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Cocaine-evoked synaptic plasticity: persistence in the VTA triggers adaptations in the NAc

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Addictive drugs hijack mechanisms of learning and memory that normally underlie reinforcement of natural rewards and induce synaptic plasticity of glutamatergic transmission in the mesolimbic dopamine (DA) system. In the ventral tegmental area (VTA), a single exposure to cocaine efficiently triggers NMDA receptor-dependent synaptic plasticity in DA neurons while in the nucleus accumbens (NAc) plasticity occurs only after repeated injections. It remains elusive whether these two forms of plasticity are independent or hierarchically organized. Here, combining *ex-vivo* electrophysiology in acute brain slices with behavioral assays modelling drug relapse in mice, we show that the duration of the cocaine-evoked synaptic plasticity in the VTA is gated by mGluR1. Overriding mGluR1 *in vivo* made the potentiation in the VTA persistent. This led to synaptic plasticity in the NAc, which contributes to cocaine-seeking behavior after protracted withdrawal. Impaired mGluR1 function in vulnerable individuals could represent a first step in the recruitment of the neuronal network that underlies compulsion and addiction.

Cocaine, one of the most addictive drugs of abuse, can induce synaptic plasticity of glutamatergic transmission in the mesolimbic DA system of rodents [1-3]. Within hours after a single cocaine injection, excitatory inputs onto DA neurons of the VTA are strengthened, which can be monitored by an increased AMPA/NMDA ratio [4]. This drug-evoked potentiation is in part mediated through an exchange of GluR2-containing for GluR2-lacking AMPA receptors, leading to EPSCs that are sensitive to polyamines and exhibit a rectifying current-voltage relationship [5,6]. This plasticity is triggered by all addictive drugs tested so far and lasts about 5 days [4,7,8]. If cocaine is self-administered repetitively for two weeks, plasticity in the VTA becomes persistent, and can be detected even months after withdrawal [9].

In the NAc, cocaine-evoked plasticity also occurs but on a slower timescale with a steeper induction threshold. A single cocaine injection is not sufficient to trigger changes in synaptic transmission. However, after five days of consecutive injections, AMPA/NMDA ratios are depressed [10]. During withdrawal from both passive exposure and self-administration, this long-term depression (LTD)-like plasticity in the NAc transforms into a potentiation through an insertion of AMPARs [10,11]. Biochemical and electrophysiological investigations suggest that the inserted receptors are GluR1 homomeric channels [12,13]. Recently it has been observed that the *in vivo* inhibition of these channels by a polyamine toxine significantly reduces cue-induced cocaine seeking after withdrawal [11]. This behavioral phenomenon, termed incubation of craving, becomes apparent following protracted withdrawal, and is believed to mimic relapse [14]. Similarly, cocaine-seeking was blocked by injections of antisense oligonucleotides of GluR1 mRNA into the NAc [15]. GluR2-lacking AMPARs seem to be involved in a receptor redistribution that contributes to the remodelling of neuronal networks underling addictive behaviors [16].

Taken together, a single injection of cocaine causes a switch-like, rapid, but transient potentiation of excitatory inputs in the VTA, while several injections are required to induce plasticity in the NAc. Here we manipulate the persistence of the plasticity in the VTA via a mGluR1-dependent mechanism, and use genetically modified mice lacking NMDAR selectively in DA neurons of the midbrain to test the effects of cocaine on the enduring forms of plasticity in the NAc and on the incubation of craving.

