SUPPLEMENTARY INFORMATION

TITLE: Synaptic depression via mGluR1 positive allosteric modulation suppresses cue-induced cocaine craving

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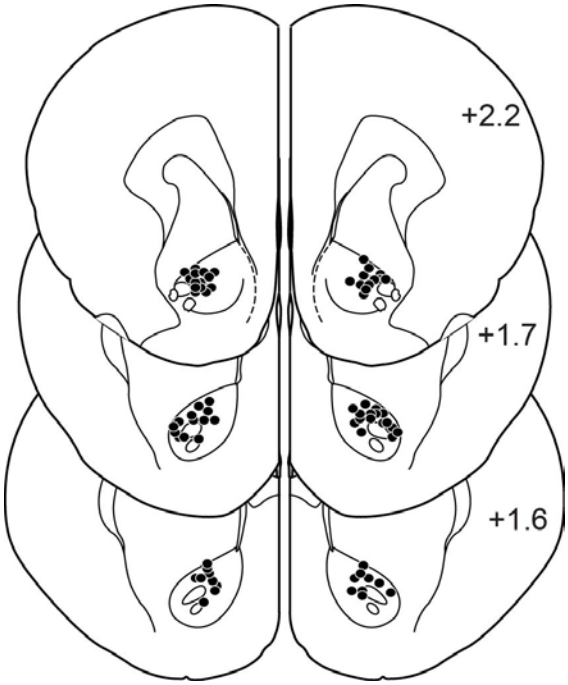
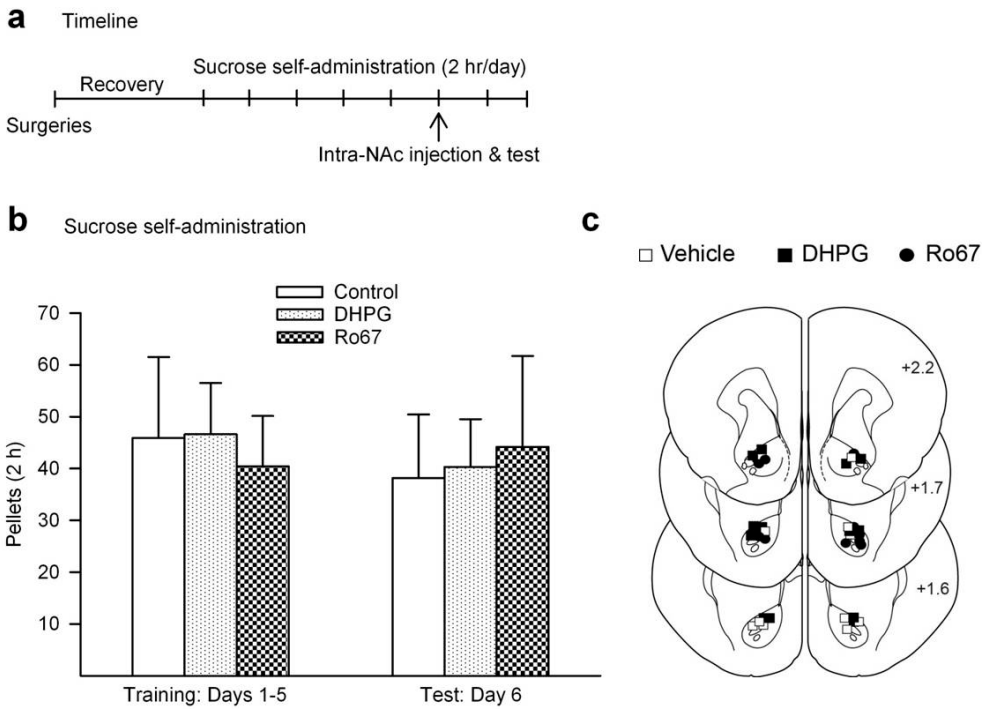
TABLE OF CONTENTS



Supplementary Figure 1. Cannulae placements for animals used to assess the effects of intra-NAc core infusions of DHPG, Ro67-7476 and SYN119 on cue-induced cocaine seeking.
Placements are depicted for animals shown in Fig. 1 of the main text. As described in the Methods, vehicle and drug injections were counterbalanced in these studies, with at least 4 days between intracranial injections. Animals with cannulae placements outside the NAc core were excluded from data analysis. Numbers represent distance anterior to bregma (mm).

