

Reversal of cocaine-evoked synaptic potentiation resets drug-induced adaptive behaviour

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Drug-evoked synaptic plasticity is observed at many synapses and may underlie behavioural adaptations in addiction¹. Mechanistic investigations start with the identification of the molecular drug targets. Cocaine, for example, exerts its reinforcing² and early neuroadaptive effects³ by inhibiting the dopamine transporter, thus causing a strong increase in mesolimbic dopamine. Among the many signalling pathways subsequently engaged, phosphorylation of the extracellular signal-regulated kinase (ERK) in the nucleus accumbens⁴ is of particular interest because it has been implicated in NMDA-receptor and type 1 dopamine (D1)-receptor-dependent synaptic potentiation⁵ as well as in several behavioural adaptations^{6–8}. A causal link between drug-evoked plasticity at identified synapses and behavioural adaptations, however, is missing, and the benefits of restoring baseline transmission have yet to be demonstrated. Here we find that cocaine potentiates excitatory transmission in D1-receptor-expressing medium-sized spiny neurons (D1R-MSNs) in mice via ERK signalling with a time course that parallels locomotor sensitization. Depotentiation of cortical nucleus accumbens inputs by optogenetic stimulation *in vivo* efficiently restored normal transmission and abolished cocaine-induced locomotor sensitization. These findings establish synaptic potentiation selectively in D1R-MSNs as a mechanism underlying a core component of addiction, probably by creating an imbalance between distinct populations of MSNs in the nucleus accumbens. Our data also provide proof of principle that reversal of cocaine-evoked synaptic plasticity can treat behavioural alterations caused by addictive drugs and may inspire novel therapeutic approaches involving deep brain stimulation or transcranial magnetic stimulation.

We first tested whether cocaine treatment interfered with activity-dependent long-term potentiation (LTP) in the nucleus accumbens. When excitatory afferents onto MSNs were challenged with a high-frequency stimulation (HFS) train, LTP of the excitatory postsynaptic currents (EPSCs) was observed (Fig. 1a). The magnitude of the LTP was halved in brain slices from mice that had received a single injection of cocaine 7 days before the recording. If the cocaine was injected a month before measuring synaptic plasticity *ex vivo*, the difference was no longer present (Fig. 1b). MSNs of the nucleus accumbens fall into two classes of about equal proportions defined by the type of dopamine receptor expressed, with a small fraction of neurons (6–17%) that express both receptors⁹. A possible explanation for the partial change in LTP magnitude is therefore that cocaine exposure abolishes plasticity selectively in one class. To test this, we attempted LTP induction *ex vivo* after cocaine exposure in bacterial artificial chromosome (BAC) transgenic mice expressing enhanced green fluorescent protein (eGFP) either in D1 receptor (D1R)- or D2R-MSNs. We identified D1R-MSNs by a crossover strategy in which we recorded from green cells in *drd1a*-eGFP mice and non-green cells in *drd2*-eGFP mice (and vice versa for D2R-MSNs). Because the two approaches to identify the cell type yielded very similar results, we pooled the data (Fig. 1c, d). The main finding of this first experiment was that HFS, which reliably

induced LTP in both types of MSNs after saline injection, became inefficient after cocaine treatment in D1R-MSNs (Supplementary Fig. 1a–d). The D2R overexpression recently reported in *drd2*-eGFP mice¹⁰ interferes with neither the reported synaptic effects nor with the acute locomotor response to cocaine (Supplementary Fig. 2). After a cocaine injection, we were unable to induce HFS LTP regardless of whether we recorded from MSNs in the shell or the core of the nucleus accumbens (Supplementary Fig. 3), but because MSNs in the shell are the immediate targets of the dopamine neurons of the medial ventral tegmental area, which undergo the most significant changes in response to a single cocaine injection¹¹, we focused on nucleus accumbens shell neurons in the present study. Without distinguishing between D1R- and D2R-MSNs, several reports have already suggested that drug-evoked synaptic plasticity in the nucleus accumbens may underlie drug-related behavioural adaptations (reviewed in ref. 12). For example, when 5–7 daily cocaine injections were followed by a 10–21-day withdrawal, an overall increase of the AMPA/NMDA receptor (AMPA/NMDAR) ratio¹³ or GluA1/2 surface expression^{14,15} was observed, and both observations were reversed by a challenge injection of cocaine.

