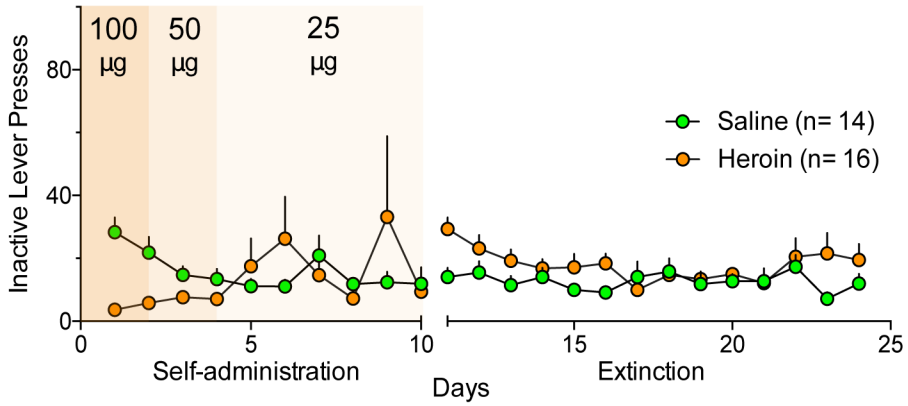
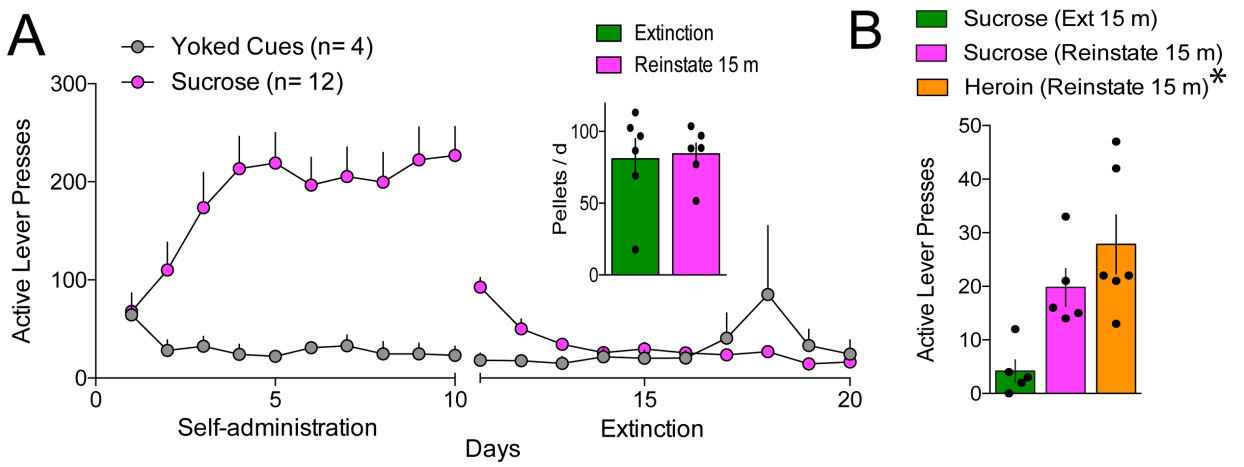
Heroin Cue-Evoked Astrocytic Structural Plasticity at Nucleus Accumbens Synapses Inhibits Heroin Seeking

