# The Role of *SlC1A3* in Alcohol Use Disorder and Alcohol-Associated Behaviors

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## Introduction

Alcohol use disorder (AUD) affects approximately 11% of the U.S. population and contributes significantly to the global burden of disease and injury. Chronic alcohol exposure disrupts glutamate homeostasis, primarily through changes in glutamate transporters, leading to persistent neuroadaptations in key brain structures that control motivated behavior [1]. The primary glutamate transporters are SLC1A2 (GLT-1) and SLC1A3 (GLAST), with SLC1A2 being more abundant (~1% of total brain protein) and GLAST approximately 4-6 times less abundant [2].

While SLC1A2 has been extensively studied in addiction and shown to regulate addiction-like behaviors across multiple substances including cocaine, opioids, ethanol, nicotine, and amphetamines [3], our research consistently finds SLC1A3 to be more prominently dysregulated following alcohol consumption. We have observed *Slc1a3*/*SLC1A3* dysregulation in various experimental paradigms. Following every-other-day two-bottle choice (EOD-2BC) drinking, *Slc1a3* was significantly downregulated in astrocyte-enriched cells but not in bulk tissue from mouse medial prefrontal cortex (mPFC)[4]. After chronic intermittent ethanol (CIE) exposure, *Slc1a3* was downregulated in both astrocyte-enriched cells and bulk tissue, but not in microglia-enriched cells from mouse mPFC [5]. In another CIE experiment, *Slc1a3* downregulation was observed 24h after the last exposure to ethanol vapor but normalized after 72h [6]. In single nucleus transcriptome profiling of dorsolateral PFC from alcohol-dependent humans, *SLC1A3* was significantly upregulated in several cell types, including astrocytes and microglia [7]. We recently identified an astrocyte-specific co-expression module upregulated in mouse mPFC following CIE, in which *Slc1a3* was a central component [8].

In this study, we aimed to specifically target *Slc1a3* in the mPFC to investigate its role in alcohol-related behaviors.

## Methods

### Viral Design and Validation

We designed an AAV5 viral vector containing four different miRNAs targeting different regions of the *Slc1a3* gene. Male C57BL/6J mice underwent stereotaxic surgery to bilaterally infuse 100nl of either the KD virus or a scrambled control (SCR) into the dmPFC (coordinates relative to bregma: AP: 1.94 mm; DV: -2.0 mm; ML: +/- 0.4 mm) and were given 3 weeks for the virus to express before tissue collection or behavioral testing. Validation of knockdown efficacy was assessed via qPCR, Western blot, and fluorescent in situ hybridization (RNAScope).

### Behavioral Testing

The 2-bottle choice (2BC) model was used to measure voluntary alcohol consumption, where mice had access to two bottles: one containing 15% ethanol and the other containing water. Drinking sessions occurred every other day (EOD-2BC), allowing measurement of both consumption and preference. Compulsive-like behavior was assessed using quinine adulteration of this model, where increasing concentrations of quinine were added to the ethanol bottle. Each concentration was given for 2 days and is presented as an average. Anxiety-like behavior was assessed using the elevated plus maze (EPM) for 5 minutes. Locomotion was measured for 15 minutes in an open field arena, with time spent in the center extracted as a secondary measurement of anxiety-like behavior.

## Results

### Viral Vector-Mediated Knockdown Specifically Targets *Slc1a3*

Our viral vector design produced robust knockdown of *Slc1a3*/SLC1A3 without affecting the expression of *Slc1a2* in the dmPFC. This infusion resulted in reduced *Slc1a3* expression in the dmPFC compared to SCR controls while the expression of *Slc1a2* remained unchanged (Fig. 1). The RNAScope of Viral vector miRNA KD confirmed the reduction *Slc1a3* expression compared to controls. *Slc1a2* is more abundant than *Slc1a3*, with varied cellular distribution patterns - some cells express neither transcript, some only *Slc1a3*, and others show co-localization.