mGluR1-dependent reversal of plasticity *in vivo*

In vitro, cocaine-evoked plasticity in the VTA can be reversed rapidly via mGluR1 activation either by synaptic glutamate or exogenous agonists [5]. Mechanistically, such mGluR-LTD exchanges the GluR2-lacking AMPARs that are inserted during cocaine-evoked plasticity with newly synthesized GluR2-containing receptors [17]. We therefore asked whether functional mGluR1 in the VTA are required for the endogenous reversal of cocaine-evoked plasticity *in vivo*. To this end, we interfered with mGluR1 function by using a dominant negative peptide that precludes the binding of mGluR1 and Homer1b/c (TAT-mGluRct) [18]. This peptide specifically inhibits mGluR-dependent synaptic plasticity in the hippocampus [19]. The peptide was TAT- and fluorescein-conjugated and stereotactically delivered bilaterally to the VTA of mice. To ensure that the peptide did not spread beyond the VTA yet remained present in DA neurons for the duration of the experiment (Fig. 1a), we visualized the fluorescence eight days after the injection (Fig. 1b) and performed recordings from fluorescent-labeled neurons. We also tested whether the dominant negative peptide reduced DHPG-evoked currents and whether it blocked mGluR-LTD *in vitro*, which was indeed the case (Fig. S1). We then measured EPSCs at -70, 0 and +40 mV to calculate the rectification index ($RI = EPSC_{-70mV}/EPSC_{+40mV}$). In animals where TAT-mGluRct was delivered, RIs measured seven days after a single cocaine injection, were significantly higher than control values or after the stereotactic injection of a TAT

control peptide (TAT-ctrl,) ($RI_{TAT\text{-}mGluRct\text{-}Cocaine}$ = 2.8 ± 0.1, $RI_{TAT\text{-}mGluRct\text{-}Saline}$ = 1.7 ± 0.1, $RI_{TAT\text{-}ctrl\text{-}Saline}$ = 1.9 ± 0.1 and $RI_{TAT\text{-}ctrl\text{-}Cocaine}$ = 2 ± 0.1; $F_{(2, 27)}$ = 22.21, ***p < 0.001; Fig. 1c, d). In the presence of the dominant negative peptide cocaine-evoked plasticity was intact, which demonstrates that mGluR-Homer binding is not required for induction (e.g. NMDA-dependence [4]) or expression (e.g. PICK1-dependent AMPAR redistribution [5], Fig. S1). Taken together, these findings suggest that the disruption of Homer 1b/c-mGluR interaction in the VTA renders the cocaine-evoked plasticity persistent.

To confirm this result pharmacologically, we blocked mGluR1 with daily systemic (i.p.) injections of the antagonist 1-aminoindan-1,5-dicarboxylic acid (AIDA) following a single injection of cocaine (Fig. 2a top panel). At the dose of 0.25 mg/kg, AIDA selectively inhibits mGluR1 receptors [20]. Since we established that seven days is normally enough for a full reversal of cocaine-evoked plasticity we cut slices on day eight and found that the current-voltage relationship (I-V) was rectifying (RI = 3.4 ± 0.2, Fig. 2b; for corresponding AMPA/NMDA ratio see Fig. S2), which was not the case if one injection of cocaine was followed by daily injections of saline (RI = 1.8 ± 0.1, Fig. 2b). As further controls, we ensured that saline or AIDA alone had no effect on RI (RI = 2.0 ± 0.1 and 1.9 ± 0.1, respectively). In contrast, with seven injections of cocaine the EPSCs were rectifying and the RI was significantly elevated compared to control values (RI = 3.0 ± 0.2). Interestingly, the increased RI following seven injections of cocaine is similar to what we observed previously 24h after a single injection, arguing that the plasticity may already be saturated by a single injection of cocaine [8]. To determine the temporal requirement of mGluR1 function, we broke down the AIDA treatment and compared an immediate treatment (days 2 and 3) with a late treatment (days 5 and 6) and prepared slices at day eight. Only the latter was efficient in maintaining synaptic plasticity, arguing that mGluR1 are activated during a narrow time window in order to reverse the cocaine-evoked plasticity (Fig. S3).

Taken together, one injection of cocaine leads to persistent plasticity when followed by daily injections of AIDA, suggesting that the endogenous reversal of cocaine-evoked plasticity depends on functional mGluR1 receptors *in vivo*.

We had previously shown that the positive mGluR1-modulator Ro 67-7674 leads to the disappearance of cocaine-evoked plasticity within 24h after one i.p. injection [5]. We therefore tested whether a positive modulation of mGluR1 receptors could reverse cocaine-evoked plasticity with a more robust induction by seven injections of cocaine, each given one hour after Ro 67-7476 (Fig. 2a, bottom panel). Indeed seven injections of cocaine when paired with Ro 67-7476 yielded a linear I-V curve (Fig. 2c; RI = 1.6 ± 0.1). As a control, Ro 67-7476 injected together with saline had no effect on rectification, while seven injections of cocaine along with saline, similar to the result above, led to significant rectification (RI = $2.2 \pm 0.1_{\text{Saline-Saline}}$, $1.9 \pm 0.1_{\text{Ro 67-7476-Saline}}$, and $2.9 \pm 0.5_{\text{Saline-Cocaine}}$). Thus, positive modulation of mGluR1 causes rapid reversal of cocaine-evoked plasticity in the VTA *in vivo*, even in response to repeated cocaine injections.

