The Role of SNr KCC2 in Opioid-Related Behavioral Adaptations

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Introduction

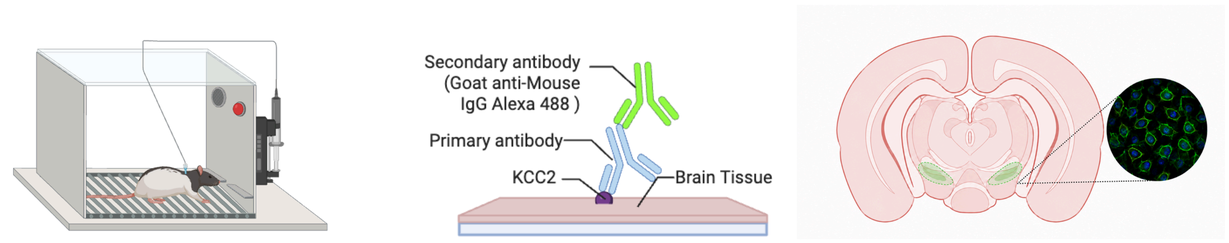
Opioid use disorder (OUD) is a chronic, compulsive disease characterized by persistent opioid use despite negative consequences. OUD affects over 16 million people globally (Dydyk et al. 2024), and 76% of all drug overdose deaths in 2023 were attributed to opioids (Centers for Disease Control and Prevention 2025). Despite this, the neural mechanisms underlying OUD are not fully understood. Recent investigations have identified **potassium chloride cotransporter isoform 2 (KCC2)** as a modulator of maladaptive inhibitory plasticity in substance use (Pearson & Ostroumov 2024). KCC2 is a neuron-specific anion transporter responsible for maintaining low intracellular chloride (Cl-). In the midbrain, KCC2 has only been detected in GABAergic neurons, therefore implicating changes specifically in inhibitory transmission. Previous investigations have shown that substance use downregulates KCC2 in GABA neurons, leading to irregular dopamine (DA) transmission across multiple reward circuits and ultimately promoting maladaptive behaviors which may perpetuate behaviors associated with OUD. When upregulated, KCC2 has been shown to restore normal behavior, making it a **promising target** for OUD (Pearson & Ostroumov 2024; Pearson et al. 2025; Thomas et al. 2018; Ostroumov et al. 2016).

Within the field of substance use disorder research, KCC2 has been characterized most heavily in the ventral tegmental area (VTA). Recent research, however, has highlighted the neighboring **substantia nigra pars reticulata (SNr)** as a promising new target area (Galaj et al. 2020). Consisting primarily of GABAergic neurons, the SNr serves as an inhibitory output nucleus for the basal ganglia, and it's understood to be critical for movement control. The SNr regulates output of the adjacent substantia nigra pars compacta (SNc), a dopamine-rich area increasingly implicated in drug reinforcement and relapse (Galaj et al., 2020; Tepper et al., 1995; Rossi et al., 2013; Wise, 1981; Corbett & Wise, 1980). Recent studies have expanded on the SNr's role in addiction to implicate OUD; notably, its GABAergic neurons have been found to express a high concentration of mu opioid receptors, and their pharmacological blockage has a more robust effect on opioid self-administration (SA) than that of analogous receptors on VTA GABAergic neurons (Galaj et al. 2020). Such findings highlight the SNr as a powerful potential modulator of opioid reward and addiction.

In the current study, we investigated **whether morphine self-administration downregulated KCC2** in SNr GABA neurons, and **whether KCC2 downregulation in morphine-naive rats was sufficient to produce behavioral alterations** associated with OUD. Our preliminary ex vivo data demonstrates that chronic morphine self-administration diminishes KCC2 expression in SNr GABA neurons. Additionally, we demonstrate that downregulation of KCC2 in SNr GABA neurons of drug-naive rats produces deficits in several behavioral tasks which may be associated with withdrawal. These findings help elucidate the potential of KCC2 in SNr GABA neurons as a novel target for therapeutic interventions aimed at OUD-induced maladaptive behaviors.