The failure of HFS to induce LTP selectively in D1R-MSNs after cocaine treatment may be due either to the impairment of LTP induction or the occlusion of LTP expression. To distinguish between the two scenarios, we recorded miniature EPSCs (mEPSCs) in both cell types (Fig. 1e and Supplementary Fig. 4a) and observed a significant increase in amplitude along with a modest change in frequency of unitary events in D1R-MSNs (whereas these parameters remained unchanged in D2R-MSNs). Given that the paired pulse ratio also remained unchanged (Fig. 1f and Supplementary Fig. 4b), a post-synaptic mechanism underlying the increase of transmission and hence an occlusion scenario is the most likely explanation.

We characterized the induction criteria for HFS LTP in MSNs *in vitro* and found that it depends on NMDAR activation (Fig. 2a). Applying the MEK (MAP ERK kinase) inhibitor U0126 for the duration of the induction protocol also led to a complete block (Fig. 2b), indicating that activation of the ERK pathway is essential for this form of LTP, akin to NMDA-dependent LTP in the hippocampus and the dorsal striatum^{16,17}, the latter also depending on D1Rs¹⁸.

Given that our results indicate an occlusion of LTP, we hypothesized that cocaine could drive synaptic potentiation selectively in D1R-MSNs via ERK activity. Indeed, a sharp increase in phosphorylated ERK is detected soon after cocaine exposure in D1R-MSNs, but not in D2R-MSNs⁹. In the nucleus ERK modulates gene expression, whereas in dendrites ERK is probably involved in the regulation of activity-dependent spine dynamics, synaptic glutamate receptor insertion and local dendritic protein synthesis¹⁹. To provide *in vivo* evidence for ERK dependence of cocaine-evoked synaptic plasticity, we treated mice with SL327, a blood–brain barrier penetrant ERK pathway inhibitor, before the saline or cocaine injection. We found that this manipulation rescued HFS LTP in D1R-MSNs one week later (Fig. 2c, d) without modification of the acute locomotor response to cocaine

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(Supplementary Fig. 5). A crossover control design with the two mouse lines again showed no difference between the green cells of one line and the non-green cells of the other (Supplementary Fig. 6).

Because inhibition of the ERK pathway blocks locomotor sensitization to cocaine^{20,21}, and we found LTP in D1R-MSNs to be dependent on ERK, we reasoned that cocaine-evoked potentiation might be a cellular correlate of the behavioural adaptation. Moreover, ERK activation is correlated both with AMPAR expression at the cell surface throughout the nucleus accumbens and with locomotor sensitization¹⁴,