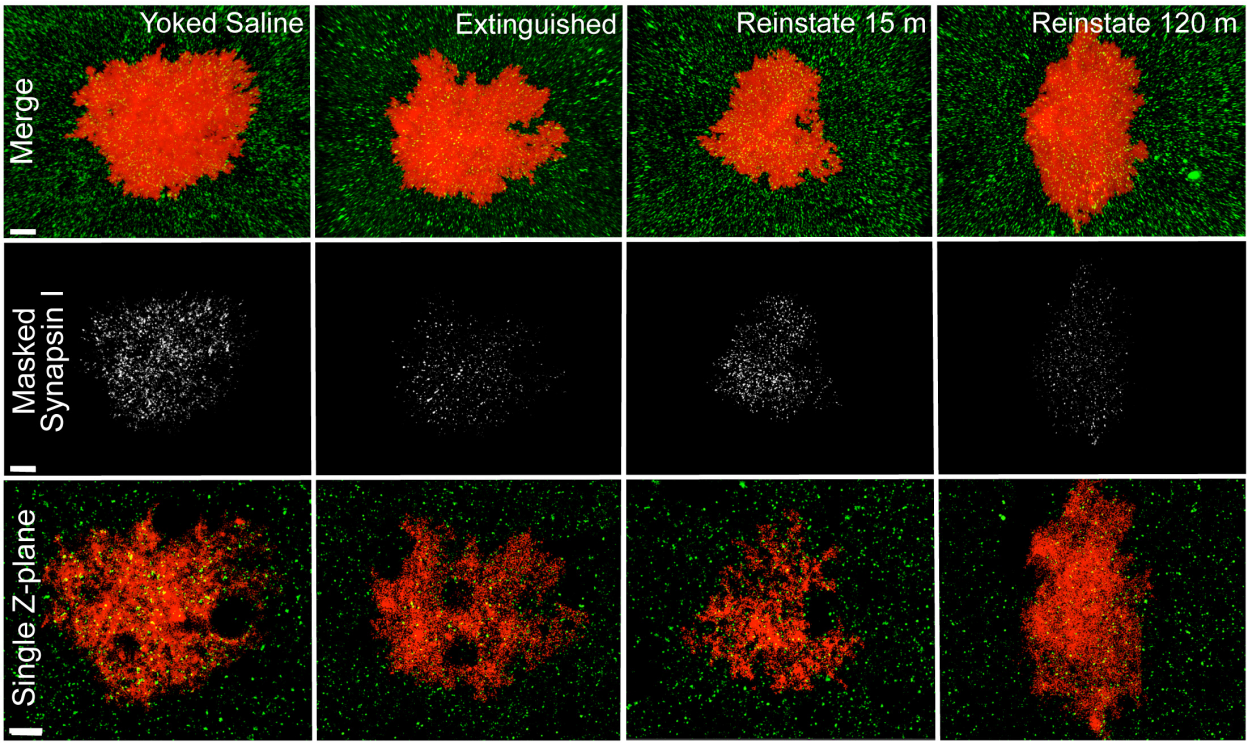
SUPPLEMENTAL INFORMATION



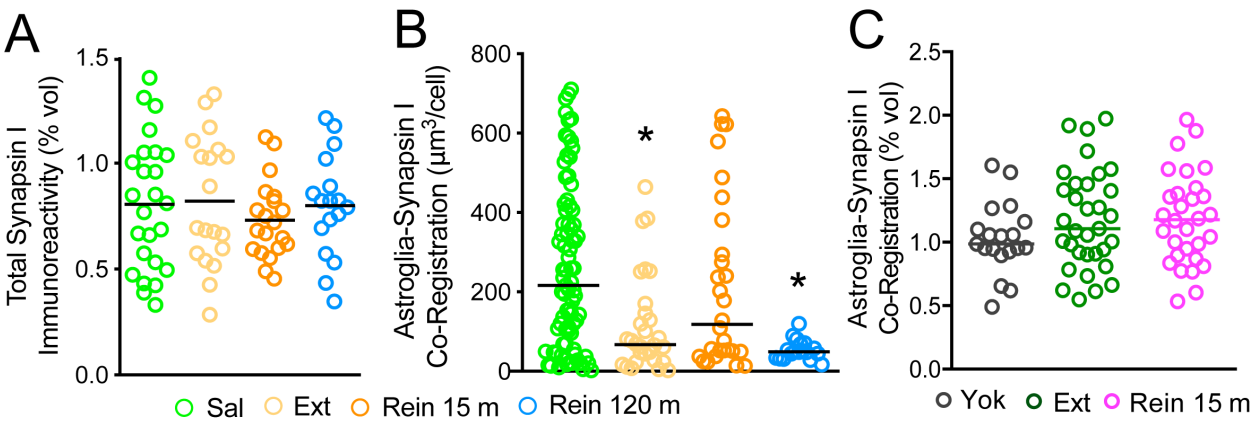
Supplemental Figure S1. (**A**) Inactive lever presses corresponding to active lever presses shown in Figure 1C.