Methods

A) Experimental Timeline: KCC2 Expression Post Chronic Morphine Self-Administration

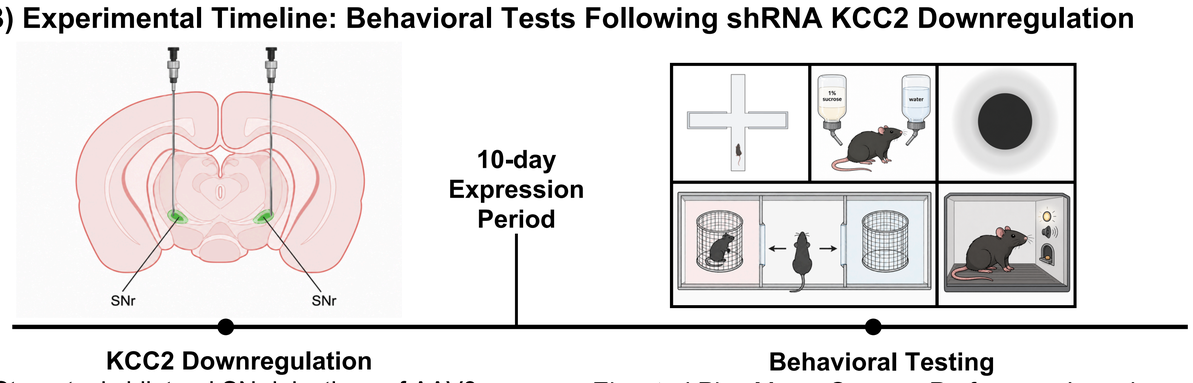


14 days of Morphine SA
Morphine Self-Administration
(12h/day Days 1-11;
6h/day Days 12-14)

Immunohistochemistry
Fluorescent labeling of KCC2
in coronal brain slices
containing the SNr

Imaging
Mica Microhub confocal
microscope at 20x

B) Experimental Timeline: Behavioral Tests Following shRNA KCC2 Downregulation



KCC2 Downregulation
Stereotaxic bilateral SNr injections of AAV8-EF1-mCherry-SICO-GFP-KCC2-shRNA

Behavioral Testing
Elevated Plus Maze, Sucrose Preference, Looming, Social Preference, and Pavlovian Reward Learning

Results

Immunohistochemistry

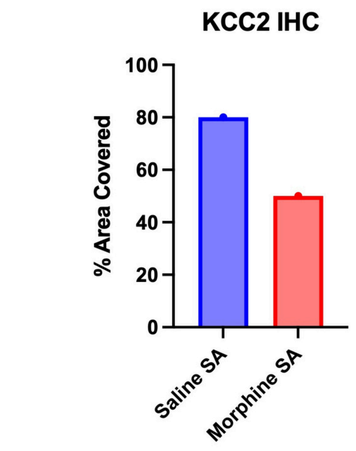


Fig. 1. Preliminary data suggests that SNr KCC2 is downregulated following chronic morphine self-administration.

Pavlovian Reward Learning

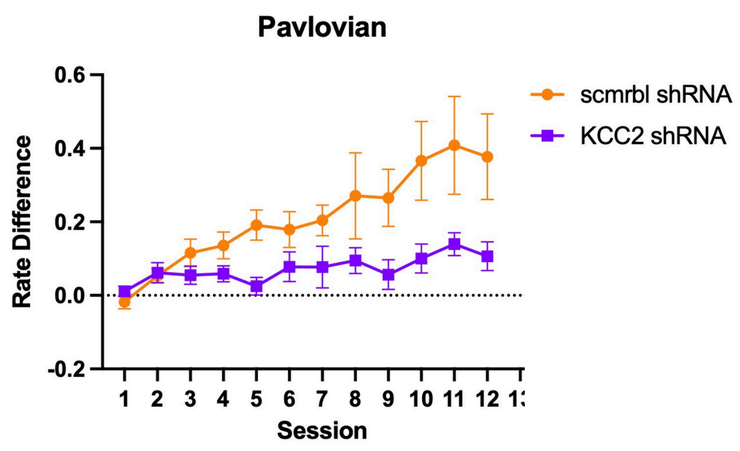


Fig. 2. KCC2 downregulation significantly decreased conditioned responding in the Pavlovian Reward Learning task.