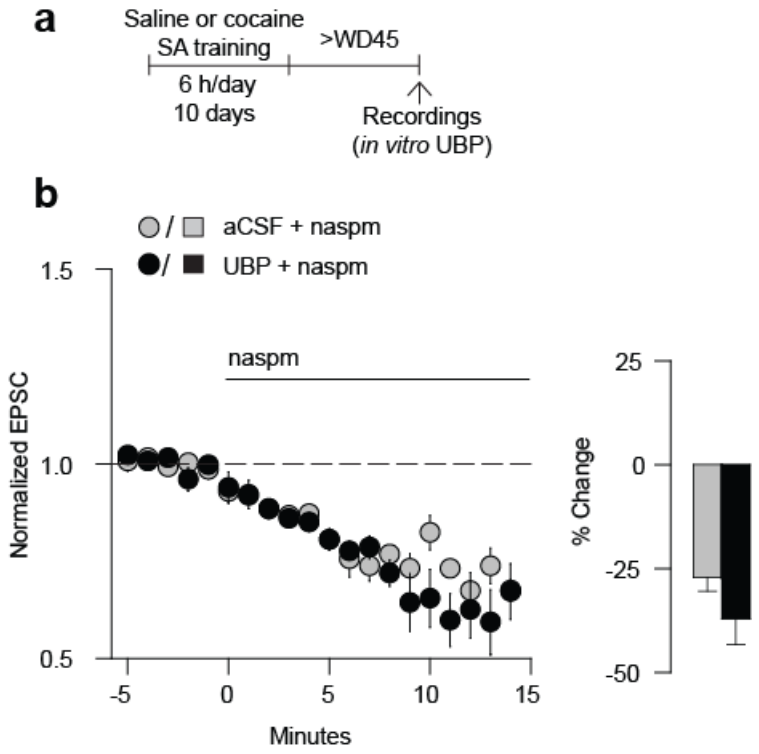


Supplementary Figure 2. Injections of DHPG or Ro67-7476 into the NAc core have no effect on sucrose self-administration. To control for nonspecific effects of DHPG and Ro67-7476 on motivated behavior, the effects of intra-NAc core injections of these compounds on sucrose self-administration were assessed.

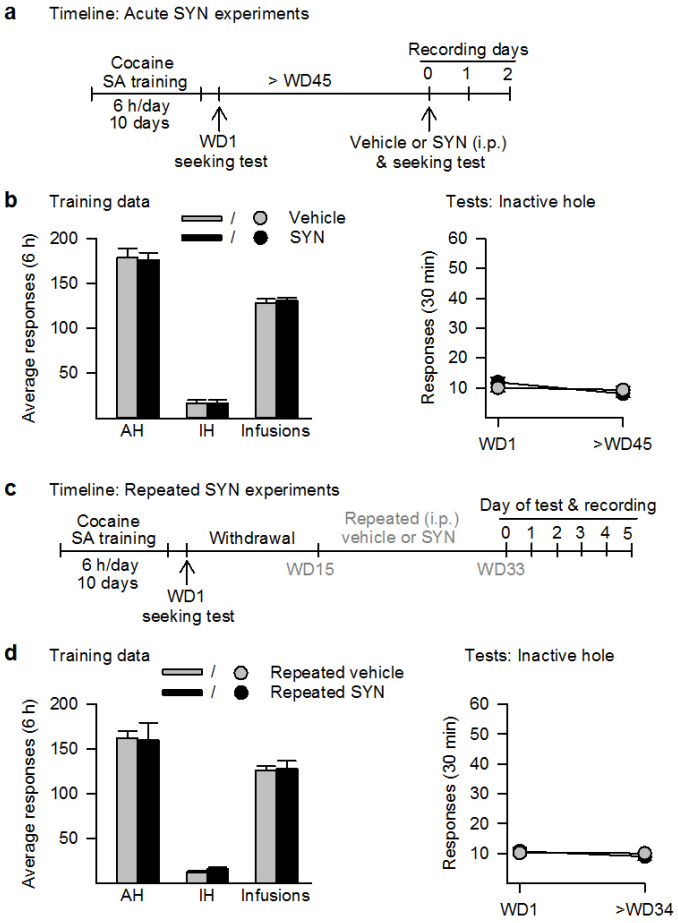
(**a**) As shown in the experimental timeline, drug-naïve rats underwent intracranial surgery to implant guide cannulae in the NAc core and, following 7 days of recovery, began daily sucrose self-administration sessions. During these 2 h sessions, a nose-poke in the active hole resulted in a sucrose pellet being dispensed in the food hopper (fixed ratio 1) and was paired with a cue-light inside the hole and a time-out period (10 sec), while responses in the inactive hole were without consequence. After 5 days of training, rats received intra-NAc core injections of vehicle, DHPG (0.25nmol/0.5µl/side), or Ro67-7476 (0.005nmol/0.5µl/side) 10 min prior to the start of the sucrose self-administration session.

