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Cocaine inverts rules for synaptic plasticity of glutamate transmission in the ventral tegmental area

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The manner in which drug-evoked synaptic plasticity affects reward circuits remains largely elusive. We found that cocaine reduced NMDA receptor excitatory postsynaptic currents and inserted GluA2–lacking AMPA receptors in dopamine neurons of mice. Consequently, a stimulation protocol pairing glutamate release with hyperpolarizing current injections further strengthened synapses after cocaine treatment. Our data suggest that early cocaine-evoked plasticity in the ventral tegmental area inverts the rules for activity-dependent plasticity, eventually leading to addictive behavior.

A widely accepted hypothesis posits that addictive drugs hijack learning processes through remodeling of neural circuits that underlie positive reinforcement, such as the mesolimbic dopamine system[1]. Cocaine, for example, induces synaptic plasticity of excitatory afferents onto dopamine neurons in the ventral tegmental area (VTA) 24 h after a single injection. This can be seen as an increase of the AMPA:NMDA ratio[2]. These synaptic changes require D1/D5 receptor and NMDA receptor (NMDAR) activation in dopamine neurons and are associated with the appearance of rectifying AMPA receptor (AMPAR) excitatory postsynaptic currents (EPSCs), suggesting that GluA2-lacking AMPARs are inserted into the synapse[2–5]. However, as GluA2-lacking AMPARs conduct very little current at +40 mV, their insertion alone cannot explain the increase in the AMPA:NMDA ratio.

How cocaine-evoked plasticity affects long-term potentiation (LTP) at the same synapses is controversial. Several studies have found that a spike timing–dependent plasticity protocol that triggers LTP in naive- and saline-injected animals is no longer efficient after cocaine treatment. This observation was interpreted as an occlusion of further LTP, when the number of AMPARs is already saturated after the drug treatment[2,6].

We used two-photon laser photolysis (2-PLP) of caged glutamate to evoke unitary (u) NMDAR and AMPAR EPSCs in dopamine neurons of the VTA (**Fig. 1a**, **Supplementary Fig. 1** and **Supplementary Methods**; animal procedures were approved by the animal welfare committee of the University of Geneva). By mapping dendrites visualized after filling the cell with Alexa-Fluor 594 through the patch pipette, uAMPAR-EPSCs could be elicited (**Fig. 1b**,**c**). On average, uAMPAR EPSCs had an amplitude of 14.7 ± 0.7 pA, were sensitive to 2,3-dihydroxy-6-nitro-7-sulfamoyl-benzo[f]quinoxaline-2,3-dione (NBQX, 20 μM, n = 3) and displayed kinetics that were similar to spontaneous EPSCs (sEPSC; **Fig. 1d**–**f**).

To characterize cocaine-induced synaptic plasticity at single putative glutamatergic synapses, we isolated uNMDAR and uAMPAR EPSCs in slices from both saline- and cocaine-treated mice (**Supplementary Fig. 1**). We then compared absolute unitary current amplitudes for AMPAR (measured at −70 mV) and NMDAR (measured at +40 mV) EPSCs in both treatment groups and observed that, after cocaine treatment, uNMDAR EPSCs were reduced and uAMPAR EPSCs were increased (**Fig. 1g**–**i**). We then pharmacologically isolated uAMPAR EPSCs and recorded their amplitudes at +40, 0 and −70 mV. This allowed us to calculate the rectification index, which increased after cocaine exposure, consistent with previous data[3,4] (**Fig. 1j**–**l**). In cells in which we were able to measure all three parameters, we noticed a strong correlation between uNMDAR EPSCs, uAMPAR EPSCs and rectification index along with an increase in AMPA:NMDA ratios (**Fig. 1m**,**n**). We found the decrease of uNMDAR EPSCs to be a predictor of a high rectification index and a large uAMPAR EPSC amplitude (**Fig. 1m**). Our results are consistent with the idea that GluA2-lacking AMPARs are inserted[3] and suggest that there is a reduction of NMDAR function.