Elevated Plus Maze

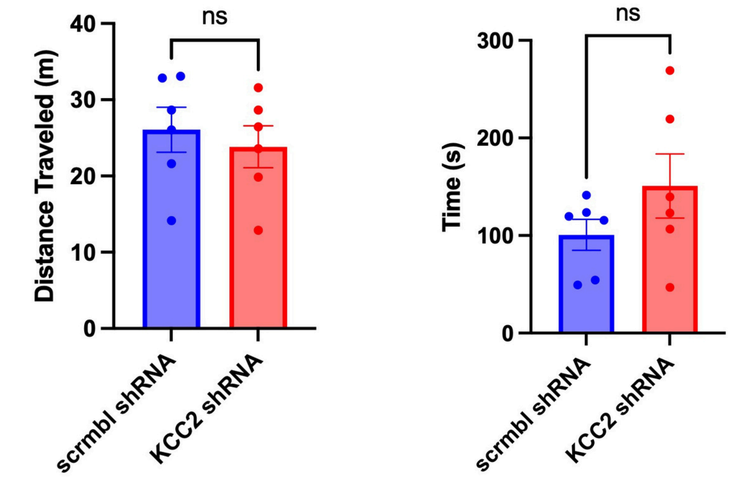


Fig. 3. KCC2 downregulation did not significantly impact Elevated Plus Maze behaviors.

Social Interaction

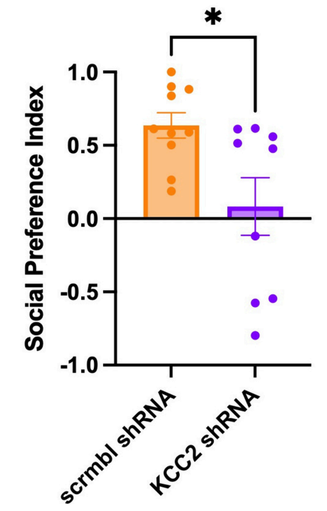


Fig. 4. KCC2 downregulation significantly decreased sociability.

Sucrose Preference

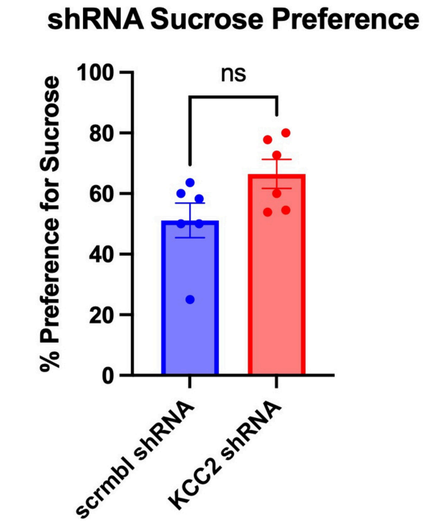


Fig. 5. KCC2 downregulation did not significantly impact sucrose preference.

Looming

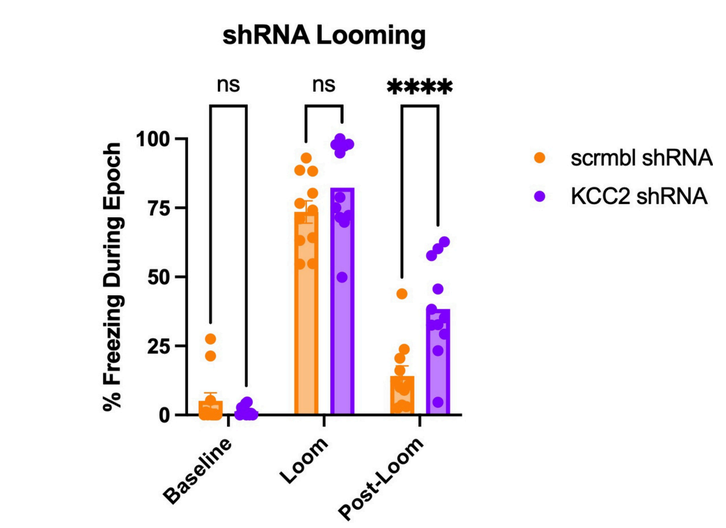


Fig. 6. KCC2 downregulation did not impact freezing before or during the stimulus, but significantly increased post-loom freezing.

Conclusion & Future Directions

The present study presents data that suggests **SNr KCC2 is downregulated by chronic morphine self-administration**. Moreover, it demonstrates that independent SNr KCC2 downregulation is sufficient to induce **learning and affective** behavioral changes including impaired cue-reward association, decreased social preference and prolonged defense responses. These findings suggest that SNr KCC2 downregulation may contribute to maladaptive molecular and behavioral adaptations in OUD.
Additional electrophysiological studies assessing changes in GABAergic SNr neuron and downstream DA neuron activity are necessary. Future studies must also investigate whether SNr KCC2 upregulation helps rescue behavioral alterations associated with morphine SA. Continued research on SNr KCC2 may provide critical insights for developing sustainable therapeutics for OUD.

References

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