(**b**) No group differences in number of pellets dispensed were observed on the test day ($F_{2,16}$=0.052, p=0.95), and the average number of pellets dispensed on the test day in each group did not differ from the average number of pellets dispensed during the 5 days of training. These data show that intra-NAc core injections of DHPG and Ro67-7476 do not affect motivation to obtain a natural reward and suggest that their effects on cue-induced cocaine seeking are due to a reduction in cocaine craving rather than non-specific alterations in motivated behavior. Rats were also tested for an additional two days following the test injection day to ensure that the injections received on day 6 had no residual effect on sucrose self-administration behavior. No group differences in number of pellets dispensed were observed on either day 7 or 8 (data not shown). Data are shown as average number of pellets dispensed (± s.e.m.) during the 2 h session. Control, n=6 rats; DHPG, n=7 rats; Ro67-7476, n=7 rats.

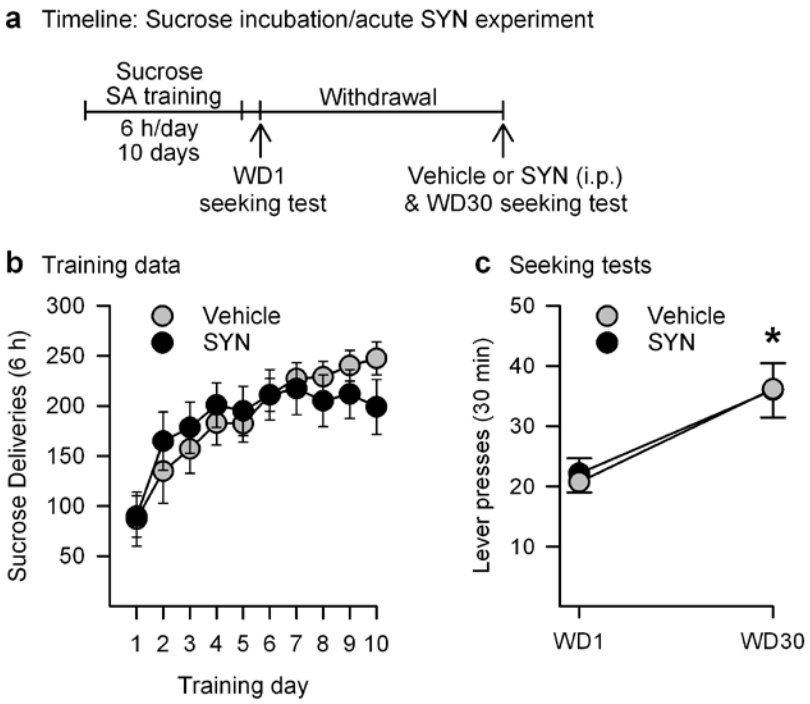
(**c**) Location of cannnulae placements in NAc core for all rats included in this study. Numbers represent distance anterior to bregma (mm).