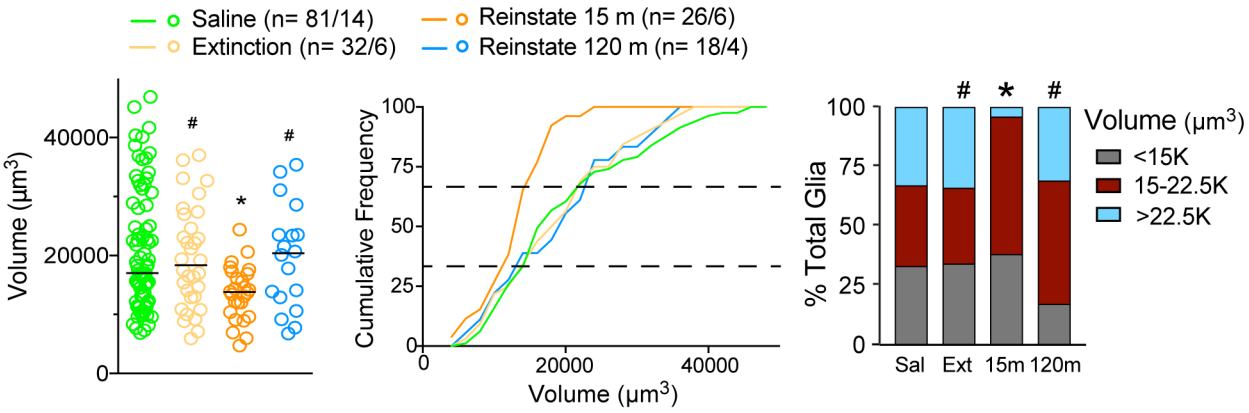
Supplemental Figure S2. (**A**) Active lever pressing during sucrose and yoked cue self-administration and extinction training. Inset shows that the treatment groups received the same number of sucrose pellets during self-administration (unpaired Student's t-test, $t_{(10)}$= 0.21, p= 0.838). (**B**) 15 min of cue-induced reinstatement in sucrose-trained rats elevated active lever pressing compared to the first 15 min of the final extinction trial, but was not different from active lever pressing in 15 min of cued heroin seeking in heroin-trained rats (1-way ANOVA, $F_{(2,13)}$= 8.154, p= 0.005, *p= 005 comparing sucrose extinction with heroin reinstated using a Sidak's post hoc test). Data shown as mean±sem.



