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Cocaine triggered AMPA receptor redistribution is reversed *in vivo* by mGluR-dependent long-term depression

Camilla Bellone[1] & Christian Lüscher[1,2]

Drugs of abuse induce long-lasting changes in neural circuits that may underlie core components of addiction. Here we focus on glutamatergic synapses onto dopamine (DA) neurons of the ventral tegmental area (VTA). Using an '*ex vivo*' approach in mice, we show that a single injection of cocaine caused strong rectification and conferred sensitivity to the polyamine Joro spider toxin (JST) of AMPAR-mediated excitatory postsynaptic currents (AMPAR EPSCs), indicating the recruitment of receptors that lack GluR2. This qualitative change in transmission was paralleled by an increase in the AMPAR:NMDAR ratio and was prevented by interfering with the protein interacting with C kinase-1 (PICK1) *in vivo*. Activation of metabotropic glutamate receptors (mGluR1s) by intraperitoneal injection of a positive modulator depotentiated synapses and abolished rectification in slices of cocaine-treated mice, revealing a mechanism to reverse cocaine-induced synaptic plasticity *in vivo*.

All addictive drugs increase dopamine concentrations in target structures of the mesocorticolimbic projections[1,2]. Therefore, plasticity at glutamatergic afferents onto DA neuron of the VTA could participate in the incentive sensitization process thought to underlie addiction[3]. It is well established that the VTA is the locus of the induction of behavioral sensitization and contributes to conditioned place preference, both of which are models of core components of addiction. The tetanic stimulation of glutamatergic afferents onto DA neurons of the VTA can induce sensitization in drug-naïve rats[4]. On the basis of this finding, the synaptic plasticity of glutamatergic transmission onto DA neurons has been implicated as being the underlying cellular substrate[5].

In fact, glutamatergic synapses onto DA neurons undergo several forms of plasticity. For example, these synapses can express NMDAR-dependent long-term potentiation (LTP)[6]. However, NMDAR-dependent LTP at these synapses is of rather modest magnitude[7,8]. In contrast, in response to a single dose of cocaine[8] and several other addictive drugs[9], the AMPAR:NMDAR ratio at excitatory synapses of the VTA strongly increases for up to 10 days[10]. Furthermore, repeated cocaine exposure over 5 days causes a loss of GABAergic inhibition that favors the induction of LTP (ref. 7). At first, these two observations seem contradictory, as an increase in the AMPAR:NMDAR ratio during cocaine administration may reflect AMPAR insertion associated with synaptic potentiation that would occlude further induction of LTP (ref. 11). In fact, using a pairing protocol to induce LTP, occlusion is observed after a single injection of cocaine[8]. This observation was recently challenged: in a spike timing–dependent plasticity protocol, and in the presence of $GABA_A$ receptor blockers, LTP was successfully induced in cocaine-treated rats[7].

Taken together, cocaine seems to drive a form of plasticity that shares some features with synaptic LTP (increase in the AMPAR:NMDAR ratio) but does not occlude further induction of LTP, which suggests that the two forms of plasticity have distinct mechanisms. A possible scenario that could account for both observations is the insertion of GluR2-lacking, calcium (Ca)-permeable AMPARs.

The possibility of a switch to Ca-permeable AMPARs at this synapse after *in vivo* cocaine exposure is further supported by a number of indirect pieces of evidence from studies in rodents. The observation that cocaine exposure increases the expression of GluR1 in DA neurons of the VTA suggests the involvement of Ca-permeable AMPARs (refs. 12,13). Furthermore, whereas the overexpression of the AMPAR subunit GluR1 is sufficient to elicit sensitization in drug-naïve rats, this behavior is reduced when GluR2 is overexpressed[12]. Finally, in mice lacking GluR1, the context-dependent associative components of sensitization and conditioned place preference are abolished[14].

Here, in acute slices of the VTA, we directly tested the hypothesis that a single exposure to cocaine drives the synaptic insertion of AMPARs that lack GluR2. Such AMPARs, in addition to their calcium permeability, also show strongly rectifying synaptic responses (that is, currents at positive potentials are smaller than currents at symmetrical negative potentials) and are sensitive to polyamine toxins[15]; therefore, their presence can be detected in acute brain slices using an *ex vivo* approach—namely, comparing AMPAR EPSCs of cocaine-treated mice to those of saline-treated or naïve mice. Our data indicated that the cocaine-evoked plasticity of excitatory synapses onto DA neurons of the VTA (refs. 8,9,14,16) involves a switch to synaptic AMPARs that lack GluR2.

[1]Department of Basic Neurosciences and [2]Clinic of Neurology, University of Geneva, 1 Michel Servet, CH-1211 Geneva, Switzerland. Correspondence should be addressed to C.L. (Christian.Luscher@medecine.unige.ch).

