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

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Cue-induced cocaine craving is a major cause of relapse in abstinent addicts. In rats, cue-induced craving progressively intensifies (incubates) during withdrawal from extended-access cocaine self-administration. After ~1 month of withdrawal, incubated craving is mediated by Ca^{2+}-permeable AMPA receptors (CP-AMPARs) that accumulate in the nucleus accumbens (NAc). We found that decreased mGluR1 surface expression in the NAc preceded and enabled CP-AMPAR accumulation. Thus, restoring mGluR1 transmission by administering repeated injections of an mGluR1 positive allosteric modulator (PAM) prevented CP-AMPAR accumulation and incubation, whereas blocking mGluR1 transmission at even earlier withdrawal times accelerated CP-AMPAR accumulation. In studies conducted after prolonged withdrawal, when CP-AMPAR levels and cue-induced craving are high, we found that systemic administration of an mGluR1 PAM attenuated the expression of incubated craving by reducing CP-AMPAR transmission in the NAc to control levels. These results suggest a strategy in which recovering addicts could use a systemically active compound to protect against cue-induced relapse.

Relapse, which is often triggered by environmental cues previously associated with cocaine use, is a major problem in treating cocaine addiction. Addicts remain vulnerable to relapse long after the acute withdrawal phase. In a rat model of this phenomenon, cue-induced cocaine craving progressively intensifies (incubates) during the first months of withdrawal from extended-access cocaine self-administration[1]. Incubation models a human scenario in which heavy drug use is interrupted by hospitalization or incarceration[2] and involves neuroadaptations in the circuitry underlying motivation and addiction[1]. The NAc is a brain region that has a central role in this circuitry and is comprised mainly of medium spiny neurons (MSNs). These MSNs mediate motivated behaviors by serving as an interface between cortical and limbic regions and the motor circuitry[3].

AMPAR transmission onto NAc MSNs is critical for drug-seeking in animal models of cocaine addiction[4]. In drug-naive rats and rats with limited cocaine exposure, AMPAR transmission in the NAc is mediated by GluA2-containing Ca^{2+}-impermeable AMPARs (CI-AMPARs)[5]. However, high-conductance CP-AMPARs accumulate in NAc synapses during withdrawal from extended-access cocaine self-administration[6,7]. After elevation of CP-AMPAR transmission has occurred, intra-NAc core injection of the selective CP-AMPAR antagonist naspm markedly reduces cue-induced cocaine-seeking[6]. These results establish that CP-AMPAR transmission in the NAc mediates the expression of incubation after prolonged withdrawal. Subsequently, we showed that the synaptic incorporation of CP-AMPARs enhances the baseline responsiveness of NAc MSNs to glutamate transmission[8]. Together, these findings suggest that, when glutamate is released in the NAc in response to presentation of cocaine-associated cues, MSNs are able to respond more robustly as a result of the presence of CP-AMPARs. This in turn leads to enhanced cocaine-seeking.

The presence of CP-AMPARs in MSNs of the NAc can be detected as an elevated rectification index for evoked AMPAR-mediated excitatory postsynaptic currents (EPSCs)[6–11]. Using this measure, we previously found that CP-AMPAR–mediated transmission becomes elevated after ~1 month of withdrawal and endures through at least withdrawal day 80 (WD80), and perhaps much longer[5]. Thus, once abstinence is achieved, the risk of relapse might be decreased if CP-AMPARs were removed from NAc synapses. Here we investigated whether this could be accomplished by increasing mGluR1 transmission. This was inspired by work in other brain regions showing that mGluR1 produces a postsynaptically expressed form of long-term depression (LTD) that relies on the removal of CP-AMPARs from synapses[12–16]. This form of mGluR-LTD had not been demonstrated in MSNs of drug-naive animals; instead, synaptic depression produced by the group I mGluR agonist DHPG depends on mGluR5 and is expressed presynaptically via CB1R-mediated inhibition of glutamate release[17,18]. However, in NAc slices prepared after incubation and CP-AMPAR accumulation, we found that DHPG-induced synaptic depression in MSN is associated with normalization of the elevated rectification index through an mGluR1 and postsynaptic PKC-dependent mechanism[10]. This indicates a marked shift in group

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I mGluR regulation of NAc synaptic transmission after incubation and points to the potential utility of mGluR1-based strategies for reducing incubated cocaine craving.

Given these findings, we examined the effect of intra-NAc and systemic administration of mGluR1 PAMs on cue-induced craving after withdrawal from the extended-access cocaine self-administration regimen leading to incubation. We also conducted measures of CP-AMPAR transmission during withdrawal and after treatment with mGluR1 PAMs or antagonists, as well as biochemical studies of group I mGluRs and Homer signaling proteins. We focused on the NAc core because this subregion is critical in cocaine-seeking elicited by cues[19] and, more specifically, blockade of CP-AMPARs in the NAc core is sufficient to prevent expression of incubation[6]. We found that mGluR1 surface expression was decreased during withdrawal, just before the onset of increased CP-AMPAR levels, suggesting that mGluR1 normally exerts an inhibitory influence on CP-AMPAR levels, which is lost during incubation. Supporting this hypothesis, we found that enhancing mGluR1 transmission during a critical period of withdrawal, using repeated mGluR1 PAM injections, blocked the emergence of enhanced CP-AMPAR transmission and incubation. Conversely, decreasing mGluR1 tone in early withdrawal accelerated CP-AMPAR accumulation. Notably, stimulating mGluR1 after incubation had occurred was sufficient to reduce CP-AMPAR transmission to control levels and attenuate the expression of incubation. The protective effects of a systemically administered mGluR1 PAM lasted ~1 d after a single injection and ~2–3 d after repeated treatment, suggesting strong translational potential.

RESULTS

mGluR1 activation in NAc core attenuates incubated seeking

All of the cocaine self-administration groups in this study underwent extended-access cocaine self-administration training (6 h d^{-1} for 10 d; **Fig. 1a**). Compared with limited-access cocaine self-administration, extended-access regimens produce different or enhanced behaviors that more closely model the compulsive drug-seeking and drug-taking characteristic of addiction[20]. Furthermore, incubation of cocaine craving is most robust after extended-access regimens, and withdrawal-associated CP-AMPAR accumulation occurs after extended-access, but not limited-access, cocaine self-administration[8].

We first asked whether enhancing NAc mGluR1 function *in vivo* could promote a reduction in CP-AMPAR transmission sufficient to prevent the expression of incubated cocaine craving. Using rats that underwent prolonged withdrawal (>WD45) from the cocaine regimen described above, hereafter referred to as incubated rats, we injected DHPG or vehicle into the NAc core just before a test for cue-induced cocaine-seeking (cannulae placements shown in **Supplementary Fig. 1**). During the seeking test, nose poking in the active hole (previously associated with cocaine and cue delivery) only delivered the cue. Thus, responding under these conditions provides a measure of cue-induced craving. As predicted from DHPG's ability to attenuate CP-AMPAR–mediated transmission *in vitro*[10], incubated rats that received intra-NAc DHPG infusion exhibited significantly reduced cocaine-seeking compared with vehicle-infused rats (t_{25} = 2.35, P = 0.03; **Fig. 1b**). The same effect was produced by intra-NAc infusion of the selective mGluR1 PAM Ro67-7476 (t_{11} = 1.95, P = 0.04; **Fig. 1c**). Neither drug altered inactive hole responding during the seeking test (**Fig. 1b**,**c**) or self-administration of a natural reward (sucrose; **Supplementary Fig. 2**), indicating that their effects on cocaine-seeking were not attributable to nonspecific motor depression or to a generalized decrease in motivation to seek reward.

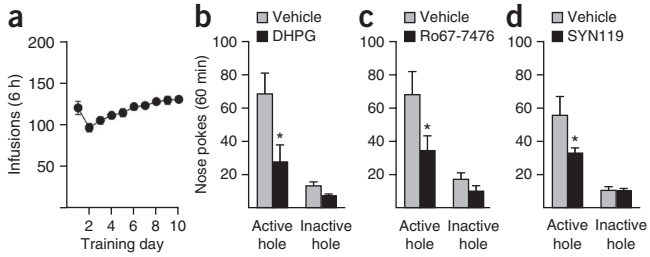

Figure 1 Incubated cocaine-seeking is attenuated by intra-NAc infusion of DHPG or mGluR1 PAMs. (**a**) Training. Nose pokes (mean ± s.e.m.) in the active hole result in cocaine infusion (0.5 mg per kg) paired with a light cue. (**b**–**d**) Seeking tests on >WD45. Shown are nose pokes (mean ± s.e.m.) in the previously active hole (a measure of cocaine-seeking) and inactive hole during a 60-min test performed under extinction conditions (nose pokes deliver cue, but not cocaine). Infusion of DHPG (500 μM; control, n = 16 rats; DHPG, n = 11 rats; **b**), Ro67-7476 (10 μM; control, n = 7 rats; Ro67-7476, n = 6 rats; **c**) or SYN119 (10 μM; control, n = 7 rats; SYN119, n = 9 rats; **d**) into the NAc core 10 min before the test decreased seeking compared with vehicle-infused controls (*P < 0.05). Seeking was also significantly reduced by a lower dose of DHPG (250 μM; 41% reduction, t_{13} = 2.23, P = 0.04 versus vehicle; control, n = 8 rats; DHPG, n = 7 rats; data not shown).

Systemic SYN119 attenuates seeking and CP-AMPAR function

If mGluR1 PAMs are to be useful therapeutics, they must exert anti-craving effects after systemic administration. Thus, we evaluated SYN119, a selective mGluR1 PAM that is behaviorally active after systemic injection[21]. We first confirmed that SYN119 reduced cue-induced cocaine-seeking after infusion into the NAc of incubated rats (t_{14} = 2.17, P = 0.048; **Fig. 1d**). We then conducted MSN recordings in NAc brain slices obtained from incubated rats and found that bath application of SYN119 normalized the elevated rectification index of AMPAR transmission (exposure × treatment interaction: $F_{1,8}$ = 18.04, P = 0.003; **Fig. 2a**) and reduced $EPSC_{-70\ mV}$ amplitude by ~25% (t_5 = 4.67, P = 0.01 versus baseline, n = 6 cells from 5 rats; data not shown). This reduction was a result of the elimination of CP-AMPAR–mediated transmission, as bath application of the CP-AMPAR antagonist naspm produced no further synaptic depression (**Fig. 2b**). In the absence of SYN119, naspm reduced $EPSC_{-70\ mV}$ amplitude by ~30% in MSNs from incubated rats (**Fig. 2b**). Furthermore, naspm-induced attenuation of $EPSC_{-70\ mV}$ amplitude was not altered by the presence of a kainate receptor antagonist (**Supplementary Fig. 3**).

Next, we assessed the effect of systemic SYN119 on cocaine craving by administering a single injection of SYN119 or vehicle (intraperitoneal) to incubated rats just before a test for cue-induced cocaine-seeking. Relative to the vehicle-injected group, cocaine-seeking was significantly blunted by systemic injection of SYN119 (withdrawal day × treatment interaction: $F_{1,32}$ = 6.21, P = 0.02; **Fig. 2c**). As with DHPG and Ro67-7476, this was not a nonspecific effect attributable to depression of motor activity, as there was no group difference in inactive hole responding during the seeking test (**Supplementary Fig. 4**). Furthermore, SYN119's effect was specific to cocaine-seeking, as rats tested after 10 d of sucrose self-administration and 30 d of withdrawal showed 'incubated' cue-induced sucrose seeking that was not reduced by SYN119 injection (**Supplementary Fig. 5**).

