Transient plasticity of perisynaptic astrocyte processes during reinstated heroin seeking

Anna Kruyer, Michael D. Scofield, Joachim D. Uys, Peter W. Kalivas

Medical University of South Carolina

ABSTRACT

Repeated 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[1-3]. Synaptic 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 retraction during extinction from cocaine self-administration[4], but it is not known how reinstatement affects these measures or whether these findings extend to other addictive drugs. We examined glial morphology and synaptic proximity of GLT-1 after heroin self-administration using confocal microscopy and found profound and transient morphological rearrangements during 15 minutes of cued 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 two distinct post-translational modifications may regulate its function after drug exposure.

METHODS

Astroglial morphological assessment: AAV5-hGFAP-hM3D-mCherry (University of Zurich) was microinjected bilaterally in the nucleus accumbens core[5]. 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 I (Abcam) and 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[4]. After deconvolution (Autoquant), astrocytes were masked to remove fluorescence signal from neighboring cells (Bitplane Imaris). Colocalization was determined using automated threshold detection settings for each channel and percent colocalization was calculated relative to astrocyte volume.

PRELIMINARY RESULTS

Nucleus accumbens astrocytes exhibit transient reductions in volume and surface area during cued heroin seeking

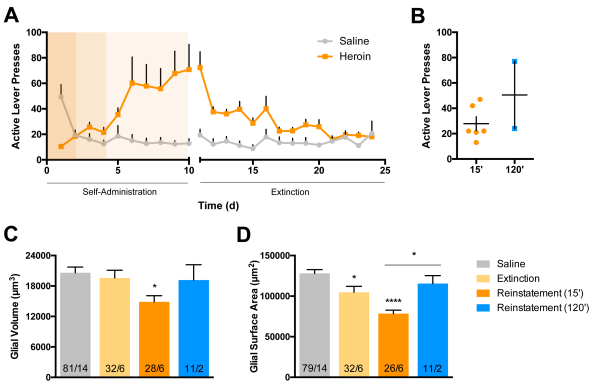

Figure 1. (A) Rats were trained to self-administer heroin for 10 days, during which time active lever presses yielded IV heroin infusion paired with light and tone cues. After 10-14 days of extinction training, in which active lever presses yielded no drug infusion and no cues, rats were either sacrificed (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 cued 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 cued seeking, when animals had begun within-trial extinction of lever pressing. In C, p = 0.0276, Saline vs. 15' Reinstatement. In D, p = 0.0187, Saline vs. Extinction; p < 0.0001, Saline vs. 15' Reinstatement; p = 0.0349 15' Reinstatement vs. 120' Reinstatement by one-way ANOVA. In C-D, N shown in bars as cells/animals.

Astrocyte fine processes increase synaptic proximity during drug seeking

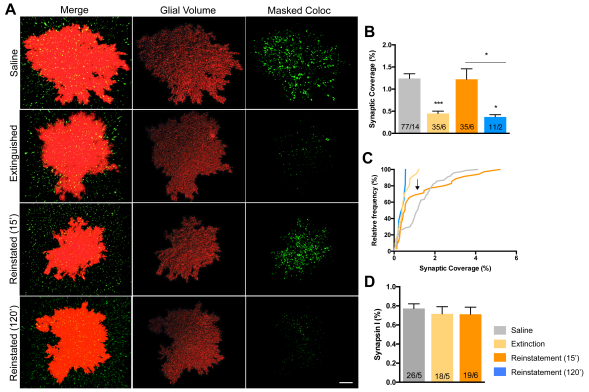

Figure 2. Astroglial fine process retraction during extinction from heroin self-administration leads to reduced colocalization of glial processes (A, red) with the pre-synaptic marker Synapsin I (A, green) relative to yoked saline controls (A-C) after normalizing to total glial volume. This colocalization is transiently restored during a 15-minute reinstatement session, but returns to extinction levels by 120 minutes. Preliminary analysis shows that total levels of Synapsin I do not differ between groups (D). In A, scale bar = 10 microns. In B, p = 0.0004, Saline vs. Extinction; p = 0.0245 Saline vs. 120' Reinstatement; p = 0.0478, 15' Reinstatement vs. 120' Reinstatement by one-way ANOVA. In B, N is shown in bars as cells/animals; in D, N is shown in bars as frames/animal.

Synaptic proximity of GLT-1 is decreased after drug exposure and is unchanged during seeking

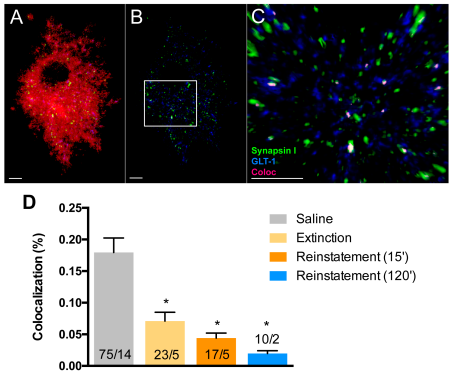

Figure 3. Despite restored synaptic contact at 15 minutes of cued reinstatement, colocalization (C, pink) of the glutamate transporter GTL-1 (A-C, blue) with Synapsin I (A-C, green) does not increase (D). Levels of GLT-1 are reduced after heroin self-administration and do not recover during extinction or reinstatement (not shown). In A-C, scale bar = 5 microns. In D, p = 0.0247, Saline vs. Extinction; p = 0.01, Saline vs. 15' Reinstatement; p = 0.0174, Saline vs. 120' Reinstatement by one-way ANOVA. All values in D are normalized to glial volume. N is shown in bars as cells/animals.

Parallel pathways modulate actin cofactor Cofilin after drug exposure

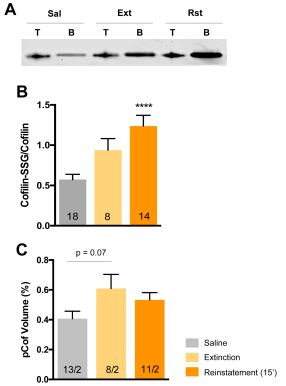

Figure 4. (A-B) Using a novel S-glutathionylation switch protocol, we have found that the actin cofactor Cofilin is glutathionylated during 15 minutes of cued cocaine seeking following the same self-administration and extinction model. S-glutathionylated Cofilin was extracted from nucleus accumbens homogenates, de-glutathionylated, and pulled down using a thiol resin (A, lanes labeled B). All samples are normalized to total levels of Cofilin from the same animal (A, lanes labeled T). Quantification is shown in B. (C) Preliminary results using confocal microscopy also point to phosphorylation of Cofilin in astrocytes after extinction from heroin self-administration. Both phosphorylation[6] and S-glutathionylation (not shown) of Cofilin serve to inhibit the actin de-polymerizing function of the protein. In B, p < 0.0001, Saline vs. 15' Reinstatement by one-way ANOVA. In B, N is shown in bars as animals; in C, N is shown in bars as cells/animals.

CONCLUSIONS

- **Astrocytes in the nucleus accumbens core exhibit transient morphological rearrangements during 15 minutes of cued 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.**

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