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AMPAR redistribution has best been studied in the hippocampus in conjunction with LTP (ref. 11) but may also occur in neurons of the VTA. The synaptic redistribution of AMPARs involves a number of protein-protein interactions with scaffolding proteins, some of which interact with the C-terminal tail of GluR2. The N-ethylmaleimide–sensitive factor (NSF)-binding site[17,18] interacts with NSF and the PDZ (postsynaptic density-95/Discs large/zona occludens-1) binding site interacts with PICK1 (refs. 19,20), glutamate receptor-interacting protein (GRIP)[21] and AMPA receptor-interacting protein (ABP)[22]. PICK1 regulates the removal and the synaptic insertion of GluR2-containing synaptic AMPARs (refs. 23–26). We therefore interfered with PICK1 function to test whether the immobilization of GluR2-containing receptors affects cocaine-driven plasticity.

If GluR-lacking receptors are inserted by cocaine exposure, a mechanism to reverse this plasticity would have to selectively remove the GluR2-lacking receptors to restore the initial GluR2-dependent transmission. Such a form of long-term depression (LTD) would depend on the presence of GluR2-lacking receptors. In fact, we have previously characterized an mGluR1-dependent LTD (mGluR-LTD)[27] that fulfills these criteria. Here we now identify mGluR-LTD as a mechanism that restores calcium-impermeable transmission in cocaine-treated mice *in vitro* and *in vivo*.

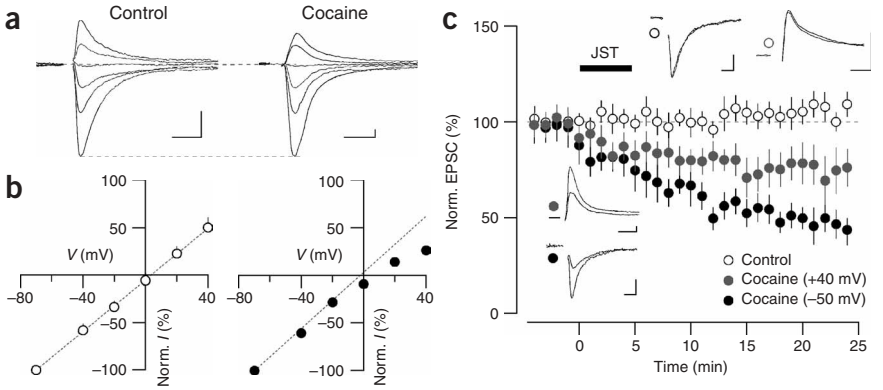

Figure 1 A single injection of cocaine induces a strong rectification and sensitivity to polyamines in AMPAR EPSCs. (**a**) Representative traces of AMPAR EPSCs recorded at +40 mV, +20 mV, 0 mV, –20 mV, –40 mV and –70 mV in neurons from control and cocaine-treated mice. (**b**) *I-V* plots of normalized and averaged AMPAR EPSCs of control (open symbols) and cocaine-treated mice (closed symbols; error bars are smaller than symbol for some data points). (**c**) JST (500 nM) depressed EPSCs recorded at –50 mV or +40 mV in mice treated with cocaine, but not in control mice (open symbols are pooled data recorded at both potentials). Insets, representative traces of control and cocaine-treated mice before and after the application of JST. Scale bars, 50 pA and 5 ms. Error bars represent s.e.m.

RESULTS

Cocaine increases rectification of AMPAR EPSCs

We characterized the AMPAR component of stimulus-evoked EPSCs in the VTA of cocaine-treated mice and found that EPSCs at negative potentials were larger than those measured at the symmetrical positive potentials (**Fig. 1a**). The rectification of EPSCs was confirmed in slices from cocaine-treated mice, but not control mice, by plotting the *I-V* relationship (**Fig. 1b**). This suggested that in cocaine-treated mice, some AMPARs were lacking the GluR2 subunit. In line with this interpretation, we found that EPSCs in cocaine-treated mice were sensitive to JST, a polyamine that blocks GluR2-lacking AMPARs. On average, JST blocked AMPAR EPSCs in cocaine-treated mice by 43.6 ± 6.6% compared to –7.0 ± 7.3% in naïve mice ($n = 6$, $P < 0.001$; **Fig. 1c**). GluR2-lacking AMPARs also contributed to EPSCs at +40 mV; we found that, on average, JST blocked AMPAR EPSCs in cocaine-treated mice by 29.2 ± 7.5% ($n = 6$, $P < 0.01$; **Fig. 1c**). Taken together, this partial inhibition at both holding potentials may reflect an incomplete diffusion of spermine or the appearance of double rectification (progressive loss of spermine block at increasingly positive potentials[15]). Although rectifying kainate receptors may, in theory, also contribute to the EPSCs measured here, their role is probably minor. Typically, kainate EPSCs have small amplitudes and slow kinetics[28]; in contrast, the synaptic responses observed here had substantial amplitudes and lasted less than 10–15 ms.