Supplementary Figure 3. The inhibitory effect of naspm on NAc MSN EPSC$_{-70mV}$ amplitude is not mediated by kainate receptors. The pharmacological action of naspm on glutamatergic synaptic transmission has been known to affect kainate receptor-mediated transmission (e.g., Sun H.Y. et al. (2009), J. Neurophysiology 101,1043-1055). We therefore conducted additional experiments in the presence of the selective kainate receptor antagonist UBP310 (5-10µM) and found that the inhibitory effect of naspm on MSN EPSC$_{-70mV}$ seen in brain slices obtained from "incubated rats" is indeed due to blockade of CP-AMPARs. (**a**) Timeline. SA, self-administration. (**b**) Naspm reduced EPSC$_{-70mV}$ amplitude to the same extent under control conditions (aCSF + naspm; 29.8 ± 3.3% reduction, 8 cells/5 rats; these data are also presented in Fig. 2c) and in the presence of UPB310 (UPB310 + naspm; 37.1 ± 6.2% reduction). n=5 cells/2 rats; t_{11}=1.17, p=0.27, aCSF + naspm vs. UBP310 + naspm during last five minutes of naspm application.



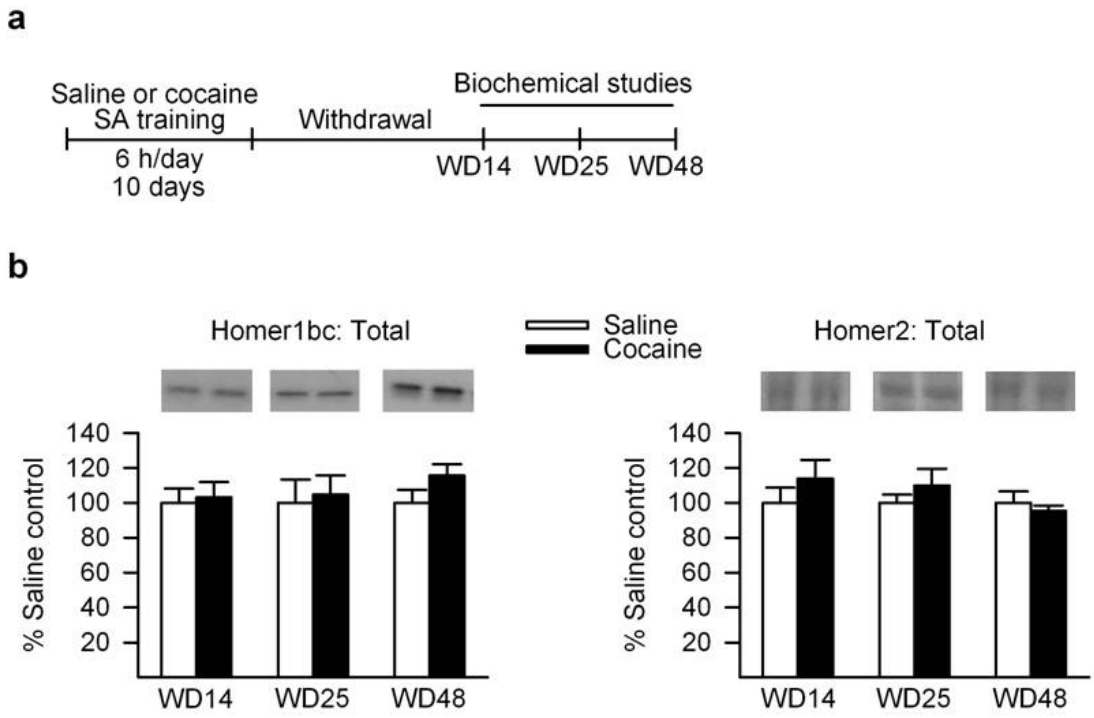
Supplementary Figure 4. Training data and additional test data for animals used to assess the effects of acute or repeated SYN119 treatment on cue-induced cocaine-seeking. Experimental timeline (**a**) and self-administration (SA) training data (**b**, left panel; averaged over days 1-10 ± s.e.m.) for all animals shown in **Fig. 2e** of the main text. During these sessions, nose-poking in the active hole (AH) resulted in a cocaine infusion (0.5 mg/kg/infusion) paired with a cue-light, while nose-poking in the inactive hole (IH) was without consequence. Rats clearly learned to discriminate between the drug-paired AH and the unpaired IH. No differences were observed in average AH or IH responding or in overall drug intake (infusions obtained) between rats destined for acute vehicle or acute SYN treatment groups. Right panel of **b**: Data shown are the mean (± s.e.m.) number of responses in the IH during two tests for cue-induced cocaine seeking. The first test was performed on withdrawal day (WD) 1. The second test was performed on WD45 or greater, 20 min after acute injection of SYN. No group differences were observed on either test day, indicating that acute SYN injection did not affect IH responding. Thus, its effects were specific to AH responding (shown in **Fig. 2e** of the main text). (**c,d**) Experimental timeline (**c**) and SA training data (**d**, left panel) for rats destined for the repeated vehicle or repeated SYN treatment groups shown in **Fig. 5** of the main text (details same as described above for panel **b**). No differences were observed between rats destined for the two treatment groups. Right panel: Data shown are mean (± s.e.m.) number of responses in the IH during two tests for cue-induced cocaine seeking. The first test was performed on WD1. The second test was performed 0-5 days after discontinuing repeated SYN injections. No group differences were observed on either test day, indicating that repeated SYN injections did not affect IH responding. Thus, effects of this treatment are specific to AH responding (shown in **Fig. 5a,b** of the main text).