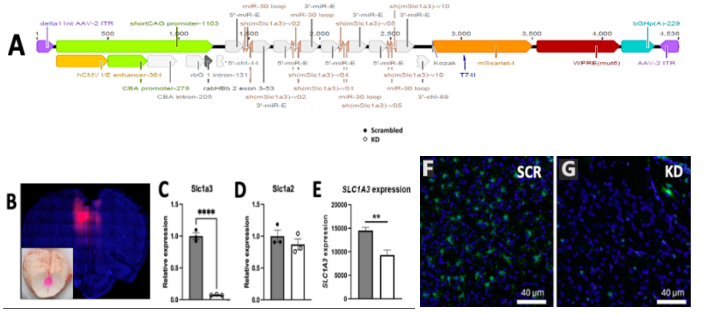


**Figure 1.** Schematic showing the viral construct. The vector was constructed and packed into an AAV5 by Zürich viral vector Facility, Switzerland (A). Viral vector infusion into the dmPFC (B) resulted in a robust KD of *Slc1a3*/SLC1A3 (C/E) without affecting the expression of *Slc1a2* (D). Representative micrographs of in-situ hybridization (RNAScope) for *Slc1a3* (green). *Slc1a3* expression in the dmPFC is reduced following viral vector infusion containing miRNA against the transcript compared to SCR (SCR: E; KD: F).

### Knockdown of *Slc1a3* in the mPFC does not significantly alter alcohol intake

For the first experiment (n=10/group), mice followed a 15% ethanol EOD-2BC drinking model. To assess compulsive-like behavior, mice were tested with quinine adulteration. The *Slc1a3* KD in the dmPFC resulted in a small but stable increase in voluntary ethanol intake and preference for alcohol over water, though these differences did not reach statistical significance (Fig. 3A-D), that persisted in the presence of quinine adulteration (Fig. 3E). Expression levels of *Slc1a3* in the prelimbic cortex (PL) inversely correlated with cumulative voluntary ethanol intake in both KD and SCR groups. This correlation was not seen in the infralimbic cortex (IL), suggesting regional specificity in how these transcripts are regulated (Fig. 4E-G).

The second experiment (n=10-11/group) repeated the procedure but excluded quinine adulteration and included

locomotion and anxiety-like behavior assessments prior to the EOD-2BC drinking model. In the third experiment (n=14/group), we performed the EOD-2BC drinking model also before the viral vector surgeries to investigate whether prior exposure to alcohol was required for *Slc1a3*-KD in the dorsomedial prefrontal cortex (dmPFC) to affect behaviors. However, KD of *Slc1a3* in the dmPFC showed no effect on consumption or preference for alcohol in either experiment (Fig. 5A-B and Fig. 6A-B), and no correlation was observed between the levels of the transcript and alcohol intake in the PL in either experiment (data not shown).

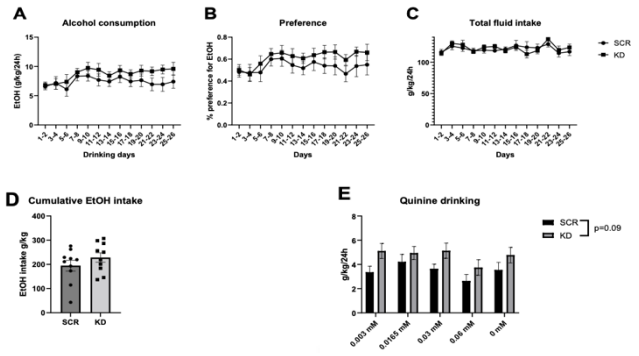


**Figure 3.** KD of *Slc1a3* in the dmPFC promotes a small, but not statistically significant, increase in voluntary ethanol intake (A) and preference for alcohol over water (B), with no observed effect of total fluid intake (C), compared to SCR. D) cumulative EtOH intake (E). The trend for increased EtOH intake, however, remained in the presence of quinine.