Rectification and AMPAR:NMDAR ratio change in parallel

Previous studies that have reported an increase in the AMPAR:NMDAR ratio using a similar *ex vivo* approach after cocaine treatment[8]. To confirm these findings, we recorded combined AMPAR-NMDAR–mediated EPSCs while holding DA neurons at +40 mV, and then recorded AMPAR EPSCs at various holding potentials in the presence of the selective NMDAR antagonist D(-)-2-amino-5-phosphonovaleric acid (D-AP5, 50 μM). This procedure allowed us to calculate the AMPAR:NMDAR ratio (by dividing the AMPAR EPSC amplitude measured either at –70 mV or +40mV by the NMDAR EPSC amplitude measured at +40 mV). We also calculated the rectification index (RI) by dividing the current at –70 mV by the amplitude at +40 mV in the same cells. The RI was 3.4 ± 0.2 (mean ± s.e.m.; $n = 12$) in cocaine-treated mice, which differed significantly from the value calculated for the control mice (**Fig. 2a**,**b**; 2.3 ± 0.2, $n = 23$, $P < 0.01$). In line with previous reports[8,9,14,16], both AMPAR:NMDAR ratios were significantly higher ($P < 0.01$) in cocaine-treated mice (+40/+40 mV: 0.77 ± 0.10, $n = 12$; and –70/+40 mV: 3.2 ± 1.1, $n = 8$) than in control mice (**Fig. 2a**,**c**; +40/+40 mV: 0.50 ± 0.03, $n = 27$; and –70/+40 mV: 0.86 ± 0.13, $n = 11$). The increase in the AMPAR:NMDAR ratio determined at +40 mV was of similar magnitude to the change in RI, which suggests a correlation between the two parameters. In fact, plotting AMPAR:NMDAR ratio as a function of RI confirmed this (**Fig. 2d**; $R = 0.63$, $P < 0.001$). These results indicate that the enhancement in AMPAR-mediated synaptic strength induced by cocaine was correlated with a change in the subunit composition of AMPARs.

AMPAR redistribution depends on PICK1-GluR2 interaction

Several possible models for this cocaine-driven AMPAR redistribution can be postulated. Redistribution may occur by inserting GluR2-lacking receptors on top of the existing pool of GluR2-containing receptors. Alternatively, GluR2-containing receptors may have to move out of the synapse to free up space for the insertion of GluR2-lacking receptors. In the latter case, the number of receptors may be limited by 'slots', where receptors are tethered to anchoring proteins of the postsynaptic density (PSD, ref. 29). To test this experimentally, we wondered whether blocking the interaction between GluR2 and PICK1 had an effect on the increased rectification and AMPAR:NMDAR ratio induced by cocaine. Because these changes can only be induced *in vivo*[16], the dominant-negative peptide Pep2-EVKI (NVYGIEEVKI), shown to efficiently and selectively block PICK1 function, and the inactive control peptide Pep2-SVKE (NVYGISVKE)[30] had to be

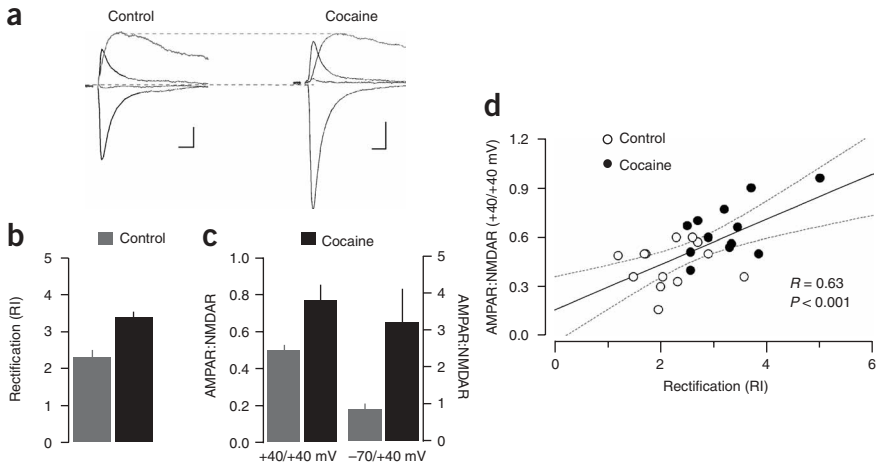

Figure 2 The increase in rectification is paralleled by an increase in the AMPAR:NMDAR ratio. (**a**) Black traces, representative AMPAR EPSCs recorded at +40 mV, 0 mV and –70 mV in neurons from control and cocaine-treated mice. Gray traces, NMDAR EPSCs recorded at +40 mV after mathematical subtraction of the AMPAR component (scale bars, 50 pA and 5 ms). (**b**,**c**) Averaged RI and AMPAR:NMDAR ratios. AMPAR EPSCs were measured at either –70 mV or +40 mV; NMDAR EPSCs were measured at +40 mV in both cases. Error bars represent s.e.m. (**d**) AMPAR:NMDAR ratio (both components measured at +40 mV) as a function of RI. Solid line, linear regression curve; dotted line, 95% confidence intervals are shown (dotted lines).

