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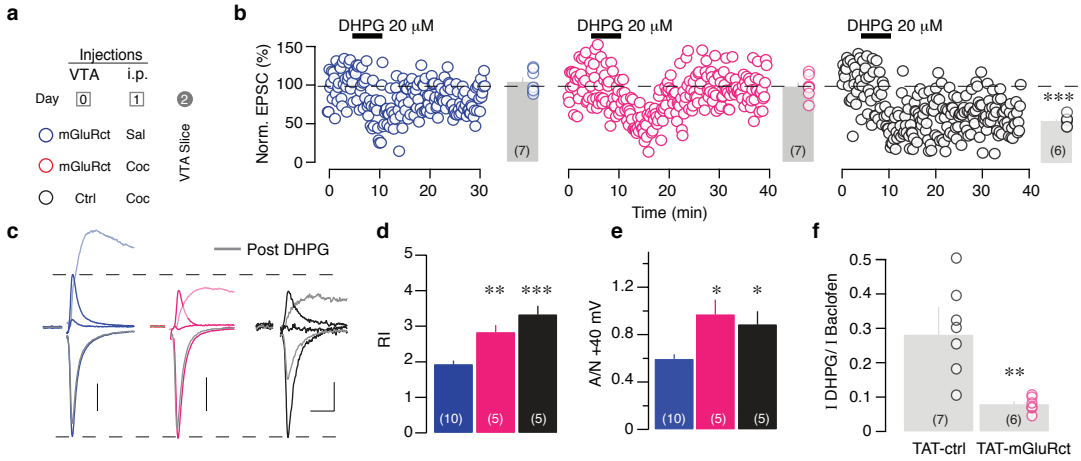

Figure S1. TAT-mGluRct inhibits mGluR function in vitro. a. Experimental protocol. b. DHPG (20 µM) application induced LTD only when cocaine (i.p.) followed the stereotactic injection of TAT-ctrl (black circles). When TAT-mGluRct was followed either by saline or cocaine injection, DHPG did not induce long-lasting changes in the AMPA EPSCs. These results suggest that TAT-mGluRct does not change the basal properties of these synapses on expressing LTD. Moreover, the fact that LTD is absent when TAT-mGluRct precede cocaine injections suggests that the function of mGluR was significantly reduced in order to block the induction of the plasticity. Relative bar graphs indicate the magnitude of LTD in each experimental group. (Significance was obtained by One-Way ANOVA, $F(2, 17) = 26.1$, $^{***}p < 0.001$). c. Sample traces of AMPA-mediated EPSC recorded at -70, 0, +40 mV and NMDA-EPSC recorded at +40 mV in each experimental group. Grey lines are averaged traces obtained in the same cell after application of DHPG. d. RI (AMPA EPSC -70 mV/EPSC +40 mV) obtained in the different experimental groups (Significance was tested by One-Way ANOVA; RI: $F(2, 17) = 21.39$; A/N: $F(2, 17) = 6.8$, $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$). Note that TAT injection does not alter the effect of cocaine on rectification index. e. Same as d but for A/N ratio. f. DA neurons of the VTA from mice injected with TAT-ctrl and TAT-mGluRct were patched by using a K-gluconate-based internal solution and maintained at a potential of -50 mV. DHPG- and baclofen-evoked currents were obtained by bath-application of DHPG (20 µM, 3 min) and baclofen (100 µM, 10 min) in the two experimental groups (Significance was tested by t-test, $t(22) = 5.7$, $^{**}p < 0.01$). DHPG- vs baclofen-evoked current ratio are plotted. Note that the I Baclofen did not change in the two groups (Baclofen: TAT-ctrl = 256.7 ± 34.5 pA; TAT-mGluRct = 287.1 ± 80.1 pA; DHPG: TAT-ctrl = 57.9 ± 8.4 pA; TAT-mGluRct = 19.9 ± 3.1 pA). All experiments were performed in presence of picrotoxin, the GABA-A antagonists. Scale bars 10 ms and 50 pA.