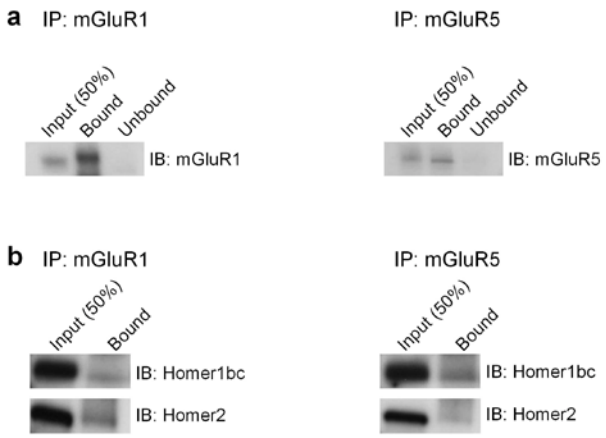
Supplementary Figure 5. Systemic SYN119 does not inhibit the expression of incubation of sucrose seeking.

As shown in the experimental timeline (**a**), rats underwent 10 days of sucrose self-administration (SA) (6 h/day). During these sessions, presses on the active lever resulted in delivery of sucrose solution into a liquid drop receptacle (10% sucrose solution, 0.75 ml per reward delivery) paired with a cue-light and tone presentation (20 sec). As shown in (**b**), no difference in sucrose deliveries during the 10 days of self-administration was observed between rats destined for acute vehicle or SYN119 (SYN) treatment groups. Data shown are the mean (± s.e.m.) number of infusions obtained during each 6 h session. (**c**) Rats in both groups then underwent withdrawal in their home cages and were tested twice for cue-induced sucrose seeking. In these tests (30 min), active lever presses resulted in the presentation of the cue-light and tone, but no sucrose delivery. The first test was on withdrawal day (WD) 1 and the second test was on WD30, 20 minutes after an acute injection of vehicle or SYN (10 mg/kg, i.p., dissolved in 10% Tween-80). Rats in both groups showed a significant increase in cue-induced sucrose seeking (i.e., incubation) from WD1 to WD30, indicating that systemic SYN administration has no effect of incubation of cue-induced sucrose craving. The ANOVA revealed no significant group effect or group X day interaction, but a significant effect of test day ($F_{1,21}$=24.43, p<0.001). Post-hoc least significant difference tests revealed a significant increase in active lever responses on WD30 compared to WD1 in both groups and no difference between groups on either test day. Data shown are the mean number of responses (± s.e.m.) on the active lever during the two tests for cue-induced sucrose seeking. *p<0.05, WD30 versus WD1 (p=0.002, Vehicle WD30 versus WD1; p=0.007, SYN119 WD30 versus WD1); Vehicle, n=11 rats; SYN119, n=12 rats.