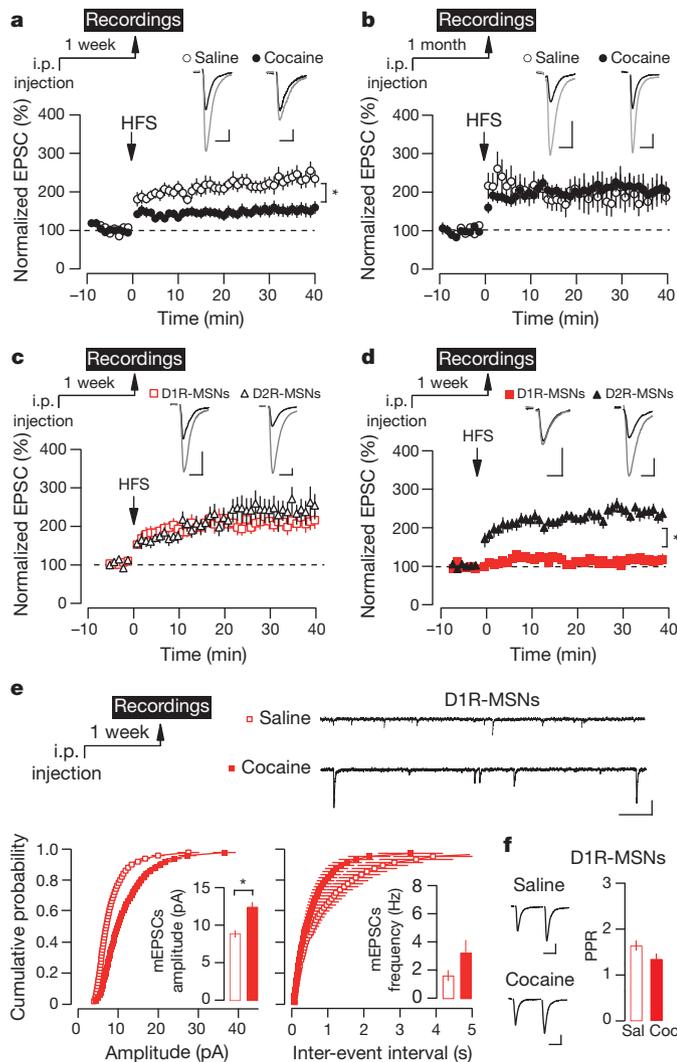


Figure 1 | Cocaine disrupts HFS-induced LTP in D1R-MSNs of the nucleus accumbens by potentiation of excitatory afferents. **a–d**, Graphs show normalized EPSCs as a function of time and the overlay of averaged (20 trials) traces of AMPAR EPSCs before (black line) and after (grey line) HFS. Symbols represent average of 6 trials. **a**, One week after cocaine injection, LTP was halved ($224 \pm 17.9\%$ to $149 \pm 14.7\%$, Student's t -test $t_{43} = 3.06$). **b**, After 1 month, no difference between treatments was detected ($204 \pm 26.1\%$ to $206 \pm 21.3\%$, $t_{21} = 0.07$). **c**, Using *drd1a*- and *drd2*-eGFP mice, HFS reliably induced LTP in D1R-MSNs and D2R-MSNs after saline injection ($206 \pm 16.0\%$ and $229 \pm 24.1\%$, respectively, $t_{27} = 0.78$). **d**, Cocaine abolished HFS LTP in D1R-MSNs but not in D2R-MSNs ($115 \pm 8.5\%$ and $239 \pm 12.7\%$, respectively, $t_{39} = 7.98$). **e**, Sample traces, cumulative probability and mean values of amplitude and frequency for mEPSCs recorded from D1R-MSNs one week after injection of saline or cocaine. Cocaine significantly increases mean amplitude of mEPSCs in D1R-MSNs ($t_{14} = 4.68$; Kolmogorov–Smirnov test: $P < 0.01$) whereas the frequency was unaffected ($t_{14} = 1.635$; Kolmogorov–Smirnov: $P > 0.05$). **f**, Paired-pulse ratio (PPR, 50 ms inter-stimulus interval) measured in D1R-MSNs was not different a week after saline (Sal) or cocaine (Coc) injection (1.7 ± 0.11 and 1.4 ± 0.1 , respectively, $t_{34} = 1.73$). Scale bars: 20 ms and 20 pA. $n = 8–30$, $*P < 0.05$. Error bars show s.e.m.

but the synapses involved have not been identified, and no causal link has been established.