delivered into DA neurons *in vivo*. To this end, we fused the peptides to an 11–amino acid sequence derived from the Tat (48–57) protein of the human immunodeficiency virus (HIV-1). This small basic peptide is an established tool to successfully deliver a variety of cargos intracellularly after systemic application[31]. To visualize the transduction of the 21-mer TAT-EVKI peptide (YGRKKRRQRRR-NVYGIEEVKI and the corresponding control peptide TAT-SVKE) into DA neurons of the VTA, the TAT-fused peptides were conjugated to N-carboxyfluorescein. We then used two-photon confocal microscopy to visualize the peptide intracellularly 4 h after intraperitoneal injection (**Fig. 3a**)[32]. As a control, we injected N-carboxyfluorescein not conjugated to the TAT sequence that did not fill the cells (data not shown).

The injection of the TAT-EVKI was followed 4 h later by the injection of cocaine, and the slices were prepared after another 24 h (**Fig. 3b**). In neurons of slices obtained under these conditions, the RI was similar to baseline (**Fig. 3c**), whereas TAT-EVKI injections without cocaine treatment did not have any effect (2.0 ± 0.2, $n = 5$ and 2.3 ± 0.2, $n = 12$; **Fig. 3d**). In contrast, when pretreated with the inactive peptide TAT-SVKE followed by cocaine injection, we observed a high RI that was significantly different from the values above (3.9 ± 0.6, $n = 9$, $P < 0.01$; **Fig. 3d**), but similar to those obtained with cocaine treatment alone (**Fig. 2b**). In parallel, TAT-EVKI also blocked the effect of cocaine on the AMPAR:NMDAR ratio (0.5 ± 0.05, $n = 10$ and 0.39 ± 0.07, $n = 7$ with TAT-EVKI alone versus 0.85 ± 0.06, $n = 6$, $P < 0.01$ for TAT-SVKE followed by cocaine; **Fig. 3e**,**f**). Taken together, these data indicate that the increase in both the RI and the AMPAR:NMDAR ratio requires the interaction of GluR2 with PICK1.

mGluRI agonists decrease rectification

If the recruitment of AMPARs is induced by cocaine, it is conceivable that the internalization of AMPARs associated with LTD may reverse the process[33]. In the present case, such an LTD would not only have to depress synaptic efficacy but also reverse rectification. In acute slices of the VTA of rats, the activation of mGluR1 in DA neurons triggers a redistribution of AMPARs, exchanging native receptors for GluR2-containing AMPARs and ultimately causing LTD that is associated with a decrease in rectification[27]. In contrast to mice, we found marked rectification in drug-naïve rats (**Supplementary Fig. 1** online). This difference between species may indicate variation in basal synaptic properties across species; alternatively, it may indicate that the GluR2 content is dynamically regulated by behavioral factors, such as stress[9], that may differ between species. Because mGluR-LTD depends on the presence of GluR2-lacking AMPARs, we predicted that the magnitude of mGluR-LTD would be larger in slices from cocaine-treated mice than from control mice. In fact, brief trains (five stimuli) at 66 Hz synaptically activate mGluRs[34]; we found that these caused a significant depression of the AMPAR EPSCs in slices of cocaine-treated mice but not in controls (**Fig. 4a**; 47.7 ± 9.1%, $n = 6$, $P < 0.01$). This depression was blocked by the selective mGluR1 antagonist 7-hydroxyimino-cyclopropa(b)chromen-1a-carboxylate ethyl ester (CPCCOEt) (3.9 ± 9.2, $P < 0.05$, data not