Bi-directional control of plasticity in the NAc

We next used the modulation of mGluR1 as a tool to test the link between cocaine-evoked potentiation in the VTA and cocaine-evoked depression in the NAc. We started by testing the effect of the mGluR1 antagonist AIDA. Similar to the experiments described above, a first dose of cocaine was followed by six daily injections of either saline, cocaine or AIDA, and on day eight we prepared coronal brain slices containing the NAc (Fig. 3a). In medium spiny neurons of the NAc shell, a day after the last injection, I-V curves were linear in all conditions (Fig. 3c). A single injection of cocaine was not sufficient to induce changes in the AMPA/NMDA ratio but transmission was significantly decreased with seven injections, consistent with previous

reports (AMPA/NMDA = 1.2 ± 0.1 and 0.7 ± 0.1, respectively; Fig. 3b, d; Ref. 10). Also consistent with previous reports, the decrease of AMPA/NMDA ratio elicited by cocaine occluded the induction of low-frequency stimulation (LFS)-induced LTD, suggesting that the two phenomena share underlying mechanisms (Fig. 3e, f; Ref. 21).

AIDA injections had no effect on synaptic transmission (AMPA/NMDA = 1.3 ± 0.2; Fig. 3b, d), but lowered the threshold for cocaine-evoked plasticity in the NAc; a single injection of cocaine was sufficient to significantly decrease the AMPA/NMDA ratio and occlude the synaptically-induced LTD if that injection of cocaine was followed by six injections of AIDA (AMPA/NMDA = 0.6 ± 0.1; Fig. 3b, d, and g). Conversely, when we applied seven injections of cocaine with Ro 67-7476 (Fig 3h), the decrease of AMPA/NMDA ratio in the NAc was abolished (AMPA/NMDA = 1.2 ± 0.1 vs 0.7 ± 0.1 in interleaved control experiments of cocaine paired with saline injections; Fig. 3i, k).

To ensure that effects on plasticity in the NAc were due to a local intervention at the level of the VTA, we carried out a series of experiments (Fig. 4a), using stereotactic injections in the VTA of the TAT-conjugated dominant negative peptide introduced above (Fig. 1a). We observed that with this selective, local disruption of mGluR1 function in neurons of the VTA, a single injection of cocaine was sufficient to trigger the depression in the NAc (AMPA/NMDA = 0.7 ± 0.1). The control peptide followed by one injection of cocaine or saline had no effect on the AMPA/NMDA ratios (AMPA/NMDA = 1.3 ± 0.1 and 1.2 ± 0.3, respectively; Fig. 4b, d). Similarly one saline injection after TAT-mGluRct delivery was also without effect (AMPA/NMDA = 1.2 ± 0.1; Fig. 4b, d). We next tested whether overriding mGluR1 in the VTA also had an effect on enduring forms of plasticity, such as the insertion of GluR2-lacking AMPARs that can be observed a month after withdrawal. To this end, we measured the RI in medium spiny neurons of the NAc 35 days after the last cocaine injection (Fig. 4e). Ten

daily injections followed by this protracted withdrawal period indeed led to strongly rectifying EPSCs in respect to controls (RI = 3.7 ± 0.6 and 2 ± 0.1 respectively; $t_{(14)}$ = 2.19, *p < 0.05; Fig. 4f, h left panel). A similarly high RI was observed after only one cocaine injection if the mouse was pre-treated with a stereotactic injection of TAT-mGluRct ($RI_{TAT\text{-}mGluRct}$ = 3.6 ± 0.5 and $RI_{TAT\text{-}ctrl}$ = 2.1 ± 0.1; $t_{(16)}$ = 3.4, **p < 0.01; Fig. 4f, h right panel). Thus, our data suggest that persistent plasticity in the VTA triggers a synaptic depression in the NAc and that a swift reversal of this plasticity may prevent synaptic alterations in the NAc. Taken together, interfering selectively with mGluR function in neurons of the VTA, controls early forms as well as enduring forms of cocaine-evoked plasticity in the NAc.