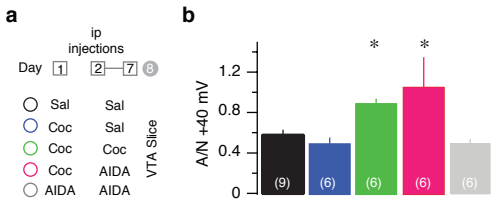

Figure S2. Modulation of mGluR1 controls the persistency of cocaine-evoked plasticity in VTA. a. Experimental protocols used for i.p. injections of AIDA (0.25 mg/kg). b. Mean AMPA/NMDA ratio for the experimental group treated with mGluR1 antagonist AIDA. Cocaine-evoked plasticity is persistent either with 7 injections of cocaine or if the first injection of cocaine is followed by 6 injections of AIDA (significance was tested using One-Way ANOVA, $F(4, 28) = 3.97$, $^*p < 0.05$).

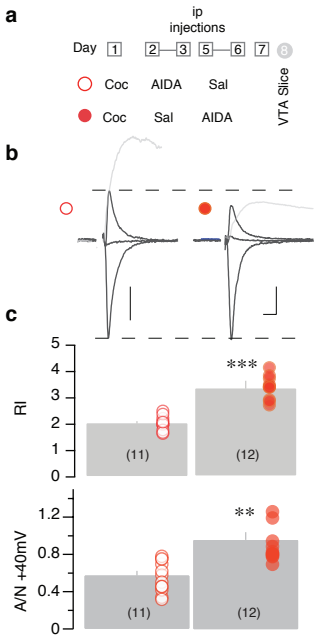

Figure S3. Timeline for the modulation of mGluR1 on the persistency of cocaine-evoked plasticity in VTA. a. Experimental protocols used for i.p. injections of AIDA (0.25 mg/kg). b. AMPAR-EPSCs obtained at -70, 0, and +40 mV and NMDA-EPSC obtained at +40 mV for the two experimental groups treated with mGluR1 antagonist AIDA at different time points. c. Mean RI (top) and AMPA/NMDA ratio (bottom) for the two experimental groups. Cocaine-evoked plasticity is persistent only when AIDA was injected at days 5-6 but not 2-3 suggesting for a narrow window of activation of mGluR1 (significance was tested using t-test; RI: $t(21) = 3.72$, $^{**}p < 0.01$; A/N: $t(22)$: 4.67, $^{***}p < 0.001$). Scale bars 10 ms and 50 pA.

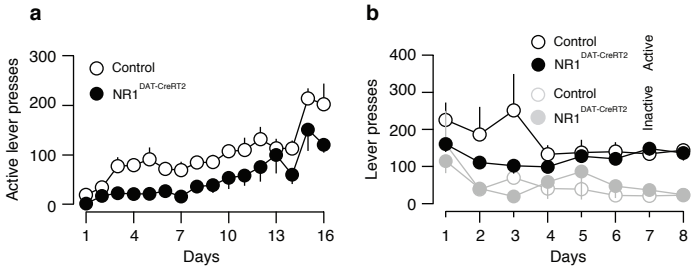

Figure S4. Lever pressing for food and cocaine in NR1DATCreERT2 mice. a. Acquisition of lever pressing for food in control and NR1DATCreERT2 mice. Mean (+s.e.m.) number of active lever presses per 90 min sessions, for the 16 sessions of food training in control (empty circles) and NR1DATCreERT2 (filled circles) mice. b. Cocaine self-administration in control and NR1DATCreERT2 mice. Mean (±s.e.m.) number of lever presses in 4 hours sessions by control (empty circles) and NR1DATCreERT2 (filled circles) mice during 8 consecutive days. Data represent presses on active (black) and inactive (grey) levers.

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Online methods

Animals

$NR1^{DATCreERT2}$ mice were generated by crossing mice carrying an inducible Cre-recombinase under the DAT-promoter with mice carrying floxed alleles for NR1 [1]. The mutation was induced in 5 months old mice by repeatedly injecting 1 mg/kg tamoxifen i.p., twice a day for 5 days. To ensure downregulation of NMDARs and a sufficient wash out period after tamoxifen treatment [2] control and $NR1^{DATCreERT2}$ mice were between 7 and 8 months old when the behavioral experiments started. They weighed 29.8 ± 1.4 g and 31.1 ± 0.8g, respectively, on the day of surgery. Mice were single housed under constant temperature (21 ± 2 °C) and humidity conditions (50 ± 5%). All experiments took place during the light-phase of the dark-light cycle, between 07:00 and 19:00. On 3 occasions during the food training phase mice received limited access to food. They were otherwise given unrestricted access to food and water throughout the whole set of experiments. Animals weight was monitored daily and kept above 85% of their initial weight. The experiments were conducted in accordance with the ethical EU guidelines for the care and use of laboratory animals, and were approved by the local animal care committee (Karlsruhe, Germany) as well the Institutional Animal Care and Use Committee of the University of Geneva.

