven synaptic depression (*astroSD*) at C-fiber synapses, both in projection- and unidentified neurons. (A) Representative CTB injection site (i) in the LPBN and biocytin-filled CTB+ neuron (ii). Schematic indicates spino-LPBN (purple) and unidentified (yellow) neurons. (B) Post hoc identification shows CTB+ spino-LPBN neurons express NK1R (top), while CTB− cells typically do not (bottom). (C–G) C-fiber-evoked EPSCs recorded from neurons in the superficial dorsal horn of acute spinal cord slices from male and female rats. EPSC amplitudes were normalized to a 3-minute baseline and plotted over time (min). Gq-DREADDs in astrocytes were activated via 5-minute bath application of CNO (10 µM). In males, Gq-DREADD activation showed *astroSD* in projection (C) as well as in unidentified neurons (D), but had no effect in Sham animals (E). In females, no significant effect was observed following Gq activation (F) or in Sham controls (G). Data are mean ± SEM. One-way ANOVA, **P < 0.001.

3 Mechanisms of astrocyte-induced plasticity



***AstroSD* requires intracellular calcium and NMDA receptor signaling but is independent of postsynaptic G-protein activation.** C-fiber-evoked EPSC amplitudes were normalized to baseline (BL, dashed line) and plotted over time. Bar graphs show BL normalized means. CNO was bath applied for five minutes from time point zero (grey area). Insets show example traces at indicated time points. *AstroSD* was blocked by intracellular calcium chelator BAPTA (A) or bath-applied NMDA receptor antagonist D-AP5 (B), but not by postsynaptic G-protein blockade with GDP-b-S (C). Data are mean ± SEM. One-way ANOVA, **P < 0.001.

4 Chemogenetic activation of astrocytes induces *astroSD* in male but not female rats in vivo



Chemogenetic activation of astrocytes *in-vivo* induces astroSD in male but not female rats. (A) C-fiber-evoked field potentials were recorded from the superficial dorsal horn (SCDH) following electrical stimulation of the sciatic nerve. (B-E) Field potential amplitudes (area under the curve) were normalized to baseline (BL, dashed line) and plotted over time. CNO was injected intravenously at time zero. Insets show representative traces at indicated time points; bar graphs display BL-normalized mean responses per time bin. (B) CNO induced astroSD in Gq-DREADD-expressing males but had no effect in Sham controls (C). No astroSD was observed in females following Gq-DREADD activation (D) or in female Sham animals (E). Data are mean ± SEM. Paired t-test, **P < 0.05.

Conclusion

Our study demonstrates that activation of astrocytic DREADDs induces a de novo and long-lasting form of synaptic plasticity - astrocyte-mediated synaptic depression (*astroSD*) - at spinal nociceptive synapses. This effect is robust, reproducible in both in vitro and in vivo settings, and sex-specific, as *astroSD* was observed exclusively in male rats. These findings point to a sexually dimorphic role of astrocytes in spinal pain modulation. Mechanistically, *astroSD* depends on NMDA receptor activation and intracellular calcium signaling, but occurs independently of postsynaptic G-protein signaling. Together, these results shift the focus toward local neuron–astrocyte interactions and highlight astrocytes as active modulators of spinal synaptic strength. This work advances our understanding of astrocyte–neuron communication in nociceptive processing and identifies astrocytes as potential therapeutic targets for chronic pain. Future studies will aim to elucidate the intracellular signaling pathways underlying this gliogenic form of synaptic plasticity.

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