Plasticity in the VTA modulates incubation of craving

It is suggested that insertion of GluR2-lacking AMPARs contributes to development of incubation of craving [11], we hypotesized that preventing cocaine-evoked plasticity in the NAc would impair drug-seeking behaviour after protracted withdrawal. We therefore tested this behavioral phenomenon in $NR1^{DATCreERT2}$ mouse. In this mouse NMDARs are ablated in DA neurons during adulthood after tamoxifen-triggered recombination and we recently showed that a single injection of cocaine no longer induces a synaptic potentiation in the VTA [22]. Following food shaping, $NR1^{DATCreERT2}$ and control mice were trained for eight days in 4 h sessions to lever press for intravenous (i.v.) cocaine self-administration that was associated with a light cue. $NR1^{DATCreERT2}$ acquired stable lever pressing for cocaine similarly to controls (Fig. 5a; for lever presses on the inactive lever see Fig. S4b). After 35 days of withdrawal, the mice were tested for cue-induced cocaine seeking. This test session lasted 90 min during which presses on the active lever under a fixed ratio schedule 1 triggered conditioned stimulus presentation without cocaine delivery. The incubation of cocaine-seeking behavior was significantly reduced in $NR1^{DATCreERT2}$ mice (Active lever 216 ± 31 vs 137 ± 12, inactive lever 56 ± 11 vs 43 ± 7; Factor Genotype, $F_{(1;28)}$ = 6.20, * p < 0.05; Fig. 5b). Slices of the midbrain and the ventral striatum were then prepared from the very same mice in order to measure AMPA/NMDA ratios and RI. In the VTA of

$NR1^{DATCreERT2}$ mice, NMDA EPSCs were absent and AMPAR EPSCs showed linear RI value. In control mice the AMPA/NMDA ratio and RI were high (AMPA/NMDA = 1.0 ± 0.1; RI = 1.9 ± 0.07 in $NR1^{DATCreERT2}$ mice vs 3.5 ± 0.6 in controls; $t_{(11)} = 2.61$, *p < 0.05; Fig. 5 c, d), confirming the presence of VTA plasticity following cocaine self-administration even after a protracted withdrawal period – a finding which was made previously also in rats [9]. Moreover, in slices of the NAc, rectification was present in control mice but not in $NR1^{DATCreERT2}$ mice (RI = 1.7 ± 0.1 $NR1^{DATCreERT2}$ mice vs 3.8 ± 0.2 in controls; $t_{(27)} = 5.68$, ***p < 0.001; Fig. 5 c, d). Plotting the RI in function of the behavioral score revealed a significant correlation (Weighted regression, $F_{(1, 14)} = 13.7$, $p = 0.003$, $r^2 = 0.533$, Fig. 5e). Taken together, preventing the induction of cocaine-evoked plasticity in DA neurons of the VTA, abolished early and enduring plasticity in the NAc and attenuated incubation of craving.

Discussion

Here we show that mGluR1 receptors on DA neurons regulate the persistence of the cocaine-evoked potentiation in the VTA, which hierarchically controls the synaptic plasticity in the NAc, leading to the insertion of GluR1 homomeric AMPARs and shaping the incubation of craving.

Mechanistically, enhanced excitation of projection neurons in the VTA may facilitate the coincident release of DA and glutamate in the NAc through a continous enhanced release of DA. This may then shift the threshold for the induction of local plasticity in the NAc by affecting circuit excitability, or by integrating biochemical signals such as intracellular calcium or CaMKII signalling [10, 23].

The hierarchical organization of cocaine-evoked plasticity may be of great relevance in the context of the emerging anatomical organization of the striatum [24, 25]. These tracing studies show that VTA and NAc are part of a reciprocal spiral in connectivity between the midbrain and the striatum. Several studies implicate the dorsal components of the DA system in cocaine-seeking habits that are observed in addiction,

which implies that the early drug effects on the VTA need to be transferred to the dorsal striatum via the NAc [26, 2]. This idea is supported by the observation that in rats development of cocaine seeking was prevented by an intrastriatal disconnection procedure, which consisted in lesioning the NAc core on one side and blocking DA receptors in the contralateral striatum [3, 27]. Under these conditions a DA antagonist injected directly into the dorsal striatum of the non-lesioned side controlled cocaine seeking. The hierarchical link of cocaine-evoked plasticity between VTA and NAc described here, may represent the first leg of the spiraling connectivity underlying compulsive habits [28].