Drug treatment

C57BL/6 mice and Pitx3-GFP mice (postnatal day 16-35) were injected i.p. with 15 mg/kg cocaine, 0.9% saline, 0.25 mg/kg AIDA or 4 mg/kg Ro 67-7476 (injected 1h prior saline or cocaine injections) using a 26G hypodermic needle (injection volume 50-100 µl) to minimize stress.

Unless specified, drugs were obtained from Tocris; spermine and picrotoxin were obtained from Sigma; cocaine was obtained from the pharmacy of the Hopitaux

Universitaires de Genève. Ro 67-7476 was a gift from F. Knoflach (F. Hoffman-La Roche Ltd, Basel, Switzerland). For the behavioral experiments, cocaine hydrochloride (Sigma-Aldrich, Chemie GmbH, Germany) was dissolved in saline. Ketamine and xylazin solutions were obtained from Pharmanovo GmbH (Hannover, Germany). All solutions injected intravenously were first filtered through sterile filters (0.2 μm).

Stereotactic TAT-fused peptide delivery

Mice were anesthetized with ketamine (100 mg/kg) and xylazine (10 mg/kg), and placed in a stereotactic frame (myNeuroLab). The TAT sequence (YGRKKRRQRRR) was fused with N-carboxyfluorescein and an homologous sequence of the carboxy terminal of Gp I mGluRs in order to prevent Homer 1b/c binding (ALTPPSPFR, Primm, Milano). As a control the TAT sequence was fused only with N-carboxyfluorescein. TAT-fused peptides were injected (0.6 μl at the concentration of 1 μM) with a glass pipette (Drummond Scientific Company) bilaterally (antero-posterior -2.4 mm, lateral 0.8 mm from Bregma, and -4.4 mm from the surface).

Electrophysiology in acute brain slices

Horizontal slices from midbrain (250 μm thick) and coronal slices containing the nucleus accumbens (NAc, 300 μm thick) were prepared following the different experimental injections protocols (see text and figures). Slices were kept in artificial cerebrospinal fluid containing (mM) 119 NaCl, 2.5 KCl, 1.3 $MgCl_2$, 2.5 $CaCl_2$, 1.0 NaH_2PO_4, 26.2 $NaHCO_3$, 11 glucose and bubbled with 95% O_2 and 5% CO_2. Whole-cell voltage-clamp recording techniques were used (30–32 °C, 2-3 ml/min, submerged slices) to measure the holding currents and synaptic responses of dopaminergic (DA) neurons of the VTA and of medium spiny neurons (MSN) of the NAc shell. The VTA is defined as the region medially to the MT (medial terminal nucleus of the accessory optical tract). DA neurons were identified either by the presence of a large hyperpolarization-activated (I_h) current

immediately after obtaining a whole-cell configuration (80%) or by using slices form a Pitx3-GFP mouse strain (20%) that express green-fluorescent protein (GFP) only in cells that express Pitx3[3], a transcription factor required for the development of DA neurons of the midbrain. The internal solution contained (mM) 130 CsCl, 4 NaCl, 2 $MgCl_2$, 1.1 EGTA, 5 HEPES, 2 Na_2ATP, 5 sodium creatine-phosphate, 0.6 Na_3GTP and 0.1 spermine. Currents were amplified, filtered at 5 kHz and digitized at 20 kHz. The liquid junction potential was small (–3 mV), and therefore traces were not corrected. All experiments were carried out in the presence of picrotoxin (100 μM) and AMPAR-EPSCs were pharmacologically isolated by application of the NMDA antagonist D,L-APV (100 μM). The NMDAR component was calculated as the difference between the EPSCs measured in the absence and presence of D,L-APV. The AMPAR to NMDAR ratio (A/N) was calculated by dividing the peak amplitudes. The rectification index (RI) was calculated by dividing the amplitude of the AMPAR-EPSC measured at –70 mV by the amplitude at +40 mV. The holding potential was –60 mV or –70 mV, and the access resistance was monitored by a hyperpolarizing step of –10 mV with each sweep, every 10 s. Experiments were discarded if the access resistance varied by more than 20%. Synaptic currents were evoked by stimuli (0.05-0.1 ms) at 0.1 Hz through bipolar stainless steel electrodes placed rostral to the VTA or, when recordings were performed in the NAc, at the prelimbic cortex−NAc border to stimulate preferentially cortical afferences. Where indicated, mGluR-LTD in DA neurons of the VTA was induced by application of the Gp I mGluR agonist DHPG (20 μM for 5 min.) while NMDA-dependent LTD in MSN of the NAc shell was induced by low frequency stimulation (LFS, 1 Hz at -40 mV for 10 min). Representative example traces are shown as the average of 10-20 consecutive EPSCs typically obtained at each potential or, in the case of plasticity protocols, during the last 5 minutes of the baseline and at least 20 minutes after the induction of plasticity.