To determine whether the reduction in cocaine-seeking after intraperitoneal SYN119 injection was attributable to reduced CP-AMPAR–mediated transmission, we performed recordings in slices prepared from a subset of SYN119- and vehicle-treated rats from the cocaine experiment (**Fig. 2d**). As expected, incubated rats injected with vehicle before the seeking test exhibited an elevated rectification

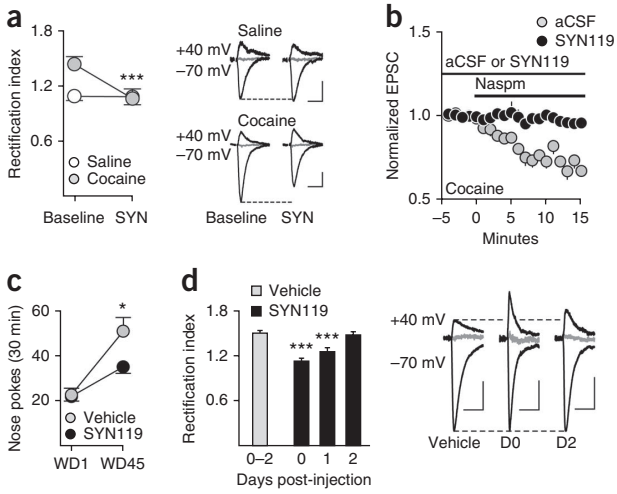

Figure 2 A single systemic injection of the mGluR1 PAM SYN119 reduces cocaine-seeking and CP-AMPAR function. (**a**) Left, following prolonged withdrawal from cocaine self-administration (>WD45), MSNs exhibited an elevated rectification index (mean ± s.e.m.) that was normalized by bath-applied SYN119 (SYN, 1 μM, 15 min). ***$P < 0.001$ versus baseline; control, $n = 4$ cells from 4 rats; SYN119, $n = 6$ cells from 5 rats. Right, example traces illustrate the effects of SYN119 in MSNs from controls and incubated rats (calibration bar = 50 pA, 40 ms). The reversal potentials for both examples are +10 mV. Overall, the reversal potentials did not differ significantly between saline and cocaine groups ($P = 0.10$). (**b**) Naspm (100 μM) significantly reduced the rectification index in the absence of SYN119 ($t_7 = 9.15$, $P < 0.001$; baseline versus last 5 min of naspm application), but this was not observed in the presence of SYN119, indicating that SYN119's ability to normalize the rectification index was a result of the elimination of CP-AMPAR transmission (control, $n = 8$ cells from 5 rats; SYN119, $n = 5$ cells from 4 rats). aCSF, artificial cerebrospinal fluid. (**c**) Nose pokes (mean ± s.e.m.) in the previously active hole during seeking tests on WD1 and >WD45. Injection of SYN119 (10 mg per kg, intraperitoneal) 20 min before the second test significantly reduced seeking compared with vehicle-injected controls (*$P = 0.048$; control, $n = 13$ rats; SYN119, $n = 21$ rats), although this reduction was partial, as WD1 and WD45 differed significantly in the SYN119 group (*$P = 0.02$). (**d**) Left, recordings from a subset of these rats revealed that SYN119 normalized the rectification index (mean ± s.e.m.) for ~24 h. These recordings were performed immediately after the test (day 0), 1 d later (day 1) or 2 d later (day 2). Right, representative traces of $EPSC_{-70\ mV}$, $EPSC_{+40\ mV}$ and the reversal potential (in gray, 0 mV) for cells from vehicle, SYN119 day 0 (D0) and SYN119 day 2 (D2) groups (calibration bars = 50 pA, 40 ms). Overall, the reversal potentials did not differ significantly between vehicle- and SYN119-treated groups ($P = 0.16$). ***$P < 0.001$ versus vehicle group; vehicle, $n = 11$ cells from 3 rats; SYN119 D0, $n = 12$ cells from 3 rats; SYN119 D1, $n = 12$ cells from 3 rats; SYN119 D2, $n = 4$ cells from 2 rats.

index regardless of whether recordings were performed immediately after the test (day 0), 1 d later (day 1) or 2 d later (day 2). In contrast, SYN119-injected rats recorded the day of the seeking test (day 0) exhibited a normalized rectification index (**Fig. 2d**) resembling that observed in saline-treated rats (**Fig. 2a**). The partial nature of SYN119's effect on seeking on day 0 (**Fig. 2c**), in contrast with the complete reversal of the elevated rectification index, could indicate that NAc synapses are strengthened not only by postsynaptic CP-AMPAR accumulation, but also by presynaptic adaptations[22] that would not be affected by SYN119. The rectification index measured in SYN119-injected rats remained significantly attenuated on day 1, but returned to the elevated level characteristic of incubation by day 2 (group effect: $F_{3,35} = 15.44$, $P < 0.001$; **Fig. 2d**). Thus, a single systemic injection of SYN119 inhibited CP-AMPAR–mediated transmission in NAc synapses for ~24 h.

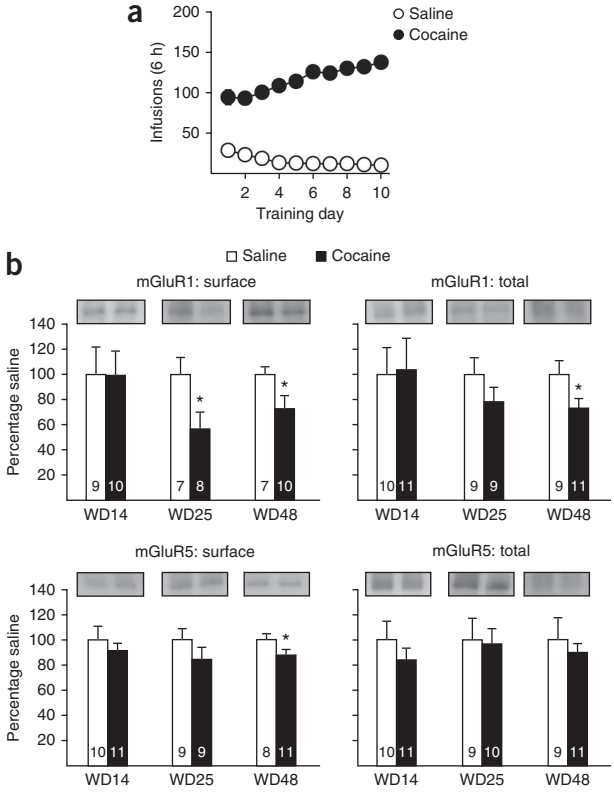

Figure 3 A withdrawal-dependent decrease in NAc mGluR1 surface expression just precedes CP-AMPAR accumulation. (**a**) Training data for saline and cocaine groups (training was as described in **Fig. 1a**). (**b**) Cell surface and total protein levels of mGluR1 and mGluR5 were measured on three withdrawal days: before (WD14), at the onset (WD25) and after (WD48) elevation of CP-AMPAR levels. Data are expressed as percent of saline group (±s.e.m.) at each time point (n values are provided in each bar). $P = 0.04$ (surface mGluR1, WD25), $P = 0.03$ (surface mGluR1, WD48; surface mGluR5, WD48) and $P = 0.03$ (total mGluR1, WD48; $t_{18} = 2.0$) versus respective saline groups (*$P < 0.05$). Differences in optical density 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. Full-length blots are presented in **Supplementary Figure 9**.

Decreased mGluR1 precedes CP-AMPAR accumulation

Given that mGluR1 activation inhibited CP-AMPAR function and thereby reduced craving (**Figs. 1** and **2**), we hypothesized that mGluR1 normally exerts a braking effect on CP-AMPAR accumulation in the NAc. This would explain why CP-AMPARs account for only 5–10% of excitatory transmission in NAc MSNs from adult, drug-naive rats[6,23]. A similar negative regulatory role for basal mGluR1 transmission has been described in other regions[7,15,24]. We further hypothesized that, during withdrawal from extended-access cocaine self-administration, a decrease in NAc mGluR1 transmission may occur that removes this brake, permitting CP-AMPARs to accumulate. One way that this could occur is through a decrease in mGluR1 surface expression. To test this possibility, we used biotinylation to measure mGluR1 surface and total protein levels during withdrawal from extended-access cocaine or saline self-administration. Given the time course of CP-AMPAR accumulation during withdrawal[5], we selected three time points for study: WD14 (before any change in AMPAR subunit

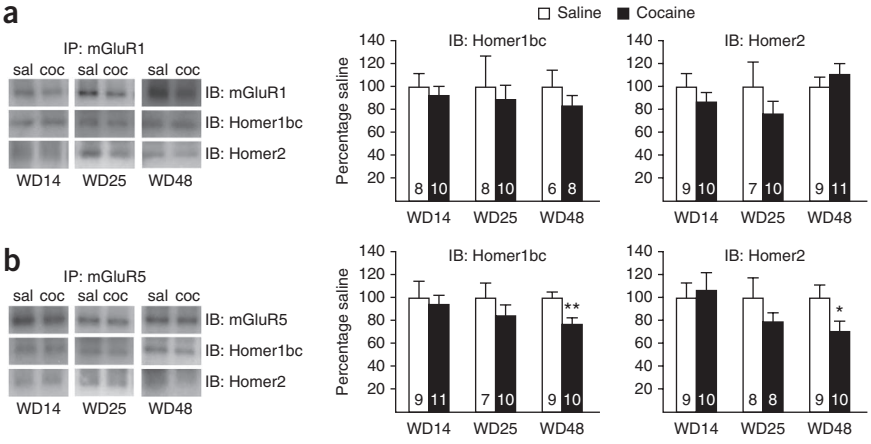

Figure 4 Prolonged withdrawal from extended-access cocaine self-administration does not alter mGluR1-Homer associations, but does decrease mGluR5-Homer associations in the NAc. (**a**) Co-immunoprecipitation experiments assessing the physical associations between mGluR1 and Homer proteins on WD14, WD25 and WD48 from extended-access cocaine (coc) or saline (sal) self-administration. No changes in association between mGluR1 and Homer proteins were observed at any of the three withdrawal time points. IB, immunoblot; IP, immunoprecipitation. (**b**) In the case of mGluR5, no change in association with Homer proteins was observed at the two earlier withdrawal time points (WD14 and WD25). However, a significant decrease in association between mGluR5 and both Homer isoforms was found on WD48 in rats that previously self-administered cocaine. Data are expressed as percent of saline group (±s.e.m.) at each time point (*n* values are provided in each bar). $^{**}P = 0.01$ (Homer1bc) and $^{*}P = 0.04$ (Homer2) versus respective saline groups. Full-length blots are presented in **Supplementary Figure 9**.

composition), WD25 (when the rectification index is just beginning to increase) and WD48 (when CP-AMPARs are stably expressed). Although the saline and cocaine groups did not differ on WD14, the cocaine group exhibited decreased mGluR1 surface expression on WD25 ($t_{13} = 2.24$, $P = 0.04$) and WD48 ($t_{15} = 1.99$, $P = 0.03$) (**Fig. 3** and **Supplementary Fig. 6**), suggesting that persistently decreased mGluR1 surface expression during cocaine withdrawal may contribute to CP-AMPAR accumulation. We speculate that the remaining mGluR1 receptors, although insufficient to maintain normal inhibitory tone on CP-AMPAR accumulation, can be stimulated pharmacologically when mGluR1-activating drugs (such as SYN119) are applied to a brain slice (**Fig. 2**) or injected directly into the NAc of an incubated rat (**Fig. 1**).

We next examined whether alterations in Homer protein expression or function might further contribute to depressed mGluR1 transmission during withdrawal. The Homer postsynaptic scaffolding proteins are critical for group I mGluR signaling, regulating their association with signaling pathways[25]. Homer proteins are the product of three genes (*Homer1*, *Homer2* and *Homer3*) that encode several transcriptional variants. Most are long forms that are constitutively expressed (Homer1b–d, Homer2ab, Homer3), but two are truncated forms (Homer1a, ania-3) that are induced by synaptic activity. In general, the association of long Homers with group I mGluRs favors receptor coupling to IP_3 receptor–dependent pathways, whereas short Homers disable this coupling, favoring regulation of G protein–dependent ion channels[26–29]. In the NAc, mGluR1 appears to elicit CP-AMPAR removal through the canonical pathway linked to PLC activation, IP_3 formation, Ca^{2+} release and PKC activation[10]. Thus, impaired signaling through the long Homers that promote this pathway could contribute, along with decreased mGluR1 surface expression, to reduced group I mGluR signaling during incubation. Indeed, there is evidence for decreased long Homer protein levels in the NAc after cocaine exposure[25], including decreases in Homer1bc and Homer 2 expression after extended-access cocaine self-administration[30]. However, when we assessed Homer1bc and Homer 2 expression during cocaine withdrawal (WD14, WD25 and WD48), we found no changes in total protein levels of these Homers (**Supplementary Fig. 7**) or their physical association with mGluR1, as determined by co-immunoprecipitation (**Fig. 4a** and **Supplementary Fig. 8**). Together, these results suggest that CP-AMPAR accumulation is likely a result of the observed decreases in mGluR1 surface expression rather than deficits in Homer signaling.