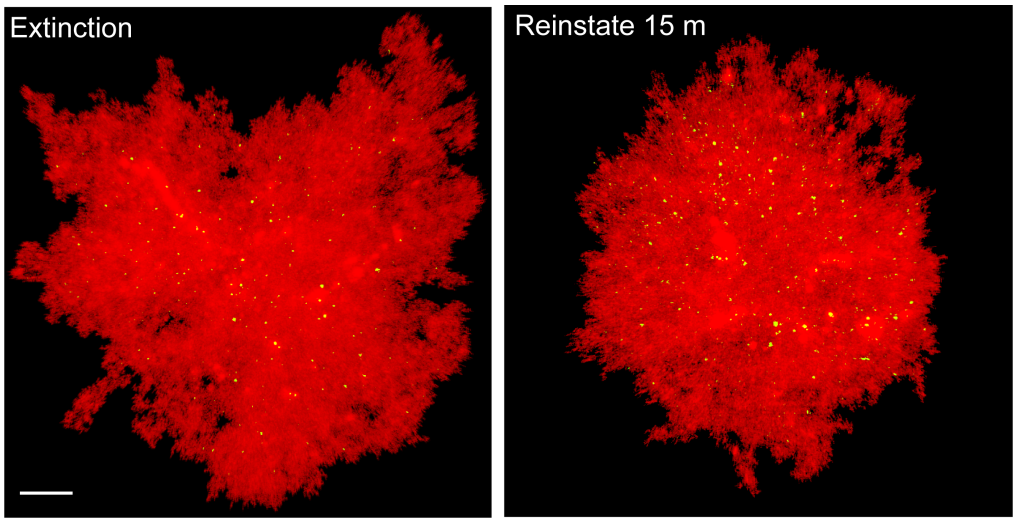
Supplemental Figure S3. Representative micrographs depicting changes in morphology and synaptic adjacency by NAcore astrocytes. Top row shows confocal images containing a single filled astrocyte (red) and immunoreactive puncta from the presynaptic marker Synapsin I (green) from yoked saline, heroin extinguished or reinstated rats. Middle row shows Synapsin I masked by the astroglial volume (white) corresponding to the Synapsin I in co-registration with the astroglial membrane. This measure of co-registration between the astroglia surface and Synapsin I was used to indicate changes in the proximity of astroglia to synapses. The bottom row shows a single Z-plane from each astrocyte and reveals the structural complexity allowing synaptic co-localization by astroglial membrane within the volume of the cell (bars= 10 μm).



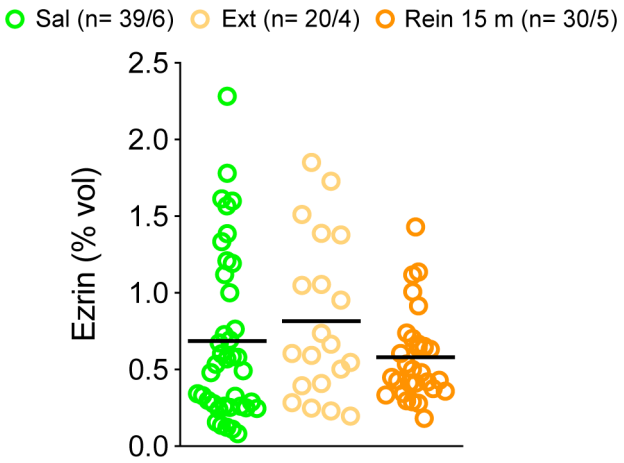
Supplemental Figure S4. (**A**) Total Synapsin I was equivalent across treatment groups. (**B**) Data not normalized to individual astroglial volume for co-registration of astroglial surface and Synapsin I (see Figure 2F for normalized data). Akin to the normalized data, synaptic co-localization showed significant differences between groups (Kruskal-Wallis= 21.7, *p<0.001 compared to Saline using a Dunn's post hoc comparison). (**C**) Co-registration between astroglia and Synapsin I from sucrose-trained rats was not different between the three treatment groups (Kruskal-Wallis= 2.15 p= 0.341). N as cells/animal = (**A**; Saline 25/7, Extinction 19/5, Reinstate 15m 20/6, Reinstate 120m 17/4), (**B**; Saline 72/14, Extinction 30/6, Reinstate 15m 26/6, Reinstate 120m 18/4), and (**C**; Yoked 19/4, Extinction 33/6, Reinstate 15m 30/5). Bars= median.