Surprisingly, recent studies suggest that the early cocaine-evoked plasticity in DA neurons of the VTA does not mediate concurrent short-term behavioral effects of the drug [22, 29], while this idea was supported by earlier experiments. For example, local application of NMDAR antagonists in the VTA abolishes conditioned place preference (CPP) [30], as does genetic knockout of the AMPA receptor subunit GluR1 ([31] but see [32]). Moreover, the viral overexpression of GluR1 enhances cocaine-induced behavioral sensitization in rats that have never been exposed to cocaine [33]. However, these approaches affect all cell types in the VTA. A selective deletion of the NMDAR subunit NR1 only in DA neurons induced in adult mice abolishes cocaine-evoked plasticity onto dopamine neurons of the VTA, but does not affect behavioral sensitization and the development of a CPP response [22]. In contrast, in the same mice later drug-associated behaviors such as reinstatement of CPP are blocked. This finding remains controversial in the light of another study using a similar genetic approach; although differences in the CPP protocol may explain this discrepancy [29]. But altogether, these results raise the possibility that cocaine-evoked plasticity in the VTA may be important behaviourally for the late-stage drug-seeking behaviors, such as described in the present study.

Our results may shed light on the mechanism underlying the progression from recreational use to compulsive abuse and relapse in drug addicts. They are in line with the observation that self-administration of cocaine causes a more persistent plasticity in the VTA than passive injections [9]. Extending the observation that mGluR1-LTD rapidly reverses cocaine-evoked plasticity *in vitro*, we show here that mGluR1-LTD in the VTA is required for the endogenous reversal of early cocaine-evoked plasticity in the VTA, as well for adaptive gating of later cocaine-evoked plasticity in the NAc. Therefore, recruitment of mGluR1 functions as a protective mechanism to counteract drug exposure. However, there is a critical time window over which mGluR1 might control cocaine-evoked plasticity. If cocaine-induced plasticity in the VTA is not reversed within days, e.g. by cocaine binging, more dorsal parts of the striatum are recruited to activate the spiral towards addiction. Thus, we think it is unlikely that this will be useful as a means to reverse previously established addiction in cocaine abusers, where persistent synaptic plasticity has already been transmitted to the striatum. However, our results do raise the possibility that individuals with deficient mGluR1-dependent LTD mechanisms may be particularly at risk of addiction. We suggest that the screening of genes controlling mGluR1 function may improve clinical efforts to assess individual vulnerability to drug addiction.

Methods summary

Horizontal slices from midbrain (250 µm thick) and coronal slices containing the nucleus accumbens (NAc, 300 µm thick) were prepared as described [17] from C57BL/6 mice (80%) and the Pitx3-GFP knockin mice (20%) [34]. To disrupt mGluR1 function *in vivo* TAT-mGluRct (YGRKKRRQRRR-ALTPPSPFR) was injected stereotactically (myNeuroLab) under anesthesia. The mGluR1 antagonist 1-aminoindan-1,5-dicarboxylic acid (0.25 mg/kg, AIDA) and the positive modulator of mGluR1 Ro 67-7476 (4 mg/kg 1h prior to cocaine/saline) were dissolved in saline (0.9% NaCl) and i.p. injected. Cocaine was dissolved in saline and injected at 15 mg/kg i.p.. $NR1^{DATCreERT2}$ mice were generated by crossing mice carrying an inducible Cre-recombinase under the DAT-promoter with mice carrying floxed alleles for NR1 [22]. The mutation was induced in 5 months old mice by repeatedly injecting 1 mg/kg tamoxifen i.p., twice a day for 5 days. Following food shaping, mice were implanted with a catheter in the right jugular vein. During the eight cocaine self-administration sessions pressing the active lever resulted in the infusion of 0.5 mg/kg cocaine and a light cue. After 35 days of withdrawal, mice were re-introduced in the operant chambers and tested for cue-induced cocaine seeking. During this 90 min session, lever pressing caused conditioned stimulus presentation but no cocaine infusion. Compiled data (3-8 mice for each condition) are expressed as mean ± s.e.m of the number of cells recorded (n). The level of significance was taken at $p = 0.05$. Data from food training and cocaine self-administration experiments were analysed using repeated measures ANOVA to investigate the effect of day, genotype and lever when appropriate. Statistical analysis for electrophysiology was performed by using One-Way ANOVA followed by Bonferroni post hoc test. For comparison of two groups the student t-test was used.