In light of our previous finding that mGluR5-mediated synaptic depression is disabled in the NAc of incubated rats[10], it was also of interest

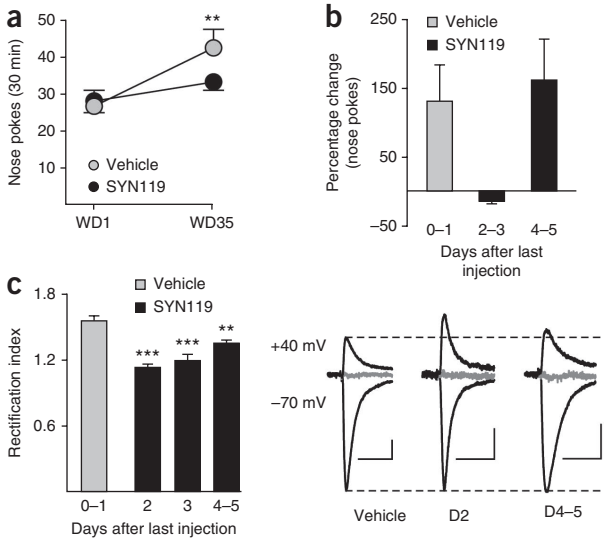

Figure 5 Repeated SYN119 injections during withdrawal interfere with CP-AMPAR accumulation and block incubation of cocaine craving. (**a**) Rats received vehicle or SYN119 injections every other day from WD15 through WD33. Repeated SYN119 injections blocked incubation of cocaine craving. Data are presented as the average number of nose pokes (±s.e.m.) during seeking tests on WD1 and WD35 ($^{**}P = 0.005$ versus WD1; $n = 13$ rats per group). (**b**,**c**) To determine the duration of SYN119-mediated attenuation of incubation and its mechanism, seeking tests and patch-clamp recordings were conducted at variable times after the last vehicle or SYN119 injection. SYN119-mediated reduction of cocaine-seeking (data expressed as percent change from WD1 test, mean ± s.e.m.; **b**) and the rectification index (mean ± s.e.m., **c**, left) persisted for 2–3 d. Representative traces are shown (**c**, right) of $EPSC_{-70\ mV}$, $EPSC_{+40\ mV}$ and the reversal potential (in gray, +5 mV) for cells from repeated vehicle, SYN119 day 2 (D2) and SYN119 day 4–5 (D4–5) groups (calibration bars = 50 pA, 40 ms). Overall, the reversal potentials did not differ significantly between vehicle- and SYN-treated groups ($P = 0.49$). $^{**}P = 0.002$, $^{***}P < 0.001$ versus vehicle controls; repeated vehicle, $n = 6$ cells from 2 rats; SYN119 D2, $n = 4$ cells from 2 rats; SYN119 D3, $n = 4$ cells from 2 rats; SYN119 D4–5, $n = 6$ cells from 3 rats.

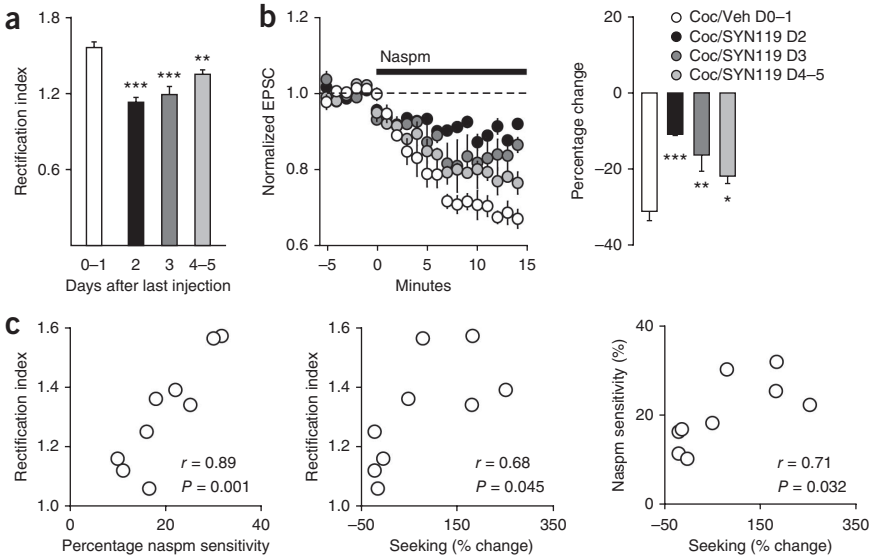

Figure 6 Correlations between effects of SYN119 on rectification index, naspm sensitivity and cue-induced cocaine-seeking. (**a**) Analysis of changes in rectification index (from **Fig. 5**, mean ± s.e.m.) following repeated SYN119 treatment. (**b**) To further assess the contribution of CP-AMPARs to the changes in rectification index shown in **a**, the CP-AMPAR antagonist naspm (100 μM) was bath-applied for 15 min and EPSC amplitude (mean ± s.e.m.) was measured at a holding potential of –70 mV ($EPSC_{-70\ mV}$, data are normalized to pre-naspm baseline; left). Right, as expected, naspm decreased $EPSC_{-70\ mV}$ by ~30% in cocaine rats that received repeated vehicle injections. In contrast, naspm sensitivity (mean ± s.e.m.) was significantly reduced in rats recorded 2–3 d (D2 or D3) after the last SYN119 injection, a time at which the rectification index was also reduced (**a** and **Fig. 5c**) and cue-induced cocaine-seeking was below incubated levels (**Fig. 5b**). By 4–5 d (D4 or D5) after the last SYN119 injection, the rectification index and naspm sensitivity had returned to levels that were more similar to those observed in cocaine/vehicle (Coc/Veh) rats (**a**,**b**), as had cue-induced cocaine-seeking (**Fig. 5b**). ***$P < 0.001$, **$P = 0.003$, *$P = 0.04$ versus Coc/Veh; Coc/Veh, $n = 5$ cells from 2 rats; SYN119 D2, $n = 4$ cells from 2 rats; SYN119 D3, $n = 4$ cells from 2 rats; SYN119 D4–5, $n = 6$ cells from 3 rats. (**c**) Analyses of correlations between cocaine-seeking (measured as percent change from active hole responses on WD1), rectification index and naspm sensitivity for all rats shown in **a**,**b** and **Figure 5b**,**c**. Left, as expected, naspm sensitivity was highly correlated with rectification index values, indicating that an elevated rectification index is a result of enhanced CP-AMPAR–mediated transmission. Right and middle, in addition, cocaine-seeking correlated with both rectification index and naspm sensitivity.

to assess mGluR5 levels and Homer coupling. We observed a small, but significant, decrease in surface mGluR5 ($t_{17} = 1.96$, $P = 0.03$; **Fig. 3b**) and a significant decrease in the association between mGluR5 and both Homer1bc ($t_{17} = 2.75$, $P = 0.01$) and Homer2 ($t_{17} = 1.91$, $P = 0.04$) on WD48 (**Fig. 4b**). We note that interpretation of the latter result is complex because uncoupling of Homers and group I mGluRs can both enhance and reduce signaling[31].

The level of mGluR1 transmission regulates incubation

Given that surface expression of mGluR1 decreased at approximately the same withdrawal time as CP-AMPARs began to accumulate (**Fig. 3**), we wondered whether potentiating mGluR1 transmission during this critical period would block CP-AMPAR accumulation and inhibit incubation of cocaine craving. Thus, after completion of cocaine self-administration training, rats were randomly assigned to receive vehicle or SYN119 injections every other day from WD15 through WD33. These rats were tested for cue-induced cocaine-seeking on WD1, before vehicle or SYN119 exposure, and again on WD35, 2 d after the last injection (**Fig. 5a**). Vehicle-treated rats exhibited increased seeking on WD35 compared with WD1, that is, incubation occurred as expected (main effect of test day: $F_{1,24} = 14.84$, $P < 0.001$). In contrast, rats treated with SYN119 during withdrawal did not exhibit incubation of cocaine-seeking between WD1 and WD35, indicating that enhancing mGluR1 transmission during cocaine withdrawal is sufficient to prevent incubation (**Fig. 5a**).

To determine whether repeated SYN119 treatment during withdrawal prevents incubation by opposing CP-AMPAR accumulation,

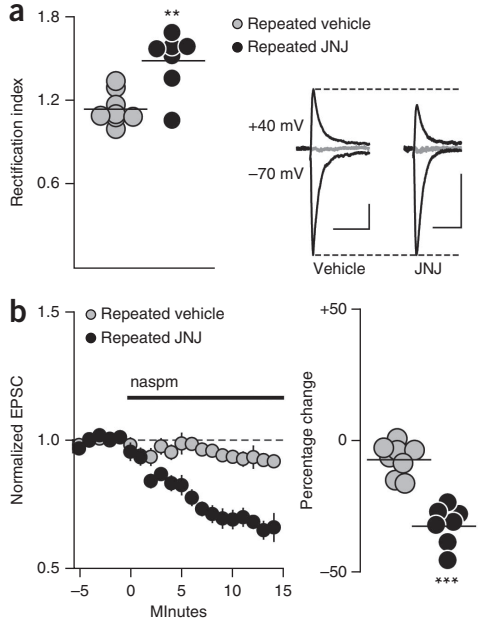

Figure 7 The onset of CP-AMPAR accumulation in the NAc is accelerated by decreasing mGluR1 tone during early withdrawal. (**a**) Rats self-administered cocaine and then received vehicle or JNJ16259685 (JNJ) injections daily on WD10–15 (2 rats) or WD11–16 (1 rat). Left, in rats treated with vehicle during withdrawal, the rectification index on WD16–17 was similar to saline controls, whereas it was elevated after repeated JNJ injections. Each circle represents one cell. Lines are means for each group. Right, representative traces of $EPSC_{-70\ mV}$, $EPSC_{+40\ mV}$ and the reversal potential (in gray, 0 mV) for cells from cocaine/vehicle (vehicle) and cocaine/JNJ (JNJ) groups (calibration bars = 50 pA, 40 ms). Overall, the reversal potentials did not differ significantly between the groups ($P = 0.18$). **$P = 0.002$ versus vehicle. (**b**) Repeated JNJ injections also increased naspm sensitivity on WD16–17 compared with the cocaine/vehicle group. Data are shown as group average $EPSC_{-70\ mV}$ amplitude (±s.e.m.) before (5 min) and during (15 min) naspm application (left) and as the average percentage change from baseline during the last 5 min of naspm application (right; each circle represents one cell; lines are means for each group) ***$P < 0.001$ versus vehicle; vehicle, $n = 8$ cells from 3 rats; JNJ, $n = 7$ cells from 3 rats.