In hippocampal interneurons that constitutively contain GluA2-lacking AMPARs, a form of long-term plasticity can be observed that relies on calcium entry through AMPARs and is independent of NMDARs[7]. Such LTP is known as anti-Hebbian because its induction requires a pairing of presynaptic activity with postsynaptic hyperpolarization[7]. We therefore compared two spike timing–dependent plasticity protocols in current-clamp mode for their efficiency to elicit LTP in slices obtained after cocaine or saline treatment. For the first protocol, the excitatory postsynaptic potential (EPSP) elicited by extracellular stimulation of the axons of the presynaptic neurons was followed by a depolarizing current injection (depolarizing spike timing–dependent (STD) protocol; **Fig. 2a**). For the second protocol a hyperpolarizing injection was applied (hyperpolarizing STD protocol; **Fig. 2b**). As reported previously[6], after a saline injection, the depolarizing STD protocol elicited robust LTP (**Fig. 2c**), whereas the hyperpolarizing STD protocol did not affect the EPSP amplitudes (**Fig. 2d**). After a single injection of cocaine, the opposite effect was observed (**Fig. 2c**,**d**). In slices obtained from mice treated with cocaine, the depolarizing STD protocol failed to induce LTP[6] (**Fig. 2c**), whereas the hyperpolarizing STD protocol induced a robust LTP (**Fig. 2d**).

To confirm the contrasting induction requirements for LTP, we determined the effects of a NMDAR blocker ((2R)-amino-5-phosphonovaleric acid, AP5, 100 μM) and an AMPAR blocker (NBQX, 20 μM) (**Fig. 2e**,**f**). After saline injection, AP5, but not NBQX, blocked the LTP that was normally induced by the depolarizing STD

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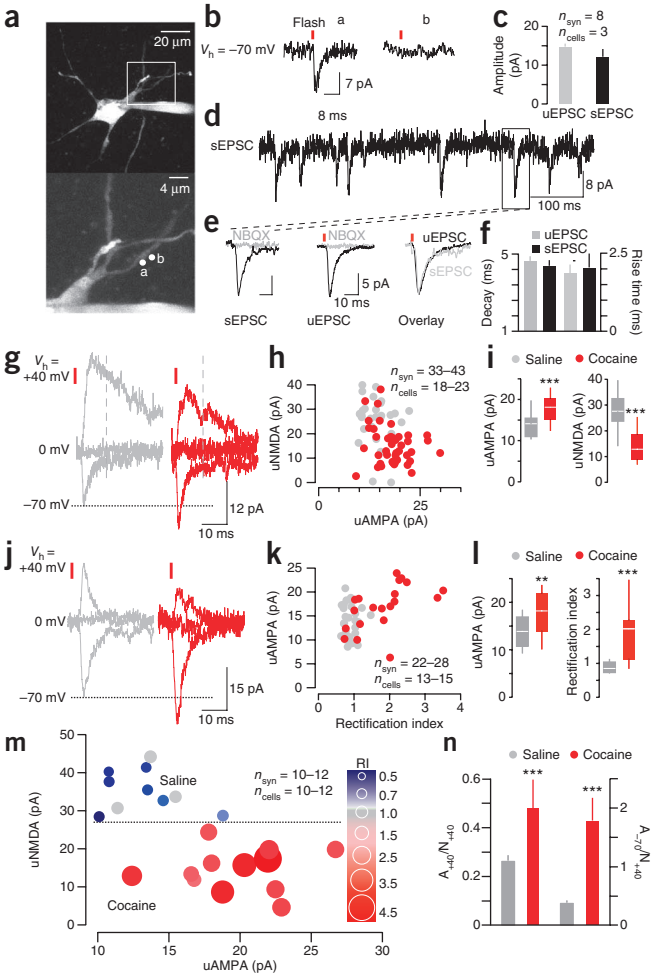

