

Upregulation of GLT-1 transporters does not prevent reinstatement to cocaine-seeking



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Introduction

- Cocaine addiction is a chronic disorder and the risk of relapse remains high even after long periods of abstinence (National Institute on Drug Abuse, 2014).
- Using the extinction-reinstatement model of relapse, synaptically-released glutamate in the nucleus accumbens (NAc) drives the reinstatement of cocaine-seeking (McFarland et al., 2003).
- After 2-3 weeks of extinction from cocaine self-administration, the expression of GLT-1 is significantly decreased in the NAc (Knackstedt et al., 2010).
- The antibiotic ceftriaxone restores GLT-1 expression and function, prevents cue-primed reinstatement of cocaine-seeking, and prevents the increase in NAc glutamate during reinstatement (Knackstedt et al., 2010; Trantham-Davidson et al., 2012).
- In order to determine whether GLT-1 upregulation in the NAc alone is sufficient to attenuate reinstatement, adeno-associated virus (AAV) was used to directly overexpress GLT-1 transporters.

Methods

- Animal subjects and surgeries:** Adult male Sprague-Dawley rats individually housed in a temperature and humidity-controlled vivarium were kept on a reverse 12-h light-dark cycle. Jugular catheters were placed and cannulas were inserted aimed at the nucleus accumbens core.

Self-Administration	Micro-Injection	Extinction Training
12 days of 2hr sessions of 10+ infusions; cocaine with light+tone	First day of extinction; GLT-1 upregulating AAV or control AAV	3 weeks of 2hr sessions; no drug, light or tone present on previously active lever

- Viral Vectors:** 1 μ l of AAV-GFAP-GLT-1 or control AAV-GFAP-eGFP bilaterally.
- Reinstatement procedures:** 1-h relapse test. Active lever was once again accompanied by the light+tone compound when pressed. No drug was present with this cue reinstatement test.
- Microdialysis:** A separate group of rats was probed for dialysis the day before reinstatement testing. 10 minute samples were collected during a 1hr cocaine primed reinstatement test.

AAV-GFAP-GLT-1 and AAV-GFAP-eGFP

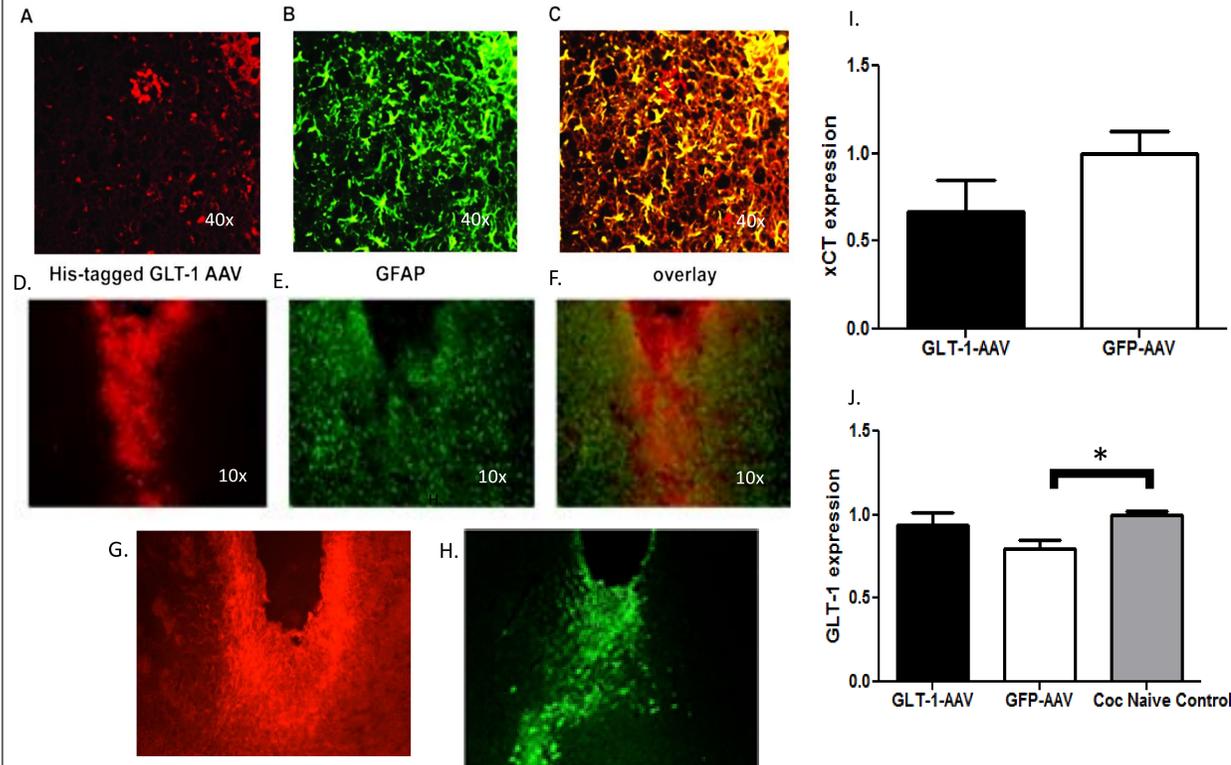


Fig.1 A/D: His-tagged GLT-1-AAV. **B/E:** GFAP stained glial cells. **C/F:** Overlap of the his-tagged GLT-1 virus and GFAP. **I:** GLT-1 AAV increased GLT-1 transporters but did not alter xCT protein amounts. **J:** GLT-1 protein expression was decreased in animals that had received GFP-AAV compared to cocaine naïve controls, while there is no difference in GLT-1 expression in animals that had received GLT-1-AAV compared to cocaine naïve controls.