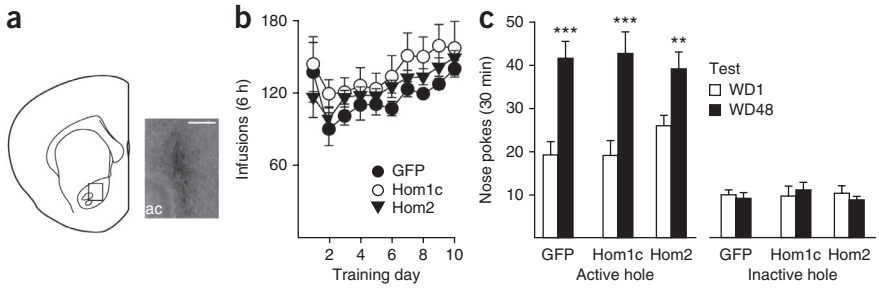

Figure 8 Homer overexpression in the NAc core does not affect incubation of cue-induced cocaine-seeking. (**a**) Staining for the hemagglutinin tag on cDNA-Homer1c verified localized overexpression in the NAc core (20× magnification, image taken ~3 months after virus injection; ac, anterior commissure). Scale bar represents 100 µm. (**b**) Following ~1 week of recovery from intra-NAc AAV injection, rats self-administered cocaine (0.5 mg per kg) for 6 h d^{-1} for 10 d. No difference in cocaine intake (average infusions obtained each day ±s.e.m.) was observed between the three groups. (**c**) All rats showed an increase in cue-induced cocaine-seeking (nose pokes, mean ± s.e.m.) on WD48 compared with WD1 (that is, incubation), regardless of which virus infusion they received. ***$P < 0.001$, **$P = 0.01$, WD1 versus WD48 (ANOVA followed by least significant difference *post hoc* comparisons); GFP, $n = 10$ rats; Homer1c, $n = 8$ rats; Homer2, $n = 10$ rats.

we subjected rats to the same regimen of repeated SYN119 or vehicle treatment (every other day, WD15–33) and then tested them for cue-induced cocaine-seeking at variable times after the last injection (0–5 d) (**Fig. 5b**). Immediately after each test, slices were prepared for whole-cell patch-clamp recording. After repeated vehicle treatment during withdrawal, rats displayed incubated cocaine-seeking compared with WD1 (**Fig. 5b**) and an elevated rectification index (**Figs. 5c** and **6a**), indicating CP-AMPAR accumulation. Notably, repeated SYN119 treatment on WD15–33 blocked incubation and reduced the rectification index when these endpoints were assessed 2–3 d after the last SYN119 injection (group effect: $F_{3,16} = 25.09$, $P < 0.001$; **Figs. 5b**,**c** and **6a**). This attenuated rectification index was a result of reduced CP-AMPAR–mediated transmission, as naspm sensitivity was significantly reduced in these rats compared with incubated rats treated with vehicle (group effect: $F_{3,15} = 11.17$, $P < 0.001$; **Fig. 6b**). Significant correlations (all P values < 0.05) were also observed between levels of cocaine-seeking, naspm sensitivity and CP-AMPAR transmission (**Fig. 6c**). However, 4–5 d after discontinuing repeated SYN119 injections, both seeking and CP-AMPAR levels recovered toward incubated levels (**Figs. 5b**,**c** and **6a**,**b**). Thus, potentiation of mGluR1 transmission during withdrawal, via repeated systemic injections of an mGluR1 PAM, reduced cocaine craving by blocking CP-AMPAR accumulation in the NAc, an effect that lasted for ~2–3 d.

If mGluR1 normally exerts inhibitory tone on CP-AMPAR accumulation, then decreasing mGluR1 transmission very early in withdrawal should accelerate CP-AMPAR accumulation. To test this, we injected rats that had self-administered cocaine with an mGluR1 antagonist (JNJ16259685, 5 mg per kg, intraperitoneal) or vehicle daily on WD10–15 (2 rats) or WD11–16 (1 rat) and carried out patch-clamp recordings 1 d after the last injection (WD16 or WD17). Normally, this is too early in withdrawal for detection of increased CP-AMPAR levels[5]. However, after repeated JNJ16259685 injections, recordings on WD16–17 revealed elevation of both the rectification index ($F_{1,13} = 15.20$, $P = 0.002$) and naspm sensitivity ($F_{1,13} = 52.12$, $P < 0.001$) (that is, a profile identical to that of incubated rats recorded after >WD45; **Fig. 7**).

Prior studies in animal models of cocaine and alcohol addiction have shown that genetically altering long Homer expression in the NAc can mimic or disrupt drug-induced alterations in group I mGluR–mediated signaling pathways and modify behavioral responding to and/or intake of the drug[25,32,33]. Given that long Homer isoforms can potentiate certain group I mGluR–mediated signaling pathways (see above), we assessed whether we could mimic the effect of repeated mGluR1 PAM treatment (**Fig. 5**) by overexpressing long Homers in the NAc. We used previously characterized adeno-associated viruses (AAVs) to overexpress Homer1c, Homer2 or GFP (control) (**Fig. 8a**). We found that rats in all three groups demonstrated equivalent cocaine self-administration (**Fig. 8b**) and incubation of cocaine craving (main effect of test day, $F_{1,25} = 47.47$, $P < 0.001$; **Fig. 8c**). These results indicate that Homer overexpression does not mimic the effects of repeated mGluR1 potentiation during withdrawal and that enhancing mGluR1 transmission using a PAM is a more promising therapeutic strategy.

DISCUSSION

We found that mGluR1 receptors in the NAc negatively regulated CP-AMPAR levels and thereby reduced cue-induced cocaine craving. First, we showed that intra-NAc infusions of the group I agonist DHPG or an mGluR1 PAM (Ro67-7476 or SYN119) attenuated the expression of incubated cue-induced cocaine-seeking. Next, in behavioral studies followed by patch-clamp recordings, we found that acute systemic administration of SYN119 reduced the expression of incubated cocaine-seeking and removed CP-AMPAR transmission from NAc synapses. This protective effect persisted for ~1 d. Control experiments revealed that the same SYN119 treatment that reduced incubation of cocaine-seeking had no effect on incubation of sucrose seeking. This is consistent with the findings of a prior study[34], indicating selectivity of GluA1-related adaptations for cocaine versus sucrose withdrawal. On the basis of these findings, we hypothesized that mGluR1 normally exerts inhibitory tone on CP-AMPAR levels in NAc synapses and that a loss of mGluR1 tone during cocaine withdrawal enables CP-AMPAR accumulation. Indeed, we found that NAc mGluR1 surface expression was normal on WD14, but was decreased by WD25 (that is, just preceding CP-AMPAR accumulation), although associations with Homer proteins were apparently not altered. Restoring mGluR1 tone during this period by administering repeated, intermittent injections of SYN119 blocked CP-AMPAR accumulation and the incubation of cue-induced cocaine-seeking, effects that persisted 2–3 d after the last PAM injection. Conversely, pharmacological reduction of mGluR1 transmission during an early withdrawal period before the decrease in mGluR1 surface expression accelerated CP-AMPAR accumulation. Together, these results suggest that a recovering addict could take an mGluR1 PAM before an anticipated encounter with cocaine-associated cues and be afforded a reasonable period of protection. Although a more persistent effect would be desirable, it is revealing that both CP-AMPARs and craving recover to incubated levels within days of discontinuing PAM exposure. This indicates that mGluR1 tone dictates whether CP-AMPARs and incubated craving can emerge, but an as yet unknown underlying mechanism must drive both phenomena. Although suppressed during repeated mGluR1 PAM exposure, this mechanism re-engages within days of discontinuing SYN119 treatment.

In the same rats that we used for analysis of mGluR1 expression during withdrawal, mGluR5 surface expression was unchanged

on WD14 and WD25; however, a modest decrease was observed on WD48, along with decreased association between mGluR5 and long Homer proteins, as measured by co-immunoprecipitation. These results provide a possible explanation for the impairment of mGluR5-dependent synaptic depression that we observed in the NAc of incubated rats at these late withdrawal times[10]. There are other reports of attenuated mGluR-LTD in the NAc after cocaine exposure[35–38], but those studies used different cocaine regimens and withdrawal times; thus, our biotinylation and co-immunoprecipitation data are not necessarily applicable.

A large number of prior studies have suggested that anti-addictive effects in animal models can be achieved by blocking group I mGluRs (primarily mGluR5)[39] rather than by enhancing mGluR1 transmission, as we observed here. However, none of these prior studies were conducted in rats that had undergone extended-access cocaine self-administration and prolonged withdrawal. Both conditions must be met for CP-AMPARs to accumulate in the NAc of adult rats. Thus, CP-AMPAR accumulation is not observed, regardless of withdrawal time, after experimenter-administered cocaine or limited-access cocaine self-administration[8,11], or even after short withdrawals from extended-access cocaine self-administration[5,6]. In the absence of CP-AMPARs, no anti-addictive effects of mGluR1 PAMs would be anticipated. Instead, mGluR1 blockade may be beneficial (for example, see ref. 40) by tapping into the same mechanisms achieved with mGluR5 blockade. In young animals, CP-AMPAR plasticity appears to be induced more readily[7]. However, based on our results in adult rodents, mGluR1 PAM–based therapies would selectively benefit addicts who have achieved abstinence and seek to sustain it, whereas different approaches would be preferred in other situations, such as when an addict is still using or just attempting to abstain[39,41]. We recognize liabilities and mixed clinical results obtained with previous glutamate-based addiction treatments[41], but mGluR1 PAMs may represent an improvement as a result of their selectivity for a single target (vis à vis nonselective ion channel blockers such as gabapentin) and duration of effect (for example, acamprosate must be taken three times per day).

Although we focused on the effects of mGluR1 PAMs mediated in the NAc, their effects in the ventral tegmental area (VTA) would also be expected to benefit cocaine users. After many types of cocaine exposure (even a single injection), CP-AMPARs are inserted into synapses onto VTA dopamine neurons in exchange for lower conductance CI-AMPARs, resulting in synaptic potentiation[13,14,42]. This is reversed by acute mGluR1 stimulation; that is, CP-AMPAR transmission is decreased and CI-AMPAR transmission is enhanced, resulting in LTD[7,12–14]. Furthermore, the level of mGluR1 tone in the VTA determines the duration of CP-AMPAR elevation in the VTA; for example, decreasing mGluR1 tone permits CP-AMPARs to persist for a longer time[7]. Notably, mGluR1 receptors in the VTA also regulate CP-AMPAR plasticity downstream in the NAc. Thus, although a single cocaine injection is not sufficient to increase CP-AMPAR levels in the NAc shell of young mice, it becomes sufficient after interruption of coupling between VTA group I mGluRs and Homer1bc[7]. Combined with our findings, these results predict that mGluR1 PAMs would oppose addiction-promoting plasticity in the VTA and NAc of human cocaine users. They would also have potential benefits with regard to cognitive deficits associated with cocaine use[43].

It is intriguing that, when CP-AMPARs are present, mGluR1 receptors exhibit a unique ability to remove them from synapses. This is true not only in the VTA and NAc (above), but also in other brain regions[44]. For example, mGluR1 stimulation results in LTD by increasing CI-AMPAR and reducing CP-AMPAR synaptic transmission in cerebellar stellate cells[15]. In the lateral amygdala, mGluR1-LTD mediated by CP-AMPAR removal has been implicated in the extinction of fear memories[16]. The cellular mechanisms underlying these effects of mGluR1 stimulation are best understood in the VTA, where strong evidence supports internalization of CP-AMPARs and translocation of CI-AMPARs from intracellular compartments to the synapse[14]. It is likely that a similar exchange occurs in the NAc[10], although it may not be driven by increased GluA2 synthesis, as seen in the VTA[5]. In stellate cells, lateral diffusion of CI-AMPARs into the synapse may contribute to enhanced CI-AMPAR transmission[45].

In light of evidence that D1 receptor– and D2 receptor–expressing MSNs in the NAc may contribute differently to cocaine-related behaviors[46,47], it may seem surprising that we observed CP-AMPAR accumulation in nearly all MSN in the NAc core of incubated rats (see also ref. 11). An important consideration is that CP-AMPAR accumulation in the NAc is first detected ~1 month after the last cocaine self-administration session. Based on this delay, we propose that CP-AMPAR accumulation is more likely to reflect withdrawal-dependent changes in glutamate transmission onto MSNs (for example, a decrease in transmission that triggers homeostatic plasticity) as opposed to differential activation of D1 and D2 receptors during the period of cocaine self-administration[5]. Nevertheless, although our recordings establish that nearly all of the MSNs in the NAc core exhibited CP-AMPAR plasticity, it remains to be determined whether CP-AMPARs accumulate at most spines or whether they are selectively positioned to respond to particular afferents. Recently, it was shown that CP-AMPAR insertion during incubation leads to the un-silencing of cocaine-generated silent synapses in the amygdala-accumbens projection[48]. It will be important to determine whether this occurs in other pathways and how mGluR1 and silent synapse–based reorganization interact to shape excitatory synaptic transmission in the NAc.