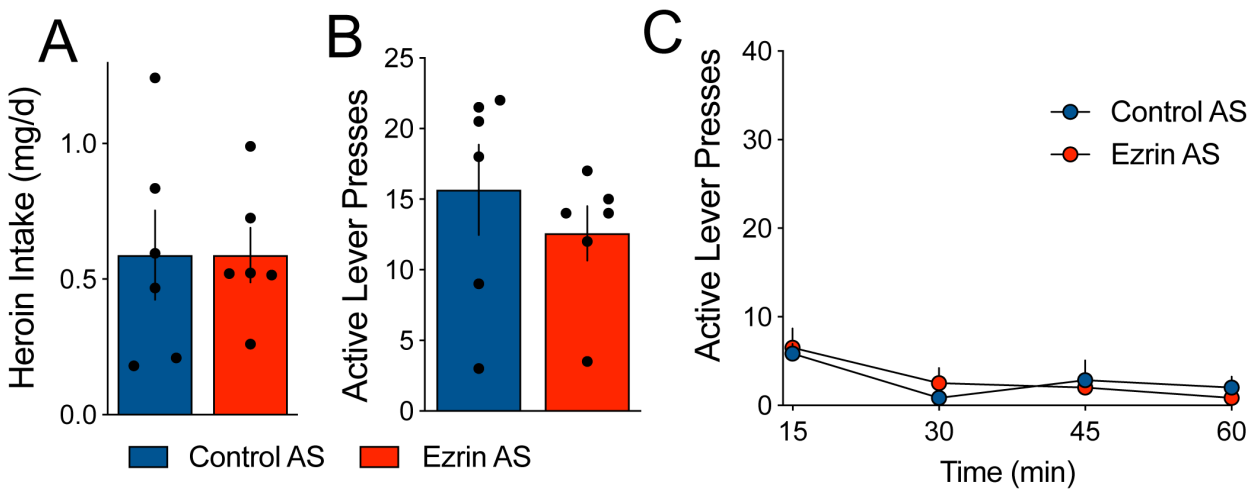
Supplemental Figure S5. The volume of NAcore astrocytes was transiently reduced by 15 min exposure to heroin cues (left panel raw data, bar= median, Kruskal-Wallis= 10.3 p= 0.016). Middle panel shows frequency distribution, note shift to the left in the reinstate 15 min group. Dashed lines indicate division according to volume of the yoked saline astroglial population into three groups. Right panel showing subpopulations ($\chi^2(6)$= 13.06, p= 0.042). *p< 0.05, compared to saline using a Dunn's post hoc (left panel) or multiple χ^2 tests with a Bonferroni adjustment for multiple comparisons (right panel), #p< 0.05, compared to reinstate 15 min.



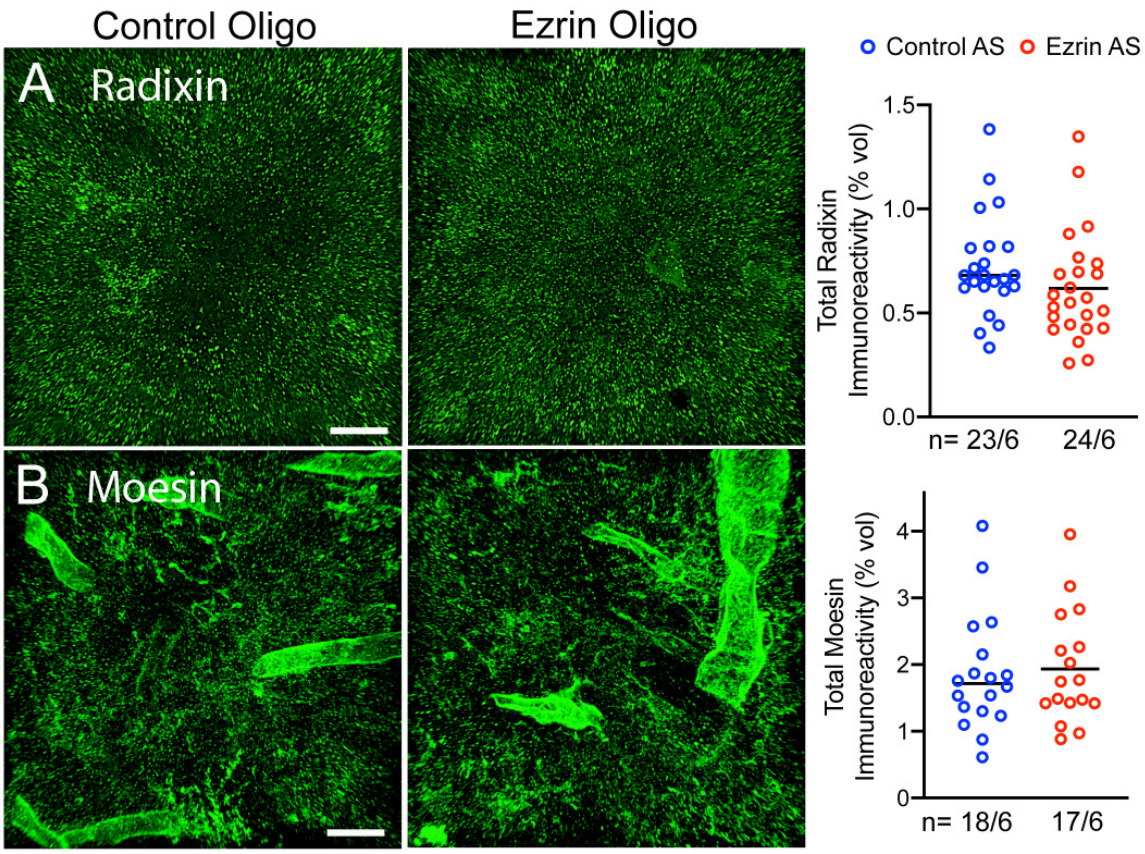
Supplemental Figure S6. Astroglial-specific p-ERM immunoreactivity was quantified in tissue extracted from extinguished rats or after 15-min of cued seeking. Heroin cues elicited a significant elevation in p-ERM levels (yellow) in NAcore astrocytes (red) compared to those observed in extinguished animals (see Figure 3B for quantification). Bar= 10 μm.