Upregulation of GLT-1 Transporters & Reinstatement

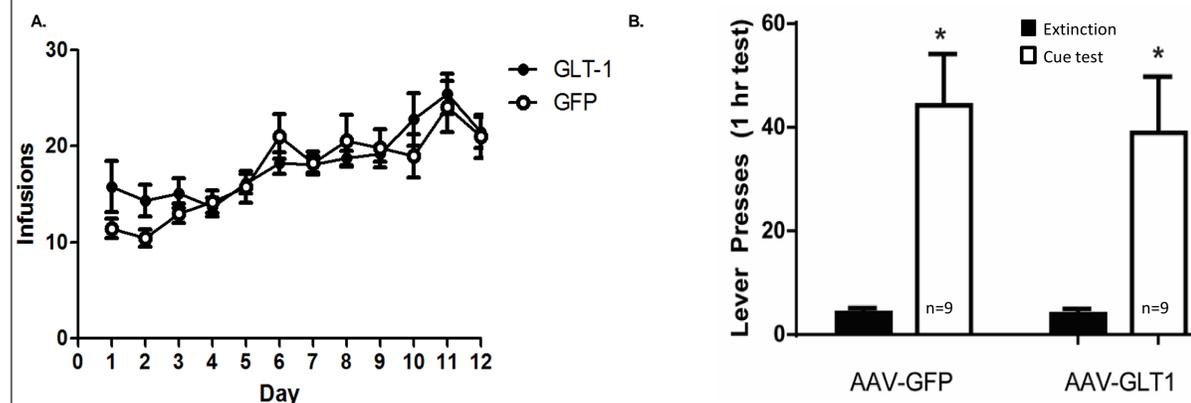


Fig.2 A. Infusions received for the 12 days of self-administration were comparable between the two groups. **B.** Lever presses during a one hour cue-primed reinstatement test compared to lever presses during extinction training. Both control animals (n=9) and GLT-1 AAV-infused animals (n=9) reinstated to cocaine-seeking ($p < .05$).

Results

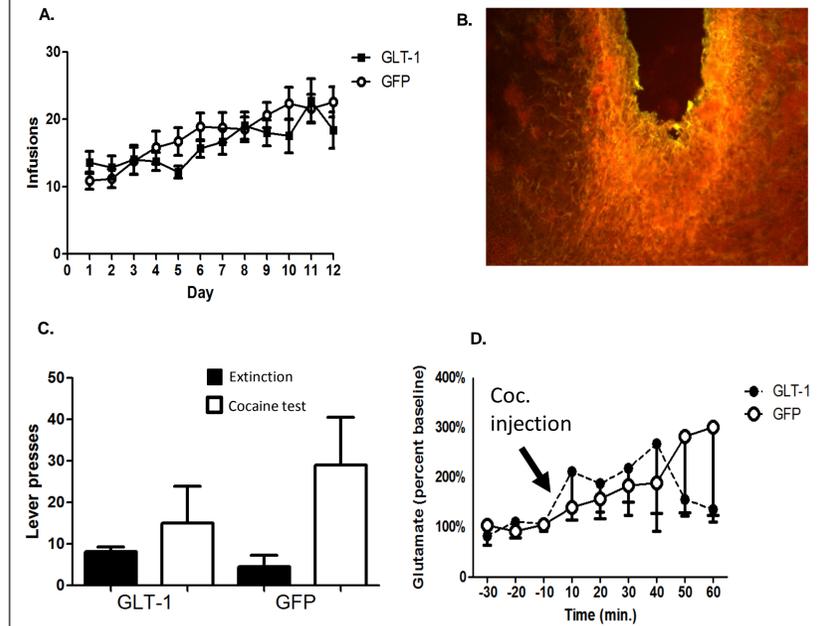


Fig.3 A: Infusions for both groups during self-administration. **B.** His-tagged virus and GFAP overlay. Dialysis probe under cannula track. **C.** Lever presses during extinction and cocaine primed reinstatement test for GLT-1 upregulated and control animals. **D.** Baseline glutamate levels for GLT-1 upregulated and control animals. An increase in glutamate levels was observed in both groups.

Conclusions

Ceftriaxone:

- Prevents reinstatement to drug-seeking behaviors when presented with a cue or cocaine;
- Upregulates GLT-1 transporters and restores the cysteine-glutamate exchange;
- Stabilizes glutamate levels during cocaine-primed reinstatement.

AAV-Mediated Upregulation of GLT-1 Transporters:

- Upregulated GLT-1 transporters;
- Did not prevent reinstatement to cue-primed cocaine-seeking;
- Perhaps attenuated cocaine-primed reinstatement;
- Increase in synaptically released extracellular glutamate levels continued to be observed even with the upregulation of GLT-1 transporters.

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