In conclusion, administration of mGluR1 PAMs to incubated rats reduced cue-induced cocaine craving by inhibiting CP-AMPAR–mediated synaptic transmission in the NAc. Furthermore, repeated mGluR1 PAM injections during a critical period of withdrawal opposed the decrease in surface mGluR1 that normally occurs, thereby maintaining mGluR1-mediated inhibitory control over CP-AMPAR accumulation and delaying incubation. These results suggest that mGluR1 PAMs could be used by recovering addicts to control cue-induced craving and prolong abstinence. This has major translational relevance, as no treatment is presently available that provides such protection.

METHODS

Methods and any associated references are available in the online version of the paper.

Note: Any Supplementary Information and Source Data files are available in the online version of the paper.

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AUTHOR CONTRIBUTIONS

J.A.L., K.Y.T. and M.E.W. were responsible for overall study design. J.A.L. and M.M. conducted the biochemical experiments. A.F.S. conducted the electrophysiological experiments, with help from E.F.-B. Surgeries, self-administration training and behavioral testing were performed by J.A.L., with help from C.T.W., X.L., K.A.F. and T.L., except for the incubation of sucrose craving study, which was performed by A.L.L. K.K.S. provided viral vectors and advice on viral vector experiments. M.F.O. provided SYN119, advised on *in vivo* SYN119 experiments, and designed

and supervised sucrose incubation studies. J.A.L., A.F.S., K.Y.T. and M.E.W. analyzed the data. J.A.L., K.Y.T. and M.E.W. wrote the paper.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

1. Pickens, C.L. *et al.* Neurobiology of the incubation of drug craving. *Trends Neurosci.* **34**, 411–420 (2011).
2. Reichel, C.M. & Bevins, R.A. Forced abstinence model of relapse to study pharmacological treatments of substance use disorder. *Curr. Drug Abuse Rev.* **2**, 184–194 (2009).
3. Sesack, S.R. & Grace, A.A. Cortico-basal ganglia reward network: microcircuitry. *Neuropsychopharmacology* **35**, 27–47 (2010).
4. Kalivas, P.W. & Volkow, N.D. The neural basis of addiction: a pathology of motivation and choice. *Am. J. Psychiatry* **162**, 1403–1413 (2005).
5. Wolf, M.E. & Tseng, K.Y. Calcium-permeable AMPA receptors in the VTA and nucleus accumbens after cocaine exposure: when, how and why? *Front. Mol. Neurosci.* **5**, 72 (2012).
6. Conrad, K.L. *et al.* Formation of accumbens GluR2-lacking AMPA receptors mediates incubation of cocaine craving. *Nature* **454**, 118–121 (2008).
7. Mameli, M. *et al.* Cocaine-evoked synaptic plasticity: persistence in the VTA triggers adaptations in the NAc. *Nat. Neurosci.* **12**, 1036–1041 (2009).
8. Purgianto, A. *et al.* Different adaptations in AMPA receptor transmission in the nucleus accumbens after short versus long access cocaine self-administration regimens. *Neuropsychopharmacology* **38**, 1789–1797 (2013).
9. Ferrario, C.R. *et al.* Alterations in AMPA receptor subunits and TARPs in the rat nucleus accumbens related to the formation of Ca^{2+}-permeable AMPA receptors during the incubation of cocaine craving. *Neuropharmacology* **61**, 1141–1151 (2011).
10. McCutcheon, J.E. *et al.* Group I mGluR activation reverses cocaine-induced accumulation of calcium-permeable AMPA receptors in nucleus accumbens synapses via a protein kinase C-dependent mechanism. *J. Neurosci.* **31**, 14536–14541 (2011).
11. McCutcheon, J.E., Wang, X., Tseng, K.Y., Wolf, M.E. & Marinelli, M. Calcium-permeable AMPA receptors are present in nucleus accumbens synapses after prolonged withdrawal from cocaine self-administration but not experimenter-administered cocaine. *J. Neurosci.* **31**, 5737–5743 (2011).
12. Bellone, C. & Lüscher, C. mGluRs induce a long-term depression in the ventral tegmental area that involves a switch of the subunit composition of AMPA receptors. *Eur. J. Neurosci.* **21**, 1280–1288 (2005).
13. Bellone, C. & Lüscher, C. Cocaine triggered AMPA receptor redistribution is reversed *in vivo* by mGluR-dependent long-term depression. *Nat. Neurosci.* **9**, 636–641 (2006).
14. Mameli, M., Balland, B., Luján, R. & Lüscher, C. Rapid synthesis and synaptic insertion of GluR2 for mGluR-LTD in the ventral tegmental area. *Science* **317**, 530–533 (2007).
15. Kelly, L., Farrant, M. & Cull-Candy, S.G. Synaptic mGluR activation drives plasticity of calcium-permeable AMPA receptors. *Nat. Neurosci.* **12**, 593–601 (2009).
16. Clem, R.L. & Huganir, R.L. Calcium-permeable AMPA receptor dynamics mediate fear memory erasure. *Science* **330**, 1108–1112 (2010).
17. Robbe, D., Kopf, M., Remaury, A., Bockaert, J. & Manzoni, O.J. Endogenous cannabinoids mediate long-term synaptic depression in the nucleus accumbens. *Proc. Natl. Acad. Sci. USA* **99**, 8384–8388 (2002).
18. Lovinger, D.M. Presynaptic modulation by endocannabinoids. *Handb. Exp. Pharmacol.* **184**, 435–477 (2008).
19. Milton, A.L. & Everitt, B.J. The persistence of maladaptive memory: addiction, drug memories and anti-relapse treatments. *Neurosci. Biobehav. Rev.* **36**, 1119–1139 (2012).
20. Vanderschuren, L.J. & Ahmed, S.H. Animal studies of addictive behavior. *Cold Spring Harb. Perspect. Med.* **3**, a011932 (2013).
21. Ngomba, R.T. *et al.* Protective role for type-1 metabotropic glutamate receptors against spike and wave discharges in the WAG/Rij rat model of absence epilepsy. *Neuropharmacology* **60**, 1281–1291 (2011).
22. Kalivas, P.W. The glutamate homeostasis hypothesis of addiction. *Nat. Rev. Neurosci.* **10**, 561–572 (2009).
23. Reimers, J.M., Milovanovic, M. & Wolf, M.E. Quantitative analysis of AMPA receptor subunit composition in addiction-related brain regions. *Brain Res.* **1367**, 223–233 (2011).
24. Bellone, C., Mameli, M. & Lüscher, C. *In utero* exposure to cocaine delays postnatal synaptic maturation of glutamatergic transmission in the VTA. *Nat. Neurosci.* **14**, 1439–1446 (2011).
25. Szumlinski, K.K., Ary, A.W. & Lominac, K.D. Homers regulate drug-induced neuroplasticity: implications for addiction. *Biochem. Pharmacol.* **75**, 112–133 (2008).
26. Xiao, B. *et al.* Homer regulates the association of group 1 metabotropic glutamate receptors with multivalent complexes of homer-related, synaptic proteins. *Neuron* **21**, 707–716 (1998).
27. Kammermeier, P.J., Xiao, B., Tu, J.C., Worley, P.F. & Ikeda, S.R. Homer proteins regulate coupling of group I metabotropic glutamate receptors to N-type calcium and M-type potassium channels. *J. Neurosci.* **20**, 7238–7245 (2000).
28. Kammermeier, P.J. & Worley, P.F. Homer 1a uncouples metabotropic glutamate receptor 5 from postsynaptic effects. *Proc. Natl. Acad. Sci. USA* **104**, 6055–6060 (2007).
29. Kammermeier, P.J. Endogenous Homer proteins regulate metabotropic glutamate receptor signaling in neurons. *J. Neurosci.* **28**, 8560–8567 (2008).
30. Ben-Shahar, O. *et al.* Extended daily access to cocaine results in distinct alterations in Homer 1b/c and NMDA receptor subunit expression within the medial prefrontal cortex. *Synapse* **63**, 598–609 (2009).
31. Hu, J.-H. *et al.* Presо1 dynamically regulates group I metabotropic glutamate receptors. *Nat. Neurosci.* **15**, 836–844 (2012).
32. Szumlinski, K.K. *et al.* Homer proteins regulate sensitivity to cocaine. *Neuron* **43**, 401–413 (2004).
33. Szumlinski, K.K. *et al.* Homer isoforms differentially regulate cocaine-induced neuroplasticity. *Neuropsychopharmacology* **31**, 768–777 (2006).
34. Edwards, S., Bachtell, R.K., Guzman, D., Whisler, K.N. & Self, D.W. Emergence of context-associated GluR1 and ERK phosphorylation in the nucleus accumbens core during withdrawal from cocaine self-administration. *Addict. Biol.* **16**, 450–457 (2011).
35. Fourgeaud, L. *et al.* A single *in vivo* exposure to cocaine abolishes endocannabinoid-mediated long-term depression in the nucleus accumbens. *J. Neurosci.* **24**, 6939–6945 (2004).
36. Moussawi, K. *et al.* N-Acetylcysteine reverses cocaine-induced metaplasticity. *Nat. Neurosci.* **12**, 182–189 (2009).
37. Grueter, B.A., Brasnjo, G. & Malenka, R.C. Postsynaptic TRPV1 triggers cell type–specific long-term depression in the nucleus accumbens. *Nat. Neurosci.* **13**, 1519–1525 (2010).
38. Huang, C.C. *et al.* Cocaine withdrawal impairs metabotropic glutamate receptor–dependent long-term depression in the nucleus accumbens. *J. Neurosci.* **31**, 4194–4203 (2011).
39. Olive, M.F. Metabotropic glutamate receptor ligands as potential therapeutics for addiction. *Curr. Drug Abuse Rev.* **2**, 83–98 (2009).
40. Dravolina, O.A., Danysz, W. & Bespalov, A.Y. Effects of group I metabotropic glutamate receptor antagonists on the behavioral sensitization to motor effects of cocaine in rats. *Psychopharmacology* (*Berl.*) **187**, 397–404 (2006).
41. Olive, M.F., Cleva, R.M., Kalivas, P.W. & Malcolm, R.J. Glutamatergic medications for the treatment of drug and behavioral addictions. *Pharmacol. Biochem. Behav.* **100**, 801–810 (2012).
42. Argilli, E., Sibley, D.R., Malenka, R.C., England, P.M. & Bonci, A. Mechanism and time course of cocaine-induced long-term potentiation in the ventral tegmental area. *J. Neurosci.* **28**, 9092–9100 (2008).
43. Olive, M.F. Cognitive effects of Group I metabotropic glutamate receptor ligands in the context of drug addiction. *Eur. J. Pharmacol.* **639**, 47–58 (2010).
44. Loweth, J.A., Tseng, K.Y. & Wolf, M.E. Using metabotropic glutamate receptors to modulate cocaine's synaptic and behavioral effects: mGluR1 finds a niche. *Curr. Opin. Neurobiol.* **23**, 500–506 (2013).
45. Gardner, S.M. *et al.* Calcium-permeable AMPA receptor plasticity is mediated by subunit-specific interactions with PICK1 and NSF. *Neuron* **45**, 903–915 (2005).
46. Lobo, M.K. *et al.* Cell type–specific loss of BDNF signaling mimics optogenetic control of cocaine reward. *Science* **330**, 385–390 (2010).
47. Bock, R. *et al.* Strengthening the accumbal indirect pathway promotes resilience to compulsive cocaine use. *Nat. Neurosci.* **16**, 632–638 (2013).
48. Lee, B.R. *et al.* Maturation of silent synapses in amygdala-accumbens projection contributes to incubation of cocaine craving. *Nat. Neurosci.* **16**, 1644–1651 (2013).