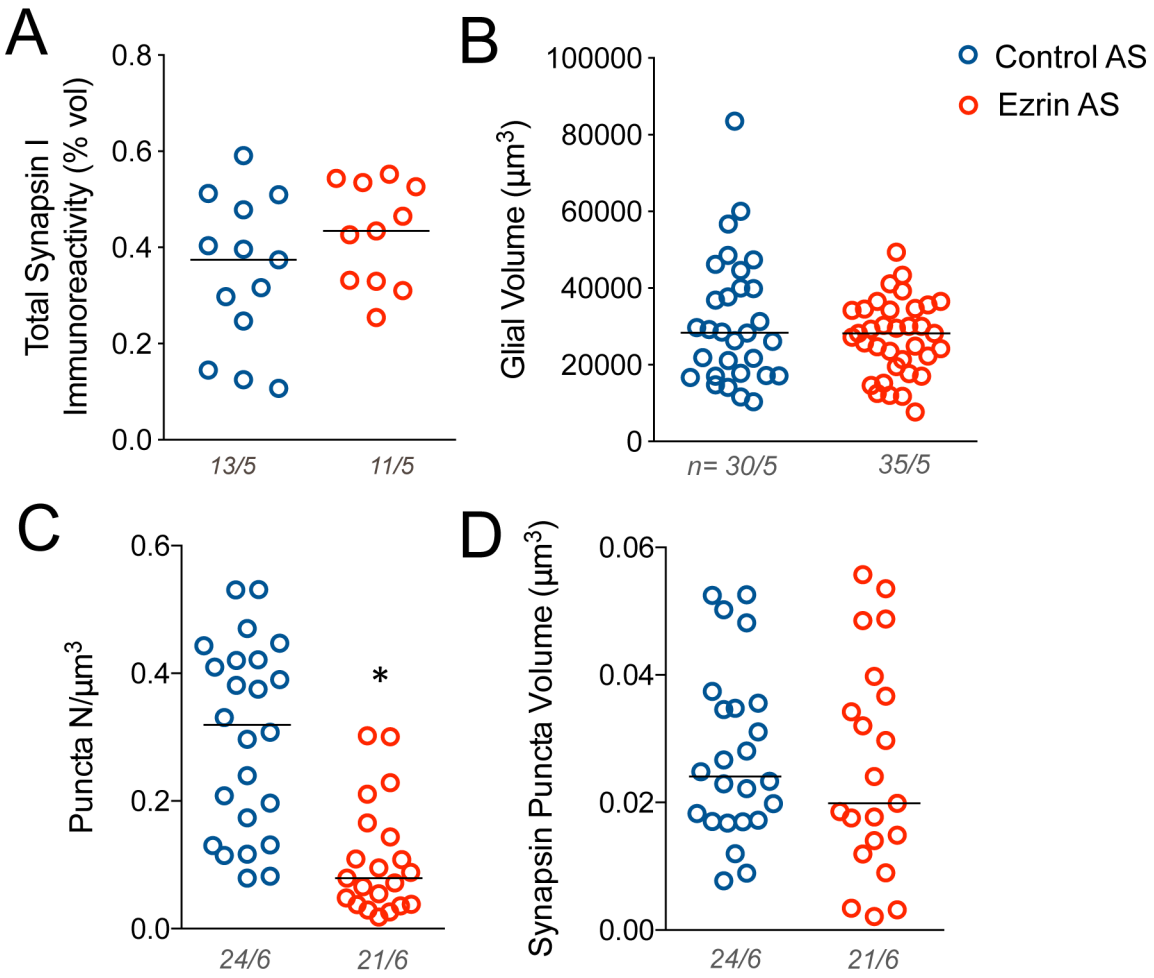
Supplemental Figure S7. No differences in total ezrin expression were found after heroin self-administration or reinstatement (Kruskal-Wallis= 1.824, p= 0.402). N shown as cells/animals, data shown as median.



