Repetitive drug use, but not repeated exposure to natural rewards, results in excess glutamate transmission within corticofugal projections to the striatum in response to reward-associated cues and contexts. Exposure to different classes of addictive drugs results in downregulation of the principal glutamate uptake transporter, GLT-1, in the nucleus accumbens, resulting in synaptic glutamate spillover and conferring relapse vulnerability.\(^5\) Dynamic proximity of astrocyte processes that contain GLT-1 affects the rate and efficiency of glutamate uptake, adding another measure of control over synaptic glutamate diffusion. Our lab has recently developed techniques to examine synaptic proximity of astrocyte fine processes and found that transient synaptic proximity of GLT-1 after heroin self-administration using confocal microscopy and found profound and transient morphological rearrangements during 10 minutes of cue-induced seeking that were reversed after 120 minutes, when active drug seeking had ceased. Since actin dynamics are known to be involved in astrocyte fine process rearrangements, we examined modifications of the actin cofactor Cofilin and found that these distinct post-translational modifications may regulate its function after drug exposure.

Astroglial morphological assessment: AA22-GFP/FITC-Molm-Cherry (University of Zurich) was microinjected bilaterally in the nucleus accumbens core. Animals were perfused transcardially with 4% PFA and 100 µm coronal sections were collected and permeabilized in 1X PBS with 2% Triton X-100 before blocking and primary antibody incubation against Synapsin (1:Acum), GLT-1 (Millipore). Z-stacks were acquired at 63X magnification using a Leica spinning disk confocal microscope and entire astrocyte cell bodies were imaged at 12-bit resolution, with 4-frame averaging, a 1024x1024 frame size and a 1-µm step size. Images were processed according to deconvolution. After deconvolution, astrocytes were masked to remove fluorescence signal from neighboring cells (Biplane Imaris). Co-localization was determined using automated threshold detection settings for each channel and percent co-localization was calculated relative to astrocyte volume.

**METHODS**

Astroglial markers and drug administration: Animals were trained to self-administer heroin for 10 days, during which time active lever presses yielded IV heroin solution paired with light and tone cues. After 10-14 days of training, in which active lever presses yielded no drug infusion and no cues, rats were either sacrificed (Extinction, C-D) or placed back in the operant chamber for 15 or 120 minutes with light and tone cues restored (B). After 15 minutes of cue reinstatement, astrocytes in the nucleus accumbens core exhibited reduced volume (C) and surface area (D) relative to astrocytes in yoked saline animals. These measures were restored to extinction levels after 120 minutes of cue-induced seeking, when animals had begun within-trial extinction of lever pressing. In C, p = 0.0278, Saline vs. 15 Reinstatement. In D, p = 0.0187, Saline vs. Extinction, p < 0.0001, Saline vs. 15 Reinstatement, p = 0.00349 15 Reinstatement vs. 120 Reinstatement by one-way ANOVA. In C-D, N is shown in bars as cell/cell interiors.

**CONCLUSIONS**

- Astrocytes in the nucleus accumbens core exhibit transient morphological rearrangements during 15 minutes of cue-induced heroin seeking that are reversed after 120 minutes.
- Synaptic proximity of fine astroglial processes is decreased after extinction from heroin self-administration and restored during active seeking.
- Synaptic proximity of the glutamate transporter GLT-1 is decreased by drug exposure and is unchanged during seeking.
- The actin cofactor Cofilin is S-glutathionylated during reinstated drug seeking, reducing its capacity to depolymerize F-actin.

**REFERENCES**


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