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Author Contributions: M.M. carried out the electrophysiology experiments. B.H. performed the behavioral experiments. D.E. generated the mutant mice. J.M.R.P. bred the mice for the behavioral experiments and injected them with tamoxifen. C.L. designed the study with M.M. and C.C. and wrote the ms with the help of M.M. and R.S.

Figure legends

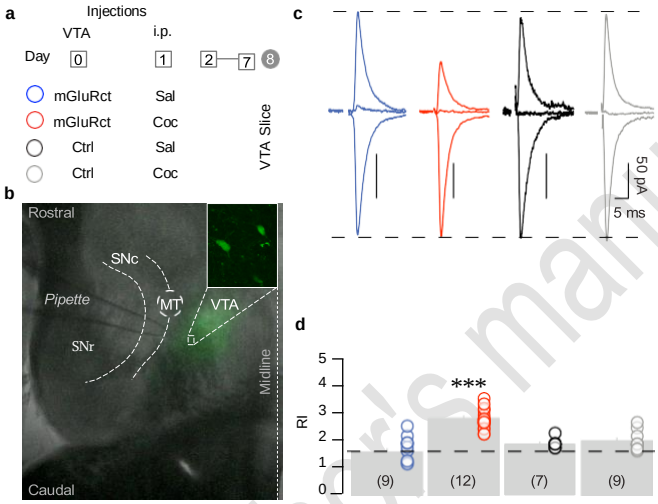
Figure 1. Disruption of Homer 1b/c-mGluR interaction in the VTA renders cocaine-evoked plasticity persistent. a. Saline (blue and black circle) or cocaine (red and grey circle) were injected i.p. 24h after stereotactic delivery of TAT-mGluRct or TAT-control (TAT-ctrl) into the VTA. Acute midbrain slices were then prepared at day eight. b. Confocal image obtained eight days after injection of TAT-mGluRct (0.6 µl at 1 µM). The fluorescence signal is superimposed to the transmitted light image. Magnification at 40X. c. Examples of AMPAR-EPSCs obtained at -70, 0, and +40 mV. d. Bar graph of averaged rectification indexes (RI) with superimposed scatter plot (symbols as above). Dashed line in the bar graph indicates the value of a linear I-V curve (70/40 = 1.75).

Figure 2. Bi-directional modulation of mGluR1 controls the persistency of cocaine-evoked plasticity in VTA. a. Experimental protocols used for i.p. injections of AIDA (top) and Ro 67-7476 (bottom). b, c. AMPAR-EPSCs obtained at -70, 0, and +40 mV and respective mean RI for the experimental group treated with AIDA. $F_{(4, 69)} = 33.6$, ***$p < 0.001$. d, e. Corresponding graphs using Ro 67-7476, the positive modulator of mGluR1. $F_{(3, 23)} = 4.7$, *$p < 0.01$.