ONLINE METHODS

Subjects and surgery. Adult male Sprague-Dawley rats (Harlan) weighing 250–275 g (~8 weeks old) on arrival were housed on a reverse 12-h light:dark cycle (lights off at 7:00 a.m.). They were acclimated to the animal facility for ~7 d before undergoing surgery for intravenous catheter implantation as described previously[6,8–11]. Following surgery, rats were singly housed. For studies combining intracranial injection with drug self-administration, rats were implanted with guide cannulae positioned 1.5 mm above the NAc core subregion[6]. During the recovery phase (~7 d) and subsequent self-administration training phase, catheters were flushed every 24–48 h with 0.9% sterile saline (wt/vol) and the antibiotic cefazolin (15 mg, intravenous; Webster Veterinary Supply). Behavioral testing began within ~1 h of the start of the dark cycle. All procedures were performed according to the US Public Health Service Guide for Care and Use of Laboratory Animals, and were approved by the Rosalind Franklin University of Medicine and Science Institutional Animal Care and Use Committee.

Drugs. Cocaine HCl was obtained from the National Institute on Drug Abuse and dissolved in 0.9% saline. The selective mGluR1 PAM SYN119 (9H-Xanthene-9-carboxylic acid (4-trifluoromethyl-oxazol-2-yl)-amide), also referred to as compound 14a (ref. 49) or Ro0711401 (ref. 21), was synthesized by EAG Labs. For systemic administration, SYN119 was dissolved in vehicle consisting of 20% 2-hydroxypropyl-β-cyclodextrin (wt/vol, #332593, Sigma-Aldrich) in 0.9% saline. For patch-clamp recordings or intracranial injection, SYN119 and Ro67-7476 (kindly provided by J. Conn, Vanderbilt University) were dissolved in 100% DMSO as a stock solution (stored at −20 °C) and used at a final concentration of ≤0.05% DMSO (vol/vol). The aCSF for intracranial injections contained 145 mM NaCl, 2.7 mM KCl, 1.0 mM $MgCl_2$, 1.2 mM $CaCl_2$, 2.0 mM NaH_2PO_4 and 2.0 mM Na_2HPO_4. DHPG (Sigma-Aldrich) was dissolved in this same aCSF solution for intracranial injection studies. JNJ16259685 (Tocris) was dissolved in the same vehicle as SYN119.

Cocaine self-administration training. Rats were randomly assigned to self-administer cocaine (0.5 mg per kg per infusion in a 100 µl kg^{-1} volume over 3 s) or saline for 10 d (6 h d^{-1}) under a fixed ratio 1 schedule as described previously[6,8–11]. Briefly, self-administration chambers (MED Associates) were located in sound-attenuating cabinets and were equipped with two holes (active and inactive) located on opposite sides of the chamber, approximately 2 cm above the floor. Nose poking in the active hole activated the pump and delivered an infusion of saline or cocaine paired with a 20-s light cue located inside the nose hole and a 20-s time-out period. Nose poking in the inactive hole was without consequence. For controls in the electrophysiology experiments, we used a combination of rats that self-administered saline and control rats that were treated identically except that they were not exposed to the self-administration chambers. Instead, after recovery from catheter implantation surgery, they were brought to holding cages away from their colony room for 6 h d^{-1} for 10 d, thereby receiving the same surgery, recovery, time away from the colony and handling as self-administration groups. Our previous work has shown that NAc AMPAR transmission in these rats is identical to that observed in saline self-administering controls[9] and in drug-naive rats (A.F. Scheyer, E. Flores-Barrera and K.Y. Tseng, unpublished observations).

Tests for cue-induced cocaine-seeking. Rats were divided into groups matched for average number of infusions and active lever responses during training. Any rats that did not learn to self-administer cocaine and/or had faulty catheters were excluded from the study (eight rats). Rats were returned to the self-administration chambers on the specified withdrawal day and tested for 30 or 60 min under extinction conditions, that is, nose pokes in the active hole resulted in presentations of the light cue previously paired with cocaine infusions, but no cocaine. The number of responses in the previously active hole was used as a measure of cocaine-seeking or craving. For studies in which vehicle or PAM was injected intracranially before a test for cue-induced cocaine-seeking (**Fig. 1**), we used a between-group design. Rats underwent withdrawal in their home cage for 45 or more d and were then injected with vehicle, DHPG (0.25 nmol per 0.5 µl per side; 500 µM), Ro67-7476 (0.005 nmol per 0.5 µl per side; 10 µM) or SYN119 (0.005 nmol per 0.5 µl per side; 10 µM) into the NAc core 10 min before the test session (60 min) as described previously[6]; during the 10-min post-injection period, they were returned to their home cage. In most cases, rats underwent two seeking tests over a 2-week period, with at least 4 d between intracranial injections. Vehicle and drug injection groups were counterbalanced in such studies. Only rats with bilateral placements in the NAc core were included in the final analysis. As a result, ten rats were excluded. For studies that evaluated the effect of systemic SYN119 treatment (**Fig. 2**), a within-subject design was used. Rats were tested for cue-induced cocaine-seeking (30 min) twice, first on WD1 (no treatment was administered) and again on or after WD45. 20 min before this second seeking test, rats received a systemic vehicle or SYN119 (10 mg per kg, intraperitoneal) injection; during the 20-min post-injection period, they were returned to their home cage. This 20-min period was determined on the basis of the half-life of SYN119 (~55 min)[49] such that the rats would be tested during the period when the drug should be most effective. For experiments in which SYN119 was administered repeatedly during withdrawal (**Figs. 5** and **6**), rats were tested for cue-induced cocaine-seeking (30 min) on WD1 (no treatment was administered) and again 0–5 d following the last repeated SYN119 or vehicle injection.

Repeated vehicle, SYN119 or JNJ16259685 during withdrawal. Rats received an injection of SYN119 (10 mg per kg, intraperitoneal) or vehicle every other day between WD15 and WD33, resulting in a total of ten injections. These injections were performed in home cages during the dark cycle. In behavioral experiments, cue-induced cocaine-seeking tests were performed 2 d following the last repeated SYN or vehicle injection, that is, WD35 (**Fig. 5a**). For experiments in which seeking tests were followed by patch-clamp recordings (**Fig. 5b**,**c**), treatments were staggered such that only one rat would complete the injection protocol on each recording day. Thus, several rats received 1–3 additional SYN119 or vehicle injections (total of 10–13 injections, every other day) so that they could be recorded on specific days after discontinuing SYN119 or vehicle treatment. In another cohort of rats (**Fig. 7**), daily injections of either vehicle or the mGluR1 antagonist JNJ16259685 (5 mg per kg, intraperitoneal) were performed on WD10–16, and the presence of CP-AMPAR transmission was determined in patch-clamp recordings conducted 1 d after the last injection, a withdrawal time before the normal onset of CP-AMPAR accumulation[5].

Biotinylation and co-immunoprecipitation. As described previously[6,9], a punch was used to obtain bilateral NAc samples consisting primarily of core (total wet weight of ~15 mg). Lateral shell is also included, but this is not believed to compromise the detection of effects relevant to CP-AMPAR accumulation because CP-AMPARs accumulate in both subregions during incubation[6,7,11]. NAc samples were biotinylated and processed to separate biotinylated proteins bound to NeutrAvidin beads (bound material, surface-expressed proteins) from the non-biotinylated (unbound) material[9]. An aliquot of starting material (NAc homogenate) from the same rats was used for co-immunoprecipitation experiments to measure changes in the association between mGluR1 or mGluR5 and Homer proteins. As described previously[6,23], 3 µg of antibody (mGluR1a, 2031, PhosphoSolutions or mGluR5, AB5675, Millipore) was incubated overnight at 4 °C with protein A agarose slurry. The pellet containing antibody-coated beads was collected by centrifugation, washed in phosphate-buffered saline and incubated overnight at 4 °C with 100 µg of NAc tissue. The agarose-bound antibody was then pelleted by centrifugation. This process produced two fractions: bound (pellet) and unbound (supernatant). The bound fraction was dissolved in 2× Laemmli sample buffer with 100mM DTT (using a volume equal to that of the input tissue volume). The process was repeated to maximize recovery.

Immunoblotting. All samples were processed and used for immunoblotting as described previously[6,9,23]. For immunoblotting of biotinylated tissue, we used antibodies to mGluR1 (1:2,000, 610965, BD Biosciences), mGluR5 (1:20,000, AB5675, Millipore), Homer1bc (1:20,000, sc-20807, Santa Cruz) and Homer2ab (1:500, sc-8924, Santa Cruz). For immunoblotting after immunoprecipitation, the same primary antibodies were used, as well as antibodies to mGluR1a (1:3,000, 2031-mGluR1a, PhosphoSolutions) and Homer2 (1:350, H00009455-B01P, Abnova). A higher concentration of Homer1bc (1:2,000) antibody was also used. In addition, we employed antibodies to rabbit or mouse IgG light-chain as secondary antibodies (211-032-171 or 115-035-174, Jackson Immuno Research Laboratories) for co-immunoprecipitation studies so that the heavy chain (~50 kDa) did not obscure the Homer band (~45 kDa). For mGluR antibodies, the mGluR1a and mGluR5 dimer band (~350 kDa) was analyzed because it represents the functional pool of these receptors[50]. Validation is provided on

the supplier's website for all antibodies used, as well as on Antibodypedia for Homer1bc (Santa Cruz) and Homer2 (Abnova). Immunoblots were analyzed with TotalLab (Life Sciences Analysis Essentials) as described previously[6,9,11,23]. Data were excluded only if there were bubbles or imperfections in the band that interfered with its analysis. The number of bands excluded varied for each antibody, fraction (surface or total), and time point. The range is as follows: WD14, 0–2 bands; WD25, 0–4 bands; WD48, 0–3 bands.

Electrophysiology. All electrophysiological procedures were conducted as previously described[10,11]. Briefly, 300-μm-thick coronal brain slices containing the NAc were cut and incubated in 95% O_2/5% CO_2 oxygenated aCSF (122.5 mM NaCl, 20 mM glucose, 25 mM $NaHCO_3$, 2.5 mM KCl, 0.5 mM $CaCl_2$, 3 mM $MgCl_2$, 1 mM NaH_2PO_4, 1 mM ascorbic acid) for at least 1 h (32–34 °C). For recordings, $CaCl_2$ was increased to 2.5 mM, $MgCl_2$ was reduced to 1 mM and 0.1 mM picrotoxin was added along with 0.05 mM (2R)-amino-5-phosphonopentanoate. All recordings were conducted at 32–34 °C using patch pipettes (6–8 MΩ) filled with a cesium-based/spermine-containing solution (140 mM CsCl, 10 mM HEPES, 2 mM $MgCl_2$, 5 mM NaATP, 0.6 mM NaGTP, 2 mM QX-314, 0.1 mM spermine) in voltage-clamp mode. A bipolar tungsten stimulating electrode placed ~300 μm from the recording site was used to elicit EPSCs in NAc MSNs. Only neurons that exhibited a stable synaptic response at the −70 mV holding potential (<15% variability in $EPSC_{-70\ mV}$ amplitude) during 15 min of baseline recording were included. Here, both the rectification index (rectification index = $[EPSC_{-70\ mV}/(-70 - E_{rev})]/[EPSC_{+40\ mV}/(+40 - E_{rev})]$) and the sensitivity to the CP-AMPAR antagonist naspm (100 μM) were assessed across treatment groups to determine the contribution of CP-AMPARs to synaptic transmission. Typically, two sets of depolarizations were applied to assess the reversal potential of the evoked response and the amplitude of the $EPSC_{+40\ mV}$ during baseline and following bath application of SYN119.