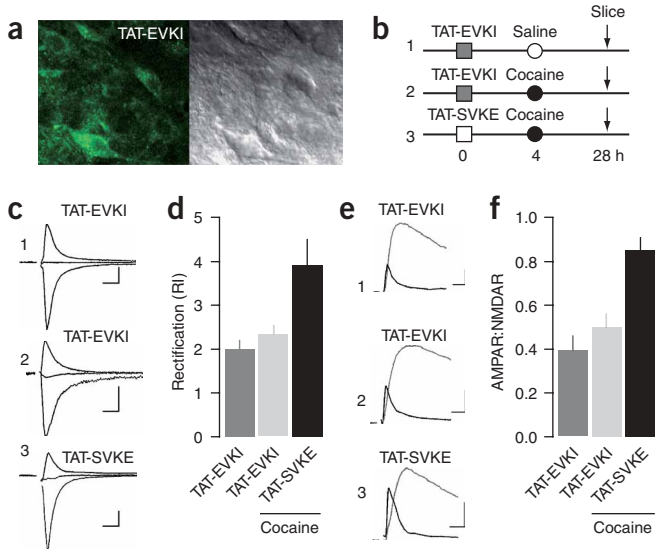

Figure 3 Disruption of PICK1-GluR2 interaction blocks the increase in both the RI and the AMPAR:NMDAR ratio. (**a**) Left, confocal image of intracellular fluorescein in VTA neurons injected with TAT-EVKI, obtained 4 h after injection (1 mg kg^{-1} i.p.). Right, transmitted light image of the same field of view as on left. The visualization of intracellular fluorescence demonstrates successful intracellular transduction of the TAT-fused peptide. Magnification, 40×. (**b**) Experimental protocol. (**c**) Representative traces of AMPAR EPSCs recorded at +40 mV, 0 mV and –70 mV in neurons from mice pretreated with TAT peptides followed by saline or cocaine (black traces). TAT-EVKI efficiently blocked the cocaine-induced increase in rectification, but had no effect on its own. In contrast, the inactive peptide TAT-SVKE did not block the increase in rectification. (**d**) Average RI. (**e**) Representative AMPAR EPSCs (black traces) and NMDAR EPSCs (gray traces), recorded at +40 mV. (**f**) Group data indicating that the increase in the AMPAR:NMDAR ratio was blocked when mice were pretreated with TAT-EVKI. Scale bars, 50 pA and 5 ms. Error bars represent s.e.m.

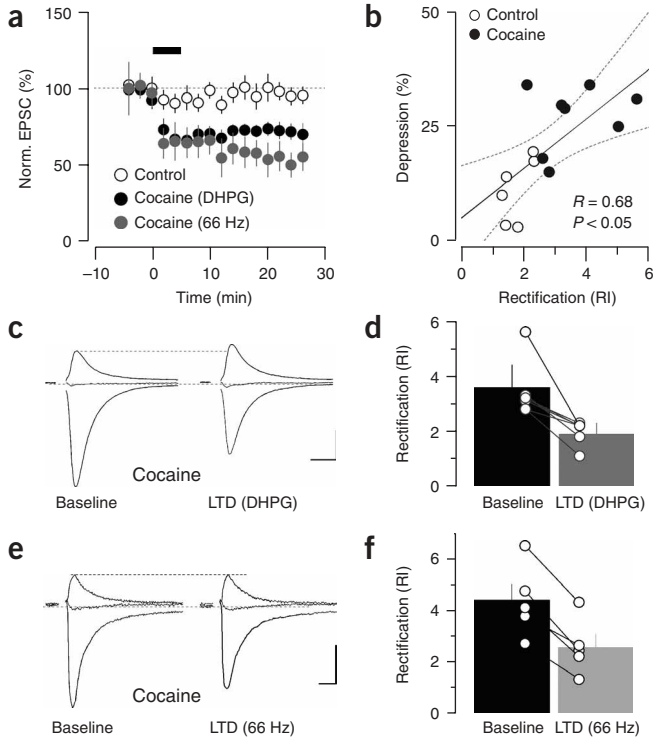

Figure 4 mGluR-LTD depends on rectification and involves a switch of the AMPAR subunit composition (**a**) The magnitude of LTD induced by mGluR activation (either by applying two trains of five stimuli at 66 Hz or by a 5-min bath application of DHPG) was significantly larger in control mice than in cocaine-treated mice. (**b**) Magnitude of LTD as a function of RI. Solid line, linear regression curve; dotted line, 95% confidence intervals. (**c**–**f**) Representative traces of AMPAR EPSCs (**c**,**e**) recorded at +40 mV, 0 mV and –70 mV before and after the chemical or synaptic induction of mGluR-LTD in cocaine-injected mice (scale bars, 100 pA and 5 ms). The rectification decreased (**d**,**f**) in each cell after the synaptic induction of mGluR-LTD, leading to significantly different mean RI. Error bars represent s.e.m.