Mouse behavior

All operant experiments were performed in mouse operant chambers model ENV-307W enclosed in light and sound-attenuating cubicles (Med-Associates, Georgia, Vermont). Each chamber was equipped with two ultrasensitive retractable levers located on each side of a food pellet dispenser during the food-shaping procedure. During i.v. self-administration, the drug delivery PVC tubing was attached to a swivel (Instech Solomon, Plymouth Meeting, PA) and connected to an infusion pump (PHM-100, Med-Associates) located outside the cubicle. Stimulus lights were located above each lever.

Food training: Sessions lasted 90min and started with the presentation of the two levers. To guarantee unbiased lever training, the side of the initially active lever was alternated between each session. Active lever presses were reinforced by the delivery of a 16 mg sweetened pellet (Bio-Serv, Frenchtown, New Jersey) under the following schedule: Fixed Ratio 1 (FR1) for 8 reinforcements, FR2 for 4 reinforcements and FR4 for at least 10 reinforcements with 80% accuracy on the active lever, which completed a cycle. When such a cycle was achieved, the side of the active lever was switched. All animals underwent 16 daily food-training sessions. On 3 occasions, sessions were separated by a day of inactivity during which animals had limited access to food. This happened between the sessions 2 and 3, 9 and 10 and between the sessions14 and 15.

Intravenous self-administration: Following food shaping mice were allowed 24 h before undergoing surgery. Mice were anesthetised with a ketamine (160 mg/kg) and xylazyne (38 mg/kg) solutions and implanted with a catheter in the right jugular vein. On one end catheters were introduced for circa 1 cm toward the heart, while the other extremity was passed subcutaneously to an exit in the mid-scapular region. Catheters were (MIVSA, CamCaths, UK) made of silicone elastomer tubing (O/D 0.63 mm x I/D 0.30 mm) attached to 26 gauge stainless steel tubing secured to a Bard mesh pad. Animals were given a minimum of 48 h recovery before intravenous self-administration sessions were initiated. Catheters were flushed daily before and after self-administration sessions with 30µl of a heparine solution in saline (20 UI). At the beginning of the cocaine self-

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administration sessions, mice were placed into the operant chambers and the two levers were presented. Presses on the active lever under an FR 1 resulted in the infusion of 0.5 mg/kg/infusion cocaine solution by activation of the infusion pump for 1.2 s. The side of the active lever was randomly assigned across animals on the first day of IV self-administration and remained constant thereafter. Each drug infusion was associated with a conditioned stimulus (CS) consisting of 10 s flashing of stimulus-lights located above the levers. To avoid accidental overdose, a 40 s time-out period followed by each infusion was given, during which active levers were recorded but had no consequence. Each session lasted 4 h and animals received a total of 8 sessions.

Incubation effect - cue-induced cocaine-seeking: At the end of the intravenous self-administration phase, animals were returned to their home cage where they remained for 35 days. After this time, they were re-introduced in the operant chambers, where they were tested for cue-induced cocaine seeking. The session lasted 90 min during which, as in the self-administration condition, presses on the active lever under an FR1 triggered CS presentation and activation of the infusion pump. However, cocaine was no longer available and CS presentation lasted 5 sec only. Such a short CS presentation was preferred in order to prevent as much as possible extinction toward its repeated exposure.

Statistical analysis

Compiled data are expressed as mean ± s.e.m. The level of significance was taken at $p = 0.05$ as determined by the non-parametric Mann-Whitney or Wilcoxon tests. 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. Data from cue-induced cocaine seeking were analysed using factorial ANOVA to investigate the effect of genotype and lever. Analyses were followed by Newman-Keuls tests when necessary.

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