AAV construction, infusion, and behavioral testing. Recombinant adeno-associated viruses (rAAV) were constructed as described previously[32,33,51,52]. Briefly, AAVs carrying equal ratios of AAV1 and AAV2 capsid proteins were used to express either hemagglutinin-tagged Homer1c or Homer2 or enhanced GFP under the control of the chicken β-actin promoter. Prior studies using these viruses have shown that transgene expression is first observed after ~7 d, reaches maximal levels by ~3 weeks post-infection, and then persists[32,33,51,52]. Thus, we injected the viruses (AAV-GFP as a control, Homer1c or Homer2) into the NAc core on the same day as the jugular catheterization surgery so that Homer expression would peak during early withdrawal (WD1 is ~17 d post-infection). Rats received stereotaxic injections of virus (0.5 μl per side) into the NAc core over 5 min. Injectors were left in place for 5 min. Approximately 1 week later, rats began cocaine self-administration training (6 h d^{-1} for 10 d). Rats were tested for cue-induced cocaine-seeking on WD1 and again on WD48. At the end of the experiment, AAV transfection was verified by immunostaining for the hemagglutinin tag and for GFP[32,33,52].

Statistical analyses. The data from the extinction tests for cue-induced cocaine-seeking were analyzed using repeated-measures analysis of variance (ANOVA) with treatment (vehicle or SYN119) as the between-subjects factor and withdrawal day (1, >34) as the within-subjects factor, followed by Sheffé *post hoc* comparisons, unless otherwise specified. For electrophysiological studies in which SYN119 was bath-applied, data were analyzed using a two-way repeated-measures ANOVA with drug exposure (saline, cocaine) as the between-subjects factor and time (before or after drug application) as the within-subjects factor, followed by least significant difference *post hoc* comparisons. Naspm sensitivity was assessed using a paired *t*-test (5 min of baseline versus last 5 min of napsm application). For electrophysiological studies conducted after *in vivo* SYN119 or JNJ16259685 administration, data were analyzed using a one-way ANOVA followed by a Dunnett's test (versus vehicle group) when appropriate. Pearson correlation calculations were used for correlational analysis of electrophysiological and behavioral data. For biochemical studies, independent sample *t* tests were used to assess group differences (cocaine versus saline) in protein levels. All *t* tests were two-tail with the exception of those conducted for **Figures 1c**, **3b** (WD48, mGluR1 total and mGluR5 surface) and **4b** (WD48, mGluR5/Homer2). Data were normally distributed in all cases, as assessed using the Kolmogorov-Smirnov test. Data collection and analysis were not performed blind to the conditions of the experiments. No statistical methods were used to predetermine sample sizes, but our sample sizes are similar to those reported in our previous studies[6,8–11] and other publications in the field[30,32,34,52].

49. Vieira, E. *et al.* Fluorinated 9H-xanthene-9-carboxlic acid oxazol-2-yl-amides as potent, orally available mGlu1 receptor enhances. *Bioorg. Med. Chem. Lett.* **19**, 1666–1669 (2009).
50. Jingami, H., Nakanishi, S. & Morikawa, K. Structure of the metabotropic gluatame receptor. *Curr. Opin. Neurobiol.* **13**, 271–278 (2003).
51. Klugmann, M. & Szumlinski, K.K. Targeting *Homer* genes using adeno-associated viral vector: lessons learned from behavioural and neurochemical studies. *Behav. Pharmacol.* **19**, 485–500 (2008).
52. Lominac, K.D. *et al.* Distinct roles for different Homer1 isoforms in behaviors and associated prefrontal cortex function. *J. Neurosci.* **25**, 11586–11594 (2005).

SUPPLEMENTARY INFORMATION

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

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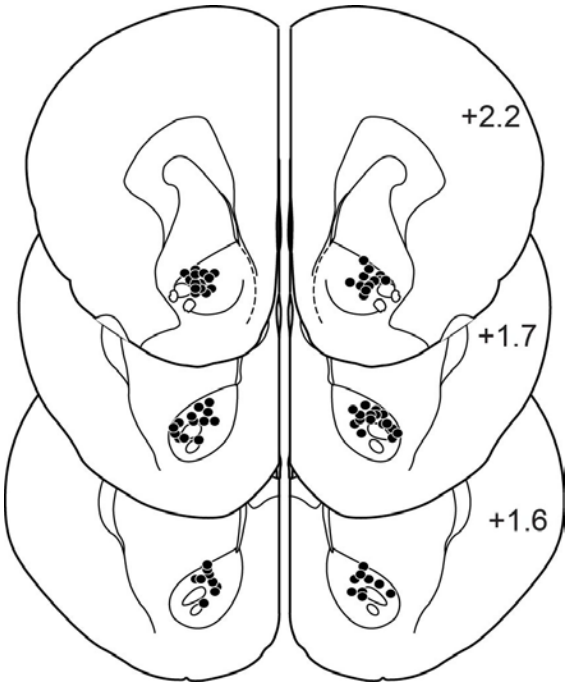

Supplementary Figure 1. Cannulae placements for animals used to assess the effects of intra-NAc core infusions of DHPG, Ro67-7476 and SYN119 on cue-induced cocaine seeking. Placements are depicted for animals shown in **Fig. 1** of the main text. As described in the Methods, vehicle and drug injections 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).

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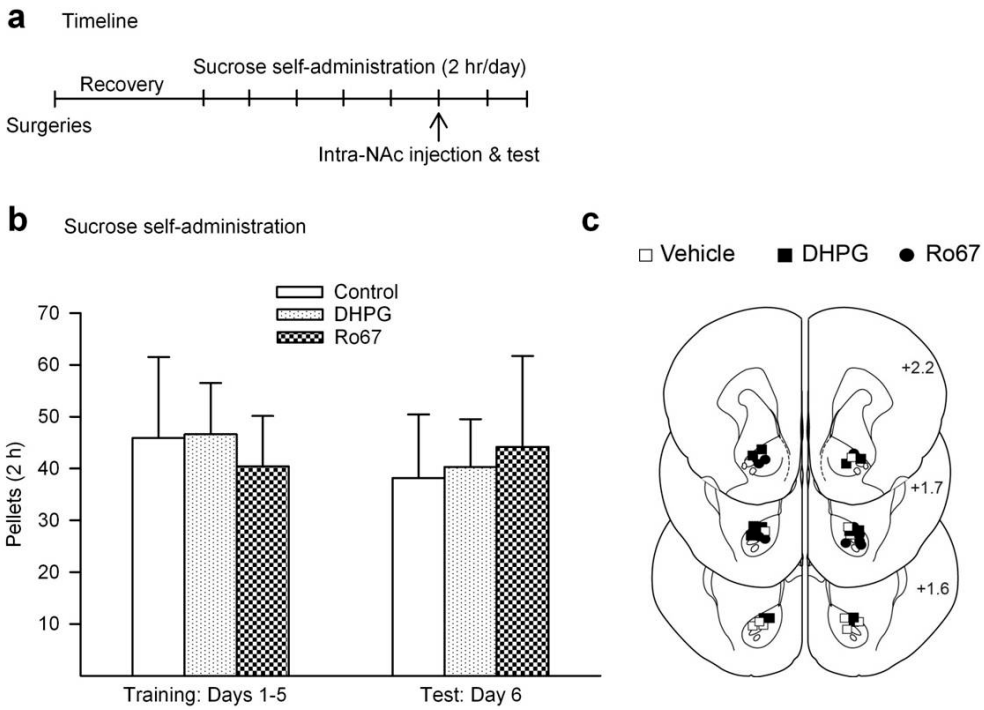

Supplementary Figure 2. Injections of DHPG or Ro67-7476 into the NAc core have no effect on sucrose self-administration. To control for nonspecific effects of DHPG and Ro67-7476 on motivated behavior, the effects of intra-NAc core injections of these compounds on sucrose self-administration were assessed.

(**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).

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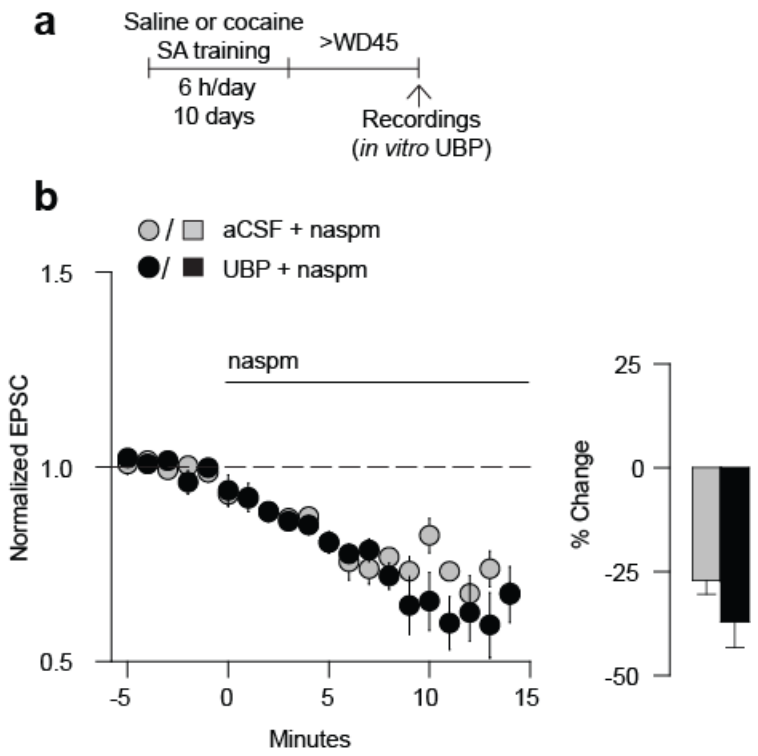

Supplementary Figure 3. The inhibitory effect of naspm on NAc MSN EPSC$_{-70mV}$ amplitude is not mediated by kainate receptors. The pharmacological action of naspm on glutamatergic synaptic transmission has been known to affect kainate receptor-mediated transmission (e.g., Sun H.Y. et al. (2009), J. 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 versus UBP310 + naspm during last five minutes of naspm application).

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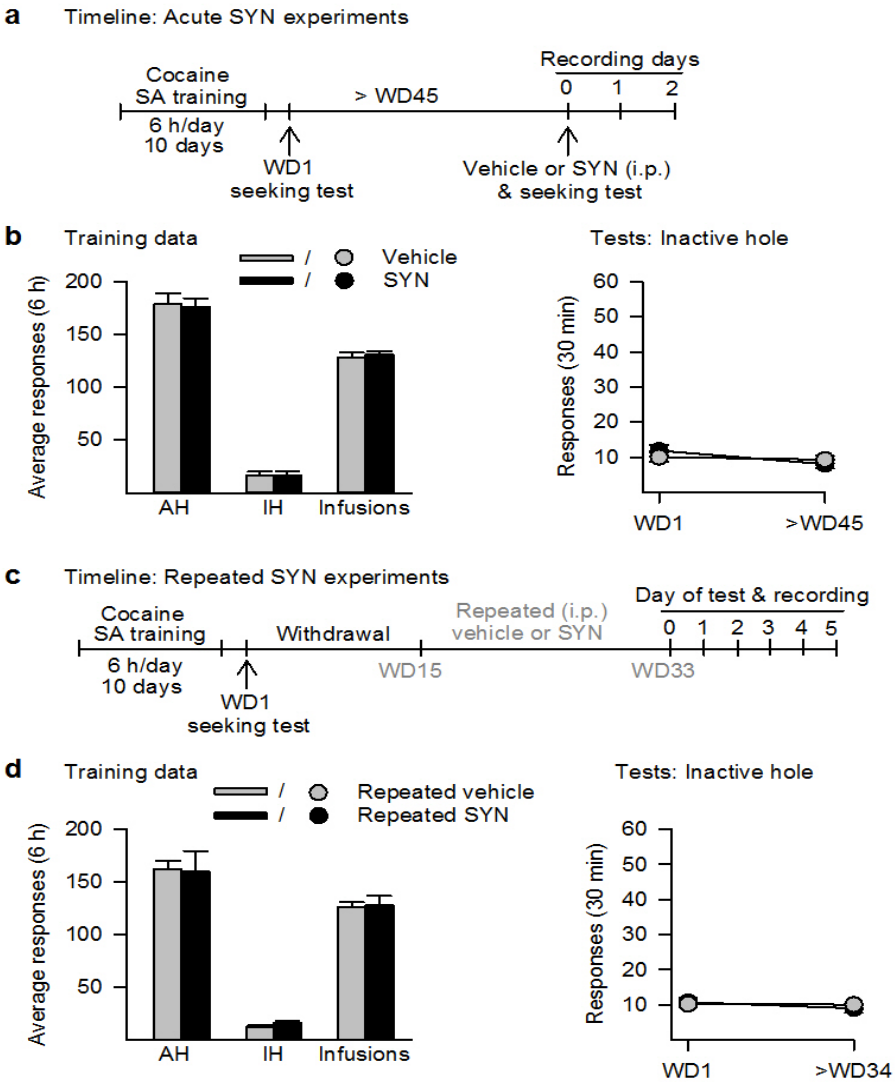

Supplementary Figure 4. Training data and additional test data for animals used to assess the effects of acute or repeated SYN119 treatment on cue-induced cocaine-seeking. Experimental timeline (**a**) and self-administration (SA) training data (**b**, left panel; averaged over days 1-10 ± s.e.m.) for 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).