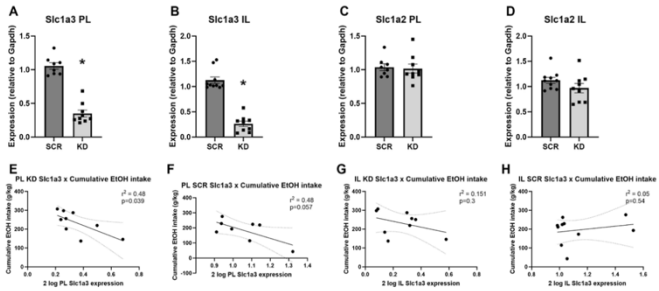


**Figure 4.** Viral vector-mediated KD of *Slc1a3* showed a robust downregulation of the transcript in the mPFC, both the prelimbic-(PL)(A) and infralimbic cortex (IL)(B), without affecting levels of *Slc1a2* (C- D). We analyzed the PL and IL separately as they are known to sometimes have opposed roles in regulating behaviors. Expression levels of *Slc1a3* inversely correlated with cumulative voluntary EtOH intake in the PL (KD: E; SCR: F) but not the IL (KD: G; SCR: H).

**Knockdown of *Slc1a3* in the mPFC has an anxiolytic effect**

While we observed no consistent effects on alcohol consumption, there was a trend toward decreased anxiety-like behavior in the elevated plus maze following *Slc1a3* KD (Fig. 5F and Fig. 6D). Animals with *Slc1a3* knockdown tended to spend more time in the open arms of the EPM compared to SCR controls, without significant differences in total arm entries, suggesting an anxiolytic effect independent of general locomotor activity. In the third experiment with prior alcohol exposure, we found a significant decrease in anxiety-like behavior in the EPM, with KD animals spending more time in the open arms ($p<0.01$), providing stronger evidence for an anxiolytic effect of *Slc1a3* reduction in the mPFC.

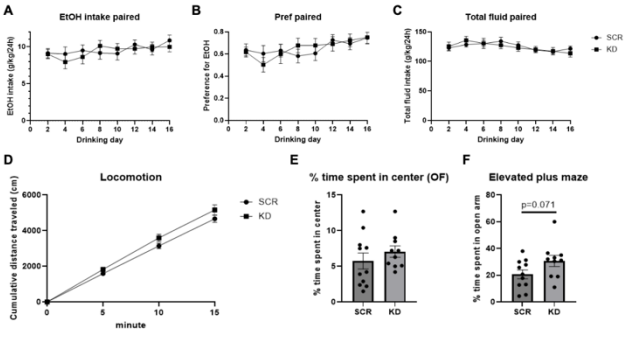


**Figure 5.** In the second experiment, no effect was observed on voluntary EtOH consumption (A), preference for alcohol over water (B) or total fluid intake (C) following *Slc1a3* KD, compared to SCR. No effect was observed on locomotion (D), however a trend for decreased anxiety-like behavior was observed in the EPM (F).

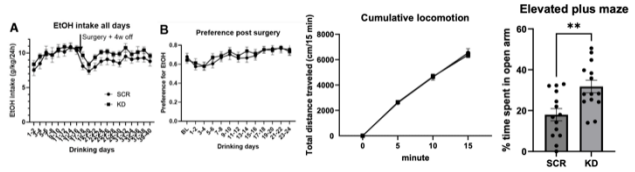


**Figure 6.** In the third experiment, no effect was observed on voluntary EtOH consumption (A) preference for alcohol over water (B) or locomotion (C)), however a significant decrease in anxiety-like behavior was observed in the EPM (D), compared to SCR.

## Discussion and conclusions

Our previous research demonstrates that *Slc1a3* is dysregulated following alcohol consumption in both humans and mice, showing more prominent changes than *Slc1a2* in our data. Here, selectively targeted *Slc1a3* in the mPFC without affecting *Slc1a2* expression. Our results show that KD of *Slc1a3* in the mPFC was not sufficient to alter alcohol-intake or preference, however, it did have an anxiolytic effect that was more robust in animals with a prior history of alcohol exposure. It is possible that Slc1a3 plays a more direct role in regulating behaviors in other drinking models and brain regions not examined in this study.

The identification of *Slc1a3* as a central component in astrocyte-specific co-expression modules that are similarly dysregulated in both mouse and human alcohol studies suggests that this transporter may act in concert with other module genes to regulate behavior, rather than function independently. Future studies may consider coordinated manipulation of multiple targets within astrocyte-specific gene networks rather than focusing on single gene knockdowns.

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