Supplementary Figure 6. Control studies for biotinylation experiments. To measure cell surface expression of mGluR1 and mGluR5, biotinylation experiments were performed as described in the **Methods** section in the main text. Briefly, NAc tissue was dissected and incubated with a membrane impermeant biotinylating reagent (1 mM sulfo-NHS-S-S biotin) to selectively modify surface-expressed proteins. Biotinylated proteins bound to NeutrAvidin beads (Bound fraction) were then separated from the non-biotinylated proteins (Unbound fraction) by centrifugation. To assess distribution of group I mGluR dimers in NAc tissue, equal amounts (15 µg) of the starting material (Input), the Bound fraction, and the Unbound fraction were analyzed by SDS-PAGE and immunoblotting for mGluR1 or mGluR5.
(**a**) The majority of mGluR1 or mGluR5 dimer was recovered in the Bound fraction, consistent with evidence that the dimer represents the functional surface-expressed receptor pool (e.g., Jingami et al., 2003). This observation was reproduced over ten times.
(**b**) Additional controls were performed to verify that intracellular proteins such as tyrosine hydroxylase (TH) are not detected in the Bound fraction. This observation was reproduced twice.



Supplementary Figure 7. No changes in total Homer protein levels are observed in the NAc during withdrawal from extended-access cocaine self-administration. (**a**) Timeline. SA, self-administration. (**b**) Homer1bc (left) and Homer2 (right) protein levels were assessed in total NAc tissue at each withdrawal day (WD) time-point (same rats as those used in **Figs. 3 and 4** of main text), and no change in either isoform was observed. Data are shown as percent saline control at each time-point. Note that, as in **Fig. 3**, differences in signal intensity for saline groups on different withdrawal days reflect differences in exposure time for different blots rather than time-dependent changes in protein levels after saline self-administration. Homer1bc, WD14: saline n=10 rats, cocaine n=11 rats. Homer 2, WD14: saline n=10 rats, cocaine n=9 rats. Homer1bc, WD25: saline n=10 rats, cocaine, n=10 rats; Homer2, WD25: saline n=10 rats, cocaine n=9 rats; Homer1bc, WD48 and Homer2, WD48: saline n=9 rats, cocaine n=11 rats.



Supplementary Figure 8. Control studies for co-immunoprecipitation (co-IP) experiments measuring association between Homer proteins and mGluR1 or mGluR5. (**a**) After IP of NAc tissue with an mGluR1 or mGluR5 antibody, immunoblotting with the same antibody verified that virtually all of the respective mGluR dimer present in the starting material (Input) is recovered in the Bound material (IP'ed material purified by Protein A/G resin) and not the Unbound material. For the comparison shown in this panel, we loaded half as much Input as Bound or Unbound material. In experiments not shown, we confirmed that the signal observed in the Bound material is not due to non-specific binding because, after IP with IgG (control condition), mGluR protein is detected in the Unbound material rather than the Bound material. (**b**) To measure changes in the association between Homer proteins and mGluR1 or mGluR5, tissue was IP'ed with mGluR1 or mGluR5 antibodies and the Bound material was probed for Homer1bc or Homer2. As shown in the representative immunoblots, only a portion of Homer protein present in the starting material (Input) is recovered in the Bound material, indicating the existence of a substantial pool of Homer proteins that is not physically associated with group I mGluRs. These findings were replicated over ten times.

Supplementary Figure 9. Supplementary Figure 9. Full-length images of the blots presented in the main figures.

To complete shortly.