Supplemental Figure S8. Rats treated with ezrin antisense or control oligo did not differ in total heroin intake during self-administration (**A**, unpaired Student's t-test, $t_{(10)}$= 0.0022, p= 0.998), in their active lever pressing during the final two days of extinction training (**B,** unpaired Student's t-test, $t_{(10)}$= 0.824, p= 0.429), or in inactive lever pressing during 60 min of cued reinstatement (**C**, 2-way ANOVA, $F_{(1, 40)}$= 0.0054, p= 0.9416, effect of treatment).



Supplemental Figure S9. Morpholino knockdown of ezrin had no impact on levels of homologous ERM proteins radixin (**A**, Mann-Whitney t-test, p=0.099) or moesin (**B**, Mann-Whitney t-test, p= 0.807) measured in a cell non-specific manner near the injection tract. Left panel, bars= 15 µm. Right panel, N shown beneath scatter plots as cells/animals, bars= median.



Supplemental Figure S10. Additional morphological measurements of NAcore astrocytes after ezrin knockdown. (**A**) Total Synapsin I immunoreactivity was unaffected in tissue from animals that underwent ezrin knockdown treatment compared to animals that received the control oligo sequence (Mann-Whitney t-test, p= 0.167). (**B**) Astroglial volume was unchanged after ezrin knockdown (Mann-Whitney t-test, p= 0.511). (**C**) Astroglia-Synapsin I co-registration was reduced after ezrin knockdown (see Figure 3E), which corresponded to a decrease in the number of co-registered Synapsin I puncta (Mann-Whitney t-test, *p<0.001). (**D**) Puncta volume was unchanged by ezrin knockdown (Mann-Whitney t-test, p= 0.612). n is shown beneath data as cells/animals, data shown as median.

Supplemental Table S1. None of the morphological measurements for yoked saline animals were normally distributed using a D'Agostino-Pearson omnibus normality test ($p<0.05$, pink). Additionally, some of the morphological measurements for the heroin groups were not normally distributed.

	SALINE		EXTINCTION		REINSTATE 15m		REINSTATE 120m	
	K2	P value	K2	P value	K2	P value	K2	P value
Volume (μm^3)	8.36	0.015	2.01	0.367	0.23	0.892	1.15	0.564
Astroglia-Synapsin I Co-Registration (% vol)	7.18	0.028	38.27	<0.001	6.00	0.049	5.03	0.081
Puncta ($N/\mu m^3$)	27.83	<0.001	1.70	0.427	8.24	0.016	15.01	<0.001
Puncta Volume (μm^3)	85.31	<0.001	31.22	<0.001	30.17	<0.001	2.74	0.254