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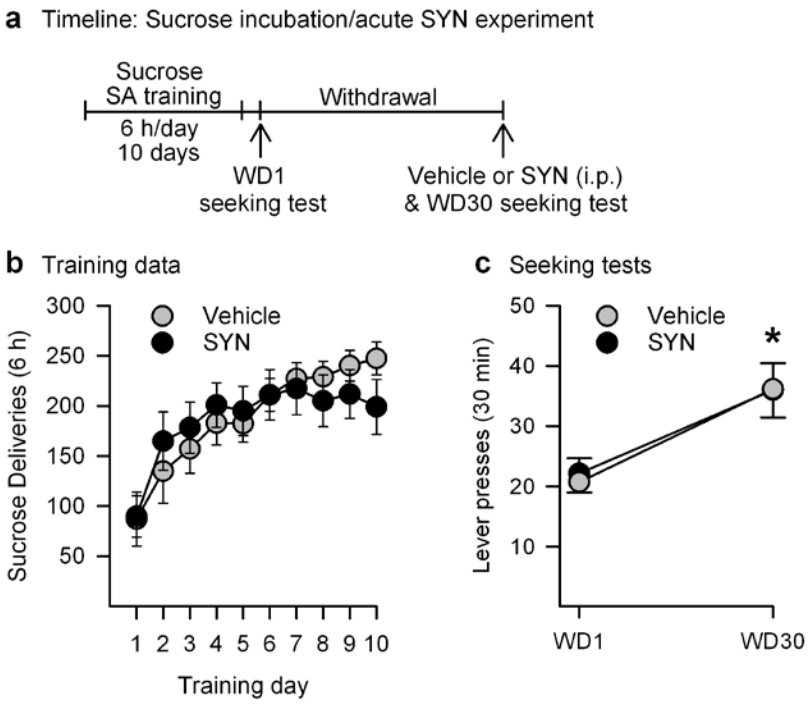

Supplementary Figure 5. Systemic SYN119 does not inhibit the expression of incubation of sucrose seeking. As shown in the experimental timeline (**a**), rats underwent 10 days of sucrose self-administration (SA) (6 h/day). During these sessions, presses on the active lever resulted in delivery of sucrose 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.

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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[50]. 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.

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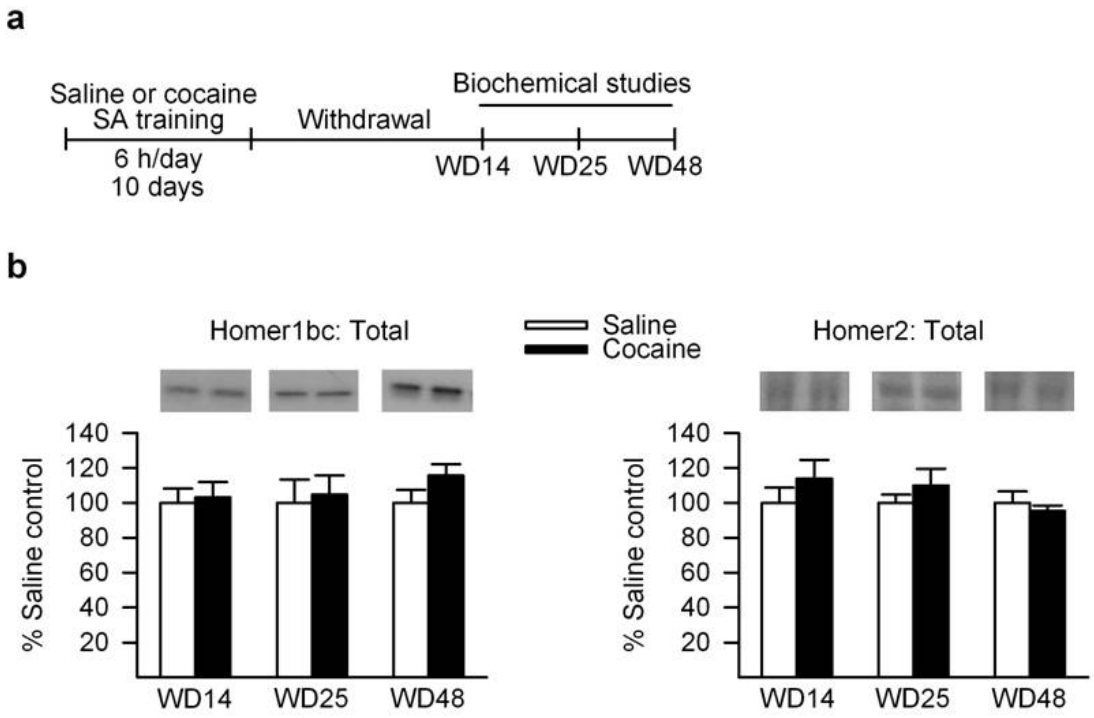

Supplementary Figure 7. No changes in total Homer protein levels are observed in the NAc during withdrawal from extended-access cocaine self-administration. (**a**) Timeline. SA, self-administration. (**b**) Homer1bc (left) and Homer2 (right) protein levels were assessed in total NAc tissue at each withdrawal 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.

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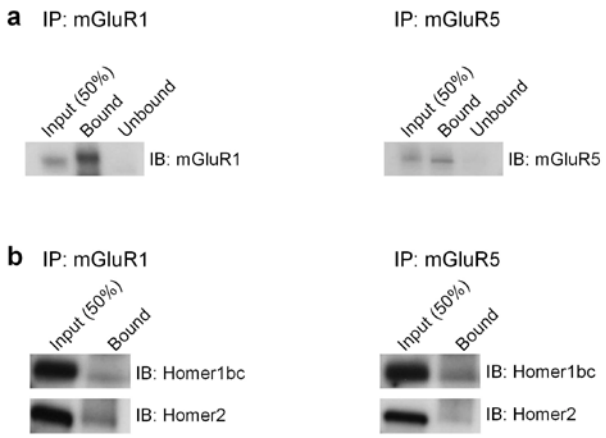

Supplementary Figure 8. Control studies for co-immunoprecipitation (co-IP) experiments measuring association between Homer proteins and mGluR1 or mGluR5. (**a**) After IP of NAc tissue with an mGluR1 or mGluR5 antibody, immunoblotting with the same antibody verified that virtually all of the respective 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.

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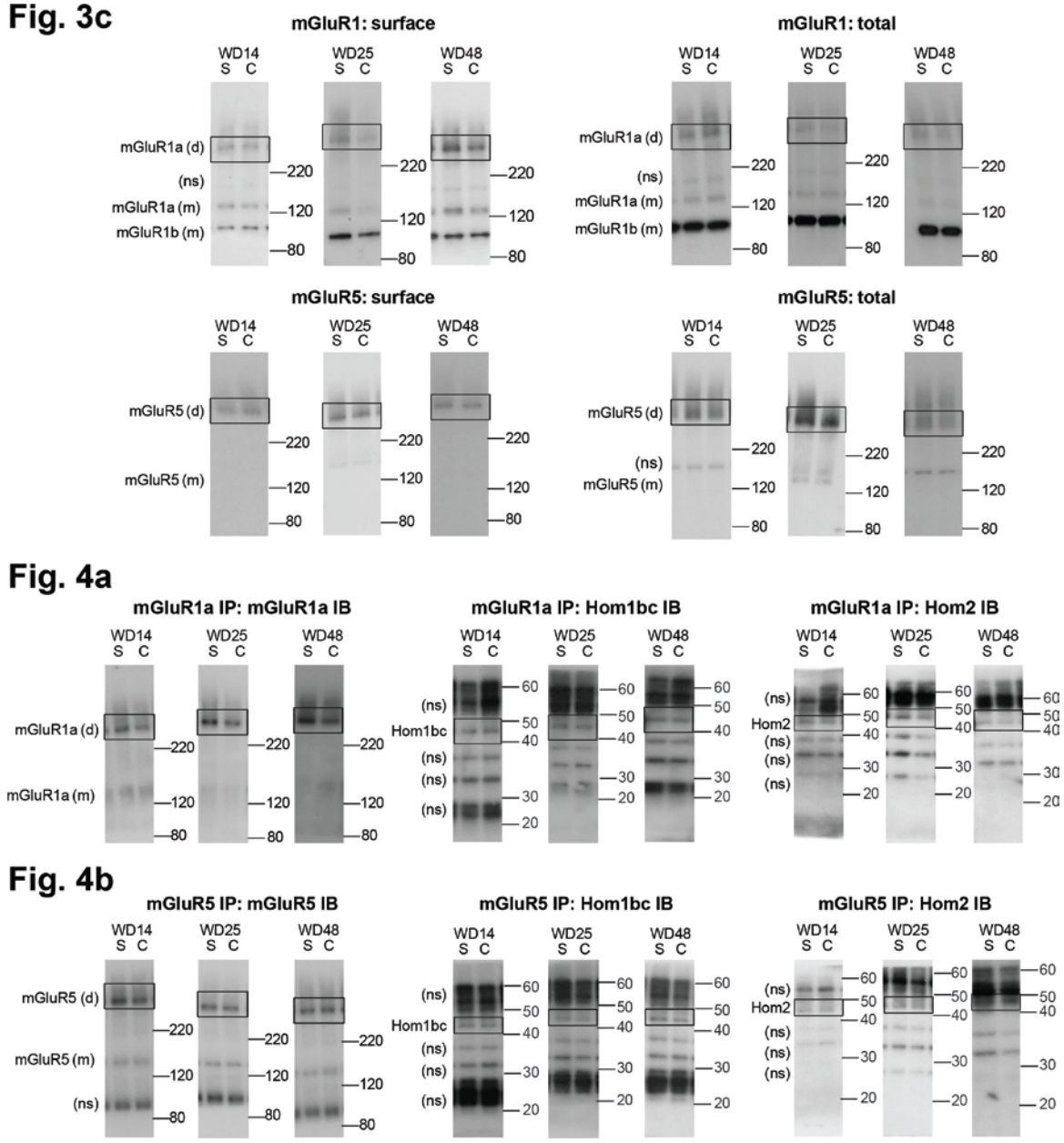

Supplementary Figure 9. Full-length images of the blots presented in the main figures. Shown are full-length images of cropped blots presented in **Fig. 3c** (top), **Fig. 4a** (middle) and **Fig. 4b** (bottom). The mGluR1 antibody used in **Fig. 3c** was an mGluR1 pan antibody, and therefore both mGluR1a and mGluR1b variants are detected. As described in Materials and Methods, the dimer band (d) was analyzed for mGluR1a and mGluR5. The monomer (m) is sometimes visible, although often times the monomer band is extremely faint or absent at the exposure times selected to best quantify the intensity of the dimer band. Blots were minimally adjusted for contrast/brightness and resized appropriately for cropped versus full-length image presentation. Boxes indicate bands presented in the cropped versions shown in the main text. Molecular weights (in kD) are shown to right of the blots. WD, withdrawal day; S, saline self-administering rats; C, cocaine self-administering rats; ns, nonspecific band; IB, immunoblot; IP, immunoprecipitation; Hom, Homer.

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