Figure 1 Cocaine alters NMDA and AMPA transmission at single synapses onto dopamine neurons of the VTA. (**a**) Two-photon laser-scanning microscopy image of a dopamine neuron (top) and a magnified dendrite (inset and bottom) showing two uncaging locations. (**b**) 2-PLP evoked a uEPSC at –70 mV at location a, but not at location b. (**c**) Summary plot of uEPSC and sEPSC amplitudes obtained in drug-naive mice (uEPSC = 14.7 ± 0.7 pA, sEPSC = 12.2 ± 2 pA, $t_6 = 1.7$, $P > 0.5$). (**d**,**e**) Sample trace of sEPSC; comparison between sEPSC and uEPSC and blockade by NBQX. (**f**) Summary plot of uEPSC and sEPSC decay (90–37%) and rise time (10–90%; decay time, uEPSC = 4.5 ± 0.3 ms, sEPSC = 4.2 ± 0.4 ms, $t_6 = 1.95$, $P > 0.5$; rise time, uEPSC = 1.8 ± 0.2 ms, sEPSC = 2 ± 0.4 ms, $t_{13} = 0.36$, $P > 0.5$). (**g**) 2-PLP–evoked uEPSC at –70, 0 and +40 mV in slices from saline- (gray) and cocaine-treated (red) mice. uNMDA amplitude was taken 10 ms after the EPSC onset (dashed line). (**h**,**i**) Scatter plot and related box plots for uEPSC AMPA and uEPSC NMDA (saline, uNMDA = 25.9 ± 1.6 pA, uAMPA = 14.2 ± 0.6 pA; cocaine, uNMDA = 15.1 ± 1.2 pA, uAMPA = 18.1 ± 0.6 pA; uAMPA saline versus cocaine, $t_{48} = 3$, $P < 0.001$; uNMDA saline versus cocaine, $t_{74} = 5.8$, $P < 0.0001$). (**j**) uAMPA EPSC evoked by 2-PLP at –70, 0, +40 mV in the presence of AP5 (100 µM; note that no current was measured 10 ms after onset; saline, 1.2 ± 0.2 pA; cocaine, 0.9 ± 0.3 pA). (**k**,**l**) Scatter plot obtained for uAMPA values and rectification index (saline, uAMPA = 13.9 ± 0.6 pA, rectification index = 0.8 ± 0.03; cocaine, uAMPA = 17.5 ± 1 pA, rectification index = 1.9 ± 0.1; uAMPA saline versus cocaine, $t_{74} = 4.3$, $P < 0.0001$; rectification index saline versus cocaine, $t_{48} = 5.9$, $P < 0.0001$). (**m**) Scatter plot combining the uNMDA, uAMPA and rectification index (color and size coded). The correlation (r^2) between AMPAR uEPSC and NMDAR uEPSC was 0.644 ($P = 0.001$), AMPAR EPSC and rectification index (RI) was –0.649 ($P = 0.001$) and NMDAR EPSC and rectification index was –0.736 ($P = 0.0001$). (**n**) Averaged AMPA/NMDA ratios; uAMPA was measured at +40 (left) or –70 (right) mV, whereas uNMDA was measured at +40 mV (A_{+40}/N_{+40}, saline = 0.26 ± 0.2, cocaine = 0.48 ± 0.1, $t_{20} = 1.9$, $P < 0.0001$; A_{-70}/N_{+40}, saline = 0. 4 ± 0.03, cocaine = 1.8 ± 0.4, $t_{20} = 3.3$, $P < 0.0001$). Compiled data are expressed as mean ± s.e.m.

protocol (**Fig. 2e**,**f**). Following cocaine treatment, the hyperpolarizing STD protocol still induced LTP in the presence of AP5, but LTP was blocked by NBQX (**Fig. 2e**,**f**). In another set of experiments, we found that AMPAR EPSPs were sensitive to 1-naphthylacetyl spermine trihydrochloride (Naspm), a selective blocker of GluA2-lacking AMPARs, only in slices from cocaine-treated mice (**Supplementary Fig. 2**). Furthermore, Naspm application blocked the induction of hyperpolarizing STD-LTP (**Fig. 2g**). Finally, to demonstrate the dependence of LTP on intracellular calcium, we filled the neurons with 1,2-bis(o-aminophenoxy)ethane-*N*,*N*,*N*′,*N*′-tetraacetic acid (BAPTA), which rendered the hyperpolarizing STD protocol inefficient (**Fig. 2h**).

Taken together, these results indicate that cocaine treatment replaces GluA2-containing AMPARs with GluA2-lacking ones, while simultaneously reducing NMDAR function, eventually altering the conditions for synaptic calcium entry. Although depolarization is required for calcium entry in drug-naive or saline-treated mice, hyperpolarization becomes an efficient manipulation after cocaine treatment, ultimately inverting the rules of LTP induction in dopamine neurons.