shown). The bath application of the mGluR group I agonist 3,5-dihydrophenylglycine (DHPG, 20 μM) also depressed AMPAR EPSCs in slices of cocaine-treated mice but not control mice (**Fig. 4a**; 28.2 ± 5.4%, $n = 7$ versus 3.5 ± 7.9%, $n = 9$; $P < 0.01$). In individual neurons, the magnitude of depression was significantly correlated with the RI measured at baseline (**Fig. 4b**; $R = 0.68$, $P < 0.05$), confirming that the composition of AMPARs determines the magnitude of depression. As evidence for the removal of receptors devoid of GluR2, we observed a decrease in RI after the induction of mGluR-LTD in slices from cocaine-treated mice (**Fig. 4c**). On average, RI was 3.6 ± 0.8 before the application of DHPG and 1.9 ± 0.4 after LTD was induced by DHPG. Likewise, synaptically evoked mGluR-LTD reduced RI from 4.4 ± 0.6 at baseline to 2.6 ± 0.5 (**Fig. 4c–f**; $P < 0.05$, $n = 5$–6). With both induction protocols, mGluR-LTD led to RIs that were not different from that of the almost linear *I-V* relationship in control mice (**Fig. 2b**; 2.3 ± 0.2, $P > 0.05$).

mGluR1s reverse AMPAR redistribution *in vivo*

We next attempted to reverse the increase in RI and AMPAR:NMDAR ratio *in vivo*, by taking advantage of the newly available, systemically active mGluR1 enhancers[35]. We first applied the selective positive modulator of mGluR1 Ro 67-7476 (3 μM, 10 min) in acute slices of cocaine-treated mice and found that it induced a depression of AMPAR EPSCs (**Fig. 5a**; 43.8 ± 6.8, $n = 7$ versus –0.4 ± 4.3, $n = 7$; $P < 0.01$ in comparison to control mice).

Mice were then injected with Ro 67-7476 24 h after a single injection of cocaine and killed 24 h later to prepare slices of the VTA (**Fig. 5b**). Both the RI and AMPAR:NMDAR ratio were significantly lower in these mice compared to mice treated with cocaine followed by an injection of saline (**Fig. 5c–f**; RI index: 2.43 ± 0.1, $n = 7$ versus 4 ± 0.6, $n = 8$, $P < 0.01$; AMPAR:NMDAR ratio: 0.53 ± 0.07, $n = 10$ versus 0.96 ± 0.1, $n = 12$, $P < 0.05$). These experiments provide a proof of principle for the use of positive modulators of mGluR1 to reverse the increases in RI and AMPAR:NMDAR ratio that occur in response to cocaine exposure.

DISCUSSION

We report that a single injection of cocaine causes an increase in rectification and the AMPAR:NMDAR ratio, thus potentiating excitatory transmission onto DA neurons but also profoundly changing the quality of transmission by rendering AMPARs calcium permeable.

In theory, an increase in the AMPAR:NMDAR ratio at depolarized potentials could reflect a replacement of inwardly rectifying AMPARs with nonrectifying AMPARs, with no effect on the synaptic strength at hyperpolarized membrane potentials. Our data, in accordance with previous reports[14], exclude this possibility, because rectification would decrease under these circumstances. On the contrary, based on the observation that rectification actually increases, we propose that cocaine triggers the insertion of AMPARs that lack GluR2, switching transmission from a calcium-impermeable to calcium-permeable synapse. Cocaine therefore not only affects synaptic efficacy but also

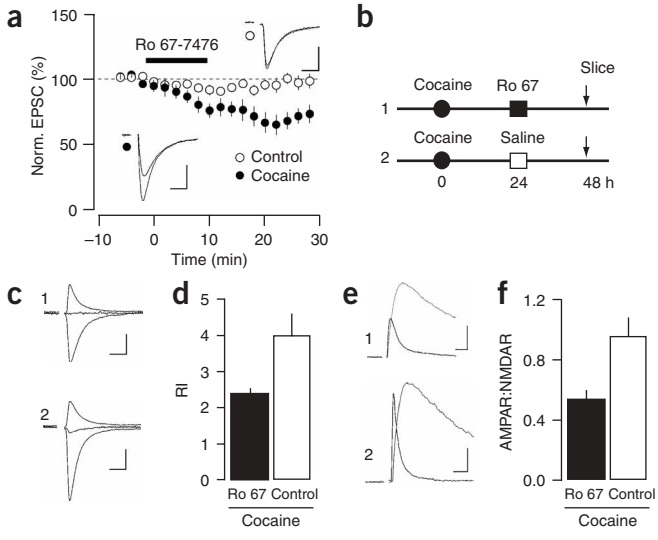

Figure 5 *In vivo* application of a positive modulator of mGluR1 reverses the increases in rectification and the AMPAR:NMDAR ratio. (**a**) In acute slices of the VTA, bath application of 3 μM of Ro 67-7476 induced a depression of AMPAR EPSCs in mice treated with cocaine but not control mice, similar to the effects of DHPG (**Fig. 4**). Insets, representative traces of EPSCs from neurons after Ro 67-7476 application. (**b**) Experimental protocol. (**c**) Representative AMPAR EPSCs recorded at +40 mV, 0 mV and –70 mV in neurons from mice treated with cocaine followed by Ro 67-7476, or cocaine followed by saline. (**d**) Average RI. (**e**) Representative AMPAR EPSCs (black traces) and NMDAR EPSCs (gray traces), recorded at +40 mV. (**f**) AMPAR:NMDAR ratio obtained from mice treated with Ro 67-7476 or saline after the initial cocaine injection. Scale bars, 50 pA and 5 ms. Numbers in **c** and **e** refer to the experimental protocols described in **b**. Error bars represent s.e.m.