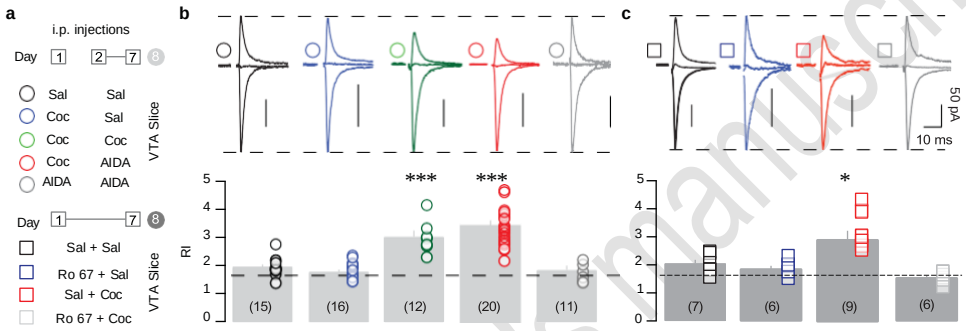
Figure 3. Modulation of mGluR1 controls cocaine-evoked plasticity in the NAc. a. Experimental protocol. b. AMPAR-EPSCs obtained at -70, 0, and +40 mV and NMDAR-EPSCs (shaded trace) obtained at +40 mV in medium spiny neurons of the NAc shell. c. I-V plot for AMPAR-EPSCs. d. Averaged AMPA/NMDA ratios obtained at +40 mV for each experimental group. $F_{(3, 31)} = 9.23$, *$p < 0.05$, **$p < 0.01$. e, f, g. LFS-LTD in the different experimental groups. h. Experimental protocol. i. AMPAR-EPSCs and NMDAR-EPSCs obtained as above in slices from animals treated with the mGluR1 enhancer Ro 67-7476 as indicated in h. j. I-V plots of AMPAR-EPSCs. k. Averaged AMPA/NMDA ratios obtained at +40 mV. $F_{(3, 28)} = 3.59$, *$p < 0.05$.

Figure 4. Early and enduring synaptic plasticity in the NAc after a single injection of cocaine. a. Disruption of mGluR1 function selectively in the VTA through stereotactic injection of TAT-mGluRct (see Fig. 1a) and preparation of NAc slices at day eight. b. AMPA- and NMDA-EPSCs obtained at -70, 0, +40 mV. c. I-V plots of AMPA-EPSCs. d. Averaged AMPA/NMDA ratio obtained at +40 mV. $F_{(2, 22)} = 6.17$, *$p < 0.05$. e. Experimental protocol as in a but preparation of NAc slices performed after 35 days of withdrawal. f. AMPA- and NMDA-EPSCs obtained at -70, 0, +40 mV. g. I-V relationship of AMPA-EPSCs. h. Averaged RI.

Figure 5. Disruption of NMDARs in midbrain DA neurons abolishes enduring plasticity in the NAc and reduces incubation of craving. a. Timeline of experimental protocol and number of cocaine infusions during self-administration in the two groups. b. Cue-induced lever pressing at day 35 was significantly reduced for the active lever in the $NR1^{DATCreERT2}$ mice. c. AMPA- and NMDA-EPSCs (grey) obtained at -70, 0, +40 mV for $NR1^{DATCreERT2}$ (red) and control mice (black) in VTA and NAc. d. Averaged AMPA/NMDA ratio and RI in the VTA and NAc for the two genotypes. e. Correlation between RI and lever presses for each neuron recorded (filled symbols represent the average RI for a given mouse).



Mameli et al., Fig. 1



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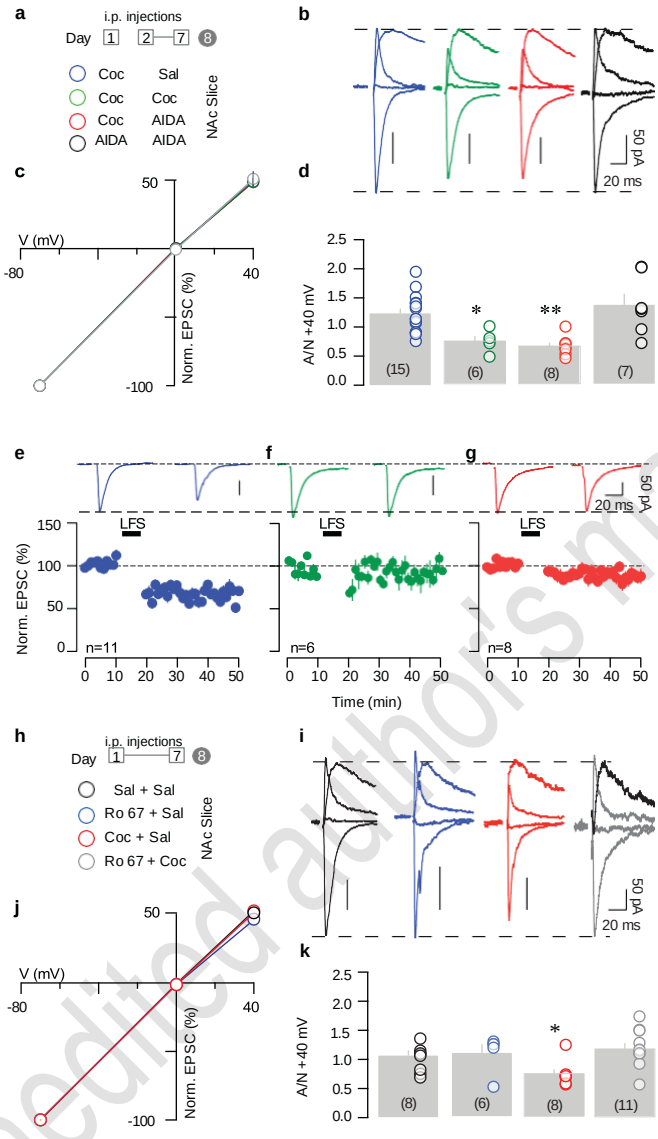