In contrast with GluA2 knockout mice, in which AMPAR EPSCs are fully rectifying[8], we found only a partial rectification following cocaine treatment, even at single synapses. This suggests that, even in the population of synapses sensitive to cocaine, a partial exchange of GluA2-containing for GluA2-lacking AMPARs occurs. This is consistent with a previous report that found that some GluA2 immunoreactivity was still present at the synapse following the drug treatment[5].

It has been argued that NMDAR function is not affected by cocaine treatment, as whole-cell currents induced by bath application of a given concentration of NMDA were not affected by cocaine exposure[2]. However, such bath application of an agonist will indiscriminately activate synaptic and extrasynaptic NMDARs[9]. As such, this finding may not be able to resolve modifications of synaptic receptor populations, especially if the overall number of synaptic and extrasynaptic receptors does not change.

A small uNMDAR EPSC is predictive of a large uAMPAR EPSC and a high rectification index, indicating that these changes occur at the same synapses. Whether they are driven by the same mechanism needs to be further investigated, but is consistent with previous findings in hippocampal interneurons that the expression of the GluA2-lacking subunit is associated with a low expression of the NR1 subunit[7,10]. Our observation is also consistent with a report showing that, at the electron microscopic level, cocaine drives the insertion of the GluA1 in only a fraction of synapses onto VTA dopamine neurons[11]. This finding infers that drug exposure might differentially affect discrete inputs from specific regions. Further studies are needed to identify these glutamatergic afferents onto dopamine neurons.

Consistent with previous reports, we found that, in dopamine neurons of naive or saline-treated mice, an NMDAR-dependent STD protocol effectively drives LTP[6] and that this protocol is ineffective after cocaine exposure[6]. Although this initial interpretation implies an occlusion between the expression mechanism of STD LTP and cocaine-evoked plasticity, our data suggest that the failure to induce further LTP after cocaine treatment is a result of a smaller synaptic NMDAR component. This suggests that the functional consequences of drug-evoked synaptic plasticity change the induction requirements for LTP from an excitation coupled with a depolarization to an excitation coupled with a hyperpolarization. Dopamine neurons can