profoundly alters the quality of excitatory transmission. Such a switch in subunit composition may be a way to control the cascades of molecular events that eventually lead to sensitization[36]. The subunit composition of synaptic AMPARs on DA neurons could thus change from mainly heteromeric GluR1/GluR2-containing AMPARs in the naïve state to GluR1 homomers (or GluR1/3 heteromers) after cocaine exposure. Such plasticity differs from hippocampal LTP, where rectification and JST sensitivity are absent at baseline and after the stable expression of LTP[37].

Under our experimental conditions, GluR2-lacking receptors contributed to the EPSC even when it was measured at positive potentials, because at +40 mV a substantial fraction of the current was still sensitive to JST. This may indicate that in spite of the intracellular presence of spermine, GluR2-lacking receptors were not fully blocked. This may also explain the incomplete rectification of the *I-V* curve. In addition, we observed a small change in the reversal potential, which may reflect the fact that the point inflection of the slope of the *I-V* curve in GluR2-lacking AMPARs is not exactly at 0 mV (ref. 38). However, arguing against this interpretation, other studies have demonstrated that there is no change in reversal potential when the polyamine block is removed[39]. In any case, because GluR2-lacking receptors have a larger single-channel conductance than GluR2-containing receptors, the insertion of only a few GluR2-lacking receptors in DA neurons of the VTA could be sufficient to cause rectification and an increase in the AMPAR:NMDAR ratio at the same time.

GluR2-lacking AMPARs could be inserted on top of or by replacing native GluR2-containing receptors. In the latter scheme, GluR2-containing receptors would have to move out of the synapse before GluR2-lacking ones can be inserted. Our data favors this scheme, as the cocaine-evoked redistribution requires PICK1 function. A similar switch has been observed in cerebellar stellate cells, in which baseline transmission is inhibited by 80% with JST and therefore must be primarily mediated by GluR2-lacking AMPARs (ref. 40). In these neurons, PICK1 is required for the delivery of GluR2-containing receptors[23,24]. In contrast, in GluR2-containing synapses of CA1 neurons, PICK1 is required for AMPAR removal by NMDAR-dependent LTD (refs. 41–43). PICK1 therefore seems to be involved in the bidirectional regulation of GluR2-lacking AMPARs (ref. 24) but does not have an effect on baseline transmission in synapses with GluR2-containing receptors[30]. This is in line with our observation that TAT-EVKI did not have an effect on rectification in slices from untreated mice but efficiently blocked cocaine-evoked plasticity.

In analogy to the calcium permeability observed with ischemia[44] and the sensitization to inflammatory pain[45], cocaine-driven plasticity may be considered a pathological plasticity. The events triggered by the Ca-permeable receptors remain to be elucidated. Perhaps the insertion of GluR2-lacking receptors is transient and GluR2-lacking receptors are subsequently replaced with GluR2-containing receptors, which would further increase the AMPAR:NMDAR ratio. However, such changes would have to be a rather slow process, as we still observed significant rectification 48 h after cocaine exposure. It is possible that, through a heterosynaptic effect, Ca-dependent signaling could eventually lead to a downregulation of $GABA_A$ receptors in DA neurons, as recently described[7].

Our data also indicate that cocaine-driven plasticity can be reversed *in vitro* and *in vivo* by activating mGluR1. This leads to a synaptic depression by removing GluR2-lacking AMPARs. Characterizing mGluR-LTD in rat slices, we have previously found substantial rectification of baseline AMPAR EPSCs[27]. In agreement with previous data[6], we report here that AMPAR EPSCs are not rectifying in naïve mice; however, the present study now demonstrates that rectification may be bidirectionally regulated. Exposure to cocaine increases rectification whereas mGluR-LTD reverses this rectification, revealing a very dynamic equilibrium in AMPAR redistribution. Because environmental factors, such as stress[9] can induce similar changes, we believe that they are at the origin of the difference. Alternatively, genuine differences between species and/or developmental regulation may be the cause.

The demonstration that mGluR-LTD is mediated by mGluR1 (ref. 27) is in line with the report that mGluR1 hypofunction leads to inherent behavioral sensitization without any previous drug exposure[46]. mGluR5 may have opposing effects, as suggested by the observation that *Grm5*$^{-/-}$ mice do not self-administer cocaine and show no increased locomotor activity[47].