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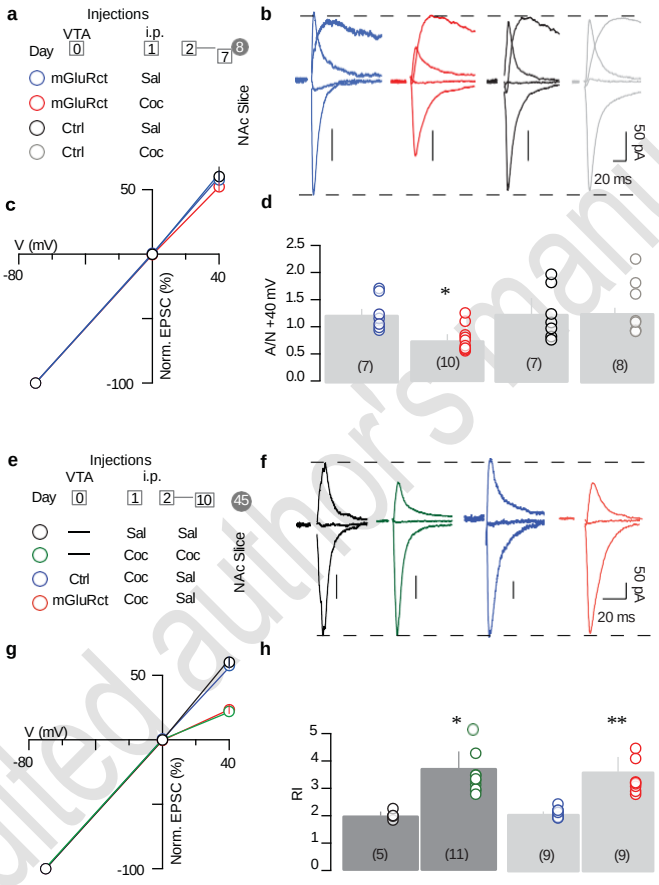

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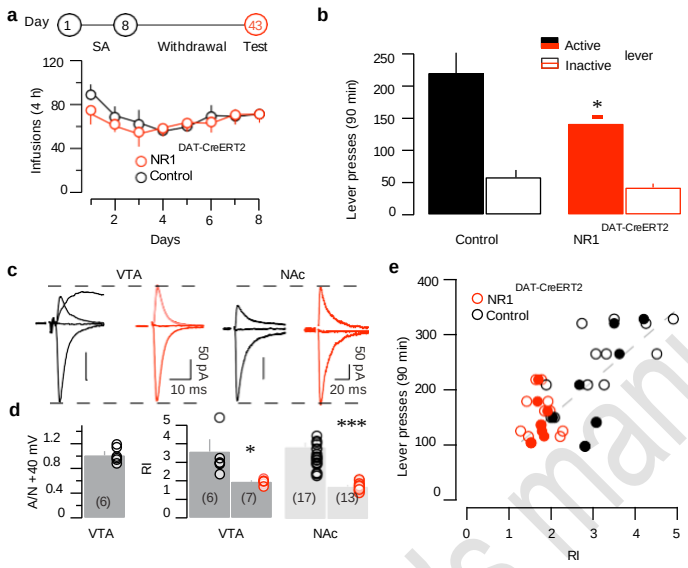

Mameli et al., Fig. 5