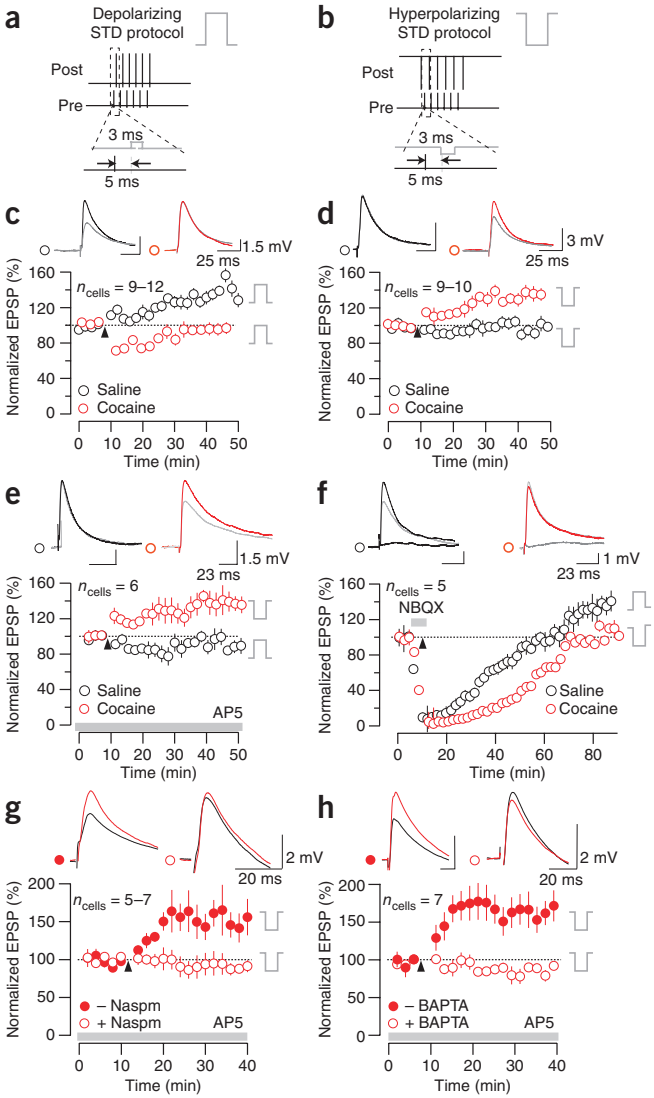

Figure 2 Cocaine treatment inverts the rules of LTP induction at excitatory inputs onto dopamine neurons of the VTA. (**a**) Depolarizing STD protocol (20 bursts at 5-s intervals; burst consisted of five stimuli at 10 Hz). A postsynaptic spike was triggered by injection of positive current (+1.5 nA for 3 ms) 5 ms after the onset of the EPSP. (**b**) Hyperpolarizing STD protocol (as above, except injection of a negative current (–1.5 nA for 3 ms)). (**c**) Time versus amplitude plots and sample traces of AMPAR EPSPs in slices obtained from saline- (black circles) and cocaine-treated (red circles) mice using the depolarizing STD protocol (saline, 134.5 ± 10%, $t_5 = 13.22$, $P < 0.001$; cocaine, 98.5 ± 6%, $t_5 = 1.4$, $P > 0.05$). (**d**) Data are presented as in **c**, but the hyperpolarizing STD protocol was used (saline, 93.9 ± 9%, $t_4 = 0.5$, $P > 0.05$; cocaine, 145.7 ± 17%, $t_5 = 7.7$, $P < 0.01$). (**e**) Time versus amplitude plots and sample traces of STD protocols used in slices from saline- (black circles) and cocaine-treated (red circles) mice when in presence of AP5 (saline, 95.9 ± 7%, $t_5 = 1.8$, $P > 0.05$; cocaine, 139.8 ± 15%, $t_5 = 12$, $P < 0.0001$). (**f**) Time versus amplitude plots and sample traces of STD protocols used in slices from saline- (black) and cocaine-treated (red) mice when in presence of NBQX (saline, 118.8 ± 9%, $t_5 = 2.9$, $P < 0.05$; cocaine, 95.1 ± 8%, $t_5 = 1.7$, $P > 0.05$). (**g**) Time versus amplitude plots and sample traces of the hyperpolarizing STD protocol in slices from cocaine-treated mice in the presence (open circles) or absence (filled circles) of Naspm (control, 153.9 ± 4%; Naspm, 83.2 ± 4%; $t_7 = 11.42$, $P < 0.001$). (**h**) Time versus amplitude plots and sample traces of the hyperpolarizing STD protocol in slices from cocaine-treated mice in the presence (open circles) or absence (filled circles) of intracellular BAPTA (control, 165.3 ± 3%; BAPTA, 80.1 ± 3%; $t_9 = 21.4$, $P < 0.001$). Compiled data are expressed as mean ± s.e.m.

be hyperpolarized in several conditions. During the acute phase of cocaine exposure, dopamine levels increase in response to DAT inhibition, which activates D2 receptors[12]. Starting with the second dose, anti-Hebbian mechanisms may therefore favor further strengthening of excitatory afferents. Dopamine neurons may also be hyperpolarized through activity of inhibitory inputs. This occurs when an expected reward is omitted or when the animals are exposed to a salient, but aversive, stimulus[13].

Taken together, our results reveal the capacity for further strengthening at drug-potentiated synapses that relies on GluA2-lacking AMPAR activation and suggest that cocaine-evoked plasticity is permissive for a specific form of further synaptic plasticity. In fact, cocaine-evoked plasticity in the VTA, when present for prolonged periods of time, can trigger synaptic changes downstream in the NAc[14,15]. Ultimately, such staged synaptic plasticity may contribute to the network reorganization believed to underlie the behavioral changes. Because addiction depends on the associations of cues, behavior and drugs, and involves the whole mesolimbic dopaminergic system, GluA2-dependent LTP in the VTA might represent an aberrant form of plasticity that drives long-term adaptations in the downstream reward circuits, eventually leading to addiction.

Note: Supplementary information is available on the Nature Neuroscience website.

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AUTHOR CONTRIBUTIONS

M.M. carried out all experiments with two–photon laser glutamate uncaging. M.M., M.T.C.B. and C.B. contributed to the long-term plasticity experiments. C.L. designed the study together with M.T.C.B., C.B. and M.M. and wrote the manuscript with the help of all of the authors.

COMPETING FINANCIAL INTERESTS

The authors declare no competing financial interests.

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