In conclusion, our results indicate that cocaine causes synaptic plasticity in the VTA through a shift in the subunit composition of AMPARs and that this effect can be reversed by mGluR1-dependent LTD. Pharmacological interventions at mGluR1, for example through positive modulation as exemplified here, may emerge as a way to determine synaptic efficacy and calcium permeability on the sliding scale between 'sensitized' and naïve neurons, with the goal of reversing context-dependent incentive saliency.

METHODS

Intraperitoneal (i.p.) injections in mice. C57BL/6 mice (postnatal day (P) 18–22, 7–9 g bodyweight) were injected i.p. with 15 mg kg^{-1} cocaine or 0.9% saline using a 26G hypodermic needle (injection volume ~50 µl) to minimize stress. To interfere with PICK1, we injected TAT-fused peptides or N-carboxyfluorescein (both at 3 µmol kg^{-1}) 4 h before the injection of cocaine. Where stated, Ro 67-7476 was dissolved and sonicated in saline (0.9% NaCl) and injected i.p. (4 mg kg^{-1}) 24 h after cocaine injections. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Geneva.

Electrophysiology in acute slices. Horizontal slices (250-µm thick) of the brain stem were prepared in cooled artificial cerebrospinal fluid containing 119 mM NaCl, 2.5 mM KCl, 1.3 mM $MgCl_2$, 2.5 mM $CaCl_2$, 1.0 mM NaH_2PO_4, 26.2 mM $NaHCO_3$ and 11 mM glucose, and bubbled with 95% O_2 and 5% CO_2. Whole-cell voltage-clamp recording techniques were used (32–34 °C, 2 ml min^{-1}, submerged slices) to measure the holding currents and synaptic responses of dopaminergic neurons of the VTA that were identified by the presence of a large hyperpolarization-activated (I_h) current[48] immediately after obtaining a whole-cell configuration. As I_h is present in ~90% of dopamine neurons[49], its presence is not an unequivocal identification criterion. However, using single-cell reverse transcription polymerase chain reaction (RT-PCR), a previous study[50] confirmed the tight correlation of I_h with tyroxine hydroxylase mRNA and found it sufficient to obtain highly significant differences between experimental and control cells. The holding potential was –50 mV, and the access resistance was monitored by a hyperpolarizing step to –60 mV with each sweep, every 10 s. Experiments were terminated if the access resistance varied by more than 20%. Synaptic currents were evoked by stimuli (0.1 ms) at 0.05 Hz through bipolar stainless steel electrodes positioned rostral to the VTA. The internal solution contained 130 mM CsCl, 4 mM NaCl, 2 mM $MgCl_2$, 1.1 mM EGTA, 5 mM HEPES buffer, 2 mM Na_2ATP, 5 mM sodium creatine-phosphate, 0.6 mM Na_3GTP and 0.1 mM spermine. Currents were amplified (Axopatch 1D), filtered at 1 kHz and digitized at 5 kHz (National Instruments Board PCI-MIO-16E4, Igor, WaveMetrics). 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). Cells were held at –50 mV for 10 min and then were depolarized at +40 mV and monitored for 15 min. To calculate the AMPAR:NMDAR ratio, we computed averaged EPSCs before and after the application of D-AP5 for 5 min (15–20 traces). The NMDA component was calculated as the difference between the EPSCs measured in the absence and presence of the antagonist. Finally, the AMPAR:NMDAR ratio was calculated by dividing the peak AMPAR-mediated EPSCs by the peak NMDAR-mediated EPSCs. The current-voltage (*I-V*) curve of the AMPAR-mediated EPSCs was then calculated in the presence of D-AP5. For the data in

Figure 1, we also looked at mice ($n = 6$) that were not treated at all (naïve mice) to ensure that the subtle stress of the i.p. injection did not affect baseline transmission. Because the data obtained from naïve and saline-injected mice did not differ, they were pooled.

Statistical analysis. Compiled data are expressed as mean ± s.e.m. For statistical comparisons we used the nonparametric Mann-Whitney or Wilcoxon matched tests; the level of significance was taken at $P = 0.05$. Example traces are averages of 20 sweeps.

Drugs. D-AP5 and DHPG were obtained from Tocris; spermine and picrotoxin (PTX) were obtained from Sigma; and cocaine was obtained from the pharmacy of the Hopitaux Universitaires de Genève. Ro 67-7476 was a gift from F. Knoflach (F. Hoffmann-La Roche Ltd, Basle, Switzerland). Peptides were custom synthesized by Primm Srl.

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

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COMPETING INTERESTS STATEMENT

The authors declare that they have no competing financial interests.

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