We then confirmed that a single injection of cocaine was sufficient to cause locomotor sensitization to a second injection of the same dose²². This was the case when challenged a week but not a month after the initial cocaine injection (Supplementary Fig. 7). The behavioural alteration therefore followed a time course similar to the cocaine-evoked synaptic potentiation (Fig. 1a, b). If cocaine-evoked potentiation is causally involved in locomotor sensitization, then depotentiating these synapses may reverse the behavioural alterations. To test this prediction experimentally, we injected channelrhodopsin (ChR2)-expressing adeno-associated virus (AAV) into the infralimbic cortex and implanted light guides into the nucleus accumbens to be able to selectively activate *in vivo* the terminals of this major excitatory input. Histological verification of the injection site did confirm robust expression in the infralimbic cortex, along with sparser expression in the prelimbic cortex (Fig. 3a and Supplementary Fig. 8). To validate further this approach, we recorded photocurrents from infected cortical neurons and prepared slices of the nucleus accumbens shell in which wide-field light exposure led to robust AMPAR-mediated EPSCs (Fig. 3b). We next applied light pulses at 1 Hz for 10 min (an established long-term depression (LTD) protocol to reduce synaptic transmission, see Methods). We found that this protocol strongly depressed transmission in nucleus accumbens slices from both saline and cocaine-treated mice (Fig. 3c). Interestingly, the magnitude of the depression was significantly larger in the latter, in line with an efficient depotentiation added to the LTD. As the LTD/depotentiation was NMDAR dependent and occurred without change of the paired pulse ratio, it is probably mediated by a postsynaptic expression mechanism and therefore constitutes an actual reversal of cocaine-evoked potentiation (Supplementary Fig. 9).

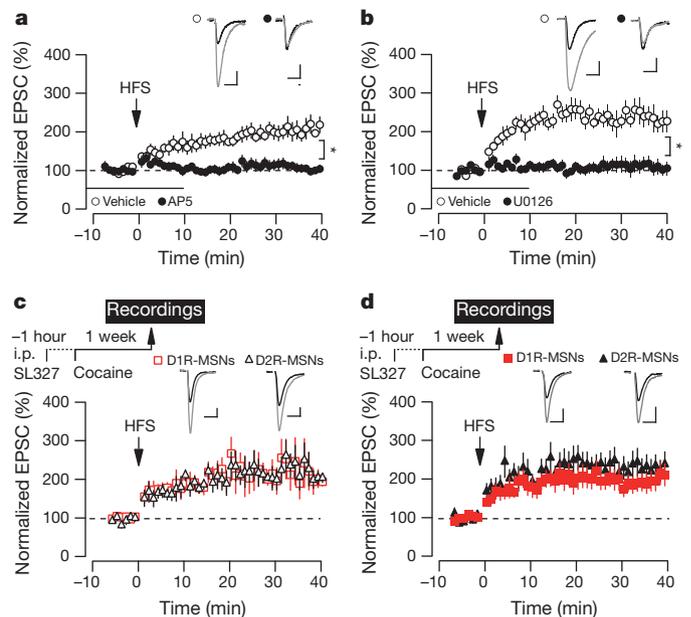


Figure 2 | HFS LTP and cocaine-evoked potentiation both depend on ERK activation. **a**, HFS LTP was blocked by an NMDAR antagonist (AP5, 100 μ M; control, $209 \pm 15\%$ versus AP5, $93 \pm 9\%$, $t_{14} = 6.78$). **b**, Bath application of the MEK inhibitor (U0126, 5 μ M, 15 min before until 10 min after HFS protocol) also blocked LTP (control, $234 \pm 17.8\%$ versus U0126, $109 \pm 16.2\%$, $t_{13} = 5.047$). **c**, Intraperitoneal administration of the MEK inhibitor (SL237, 40 mg kg⁻¹) in *drd1a*-eGFP mice or *drd2*-eGFP mice 1 h before saline did not modify the magnitude of HFS LTP in D1R- or D2R-MSNs when assessed one week later ($224 \pm 31\%$ and $221 \pm 27\%$, respectively, $t_{10} = 0.09$). **d**, SL237, administered 1 h before cocaine restored HFS LTP in D1R-MSNs ($200 \pm 22\%$ versus $232 \pm 27\%$ for D2R-MSNs, $t_{22} = 0.93$). Scale bars: 10 ms and 20 pA. $n = 6–13$, $*P < 0.05$. Error bars show s.e.m.

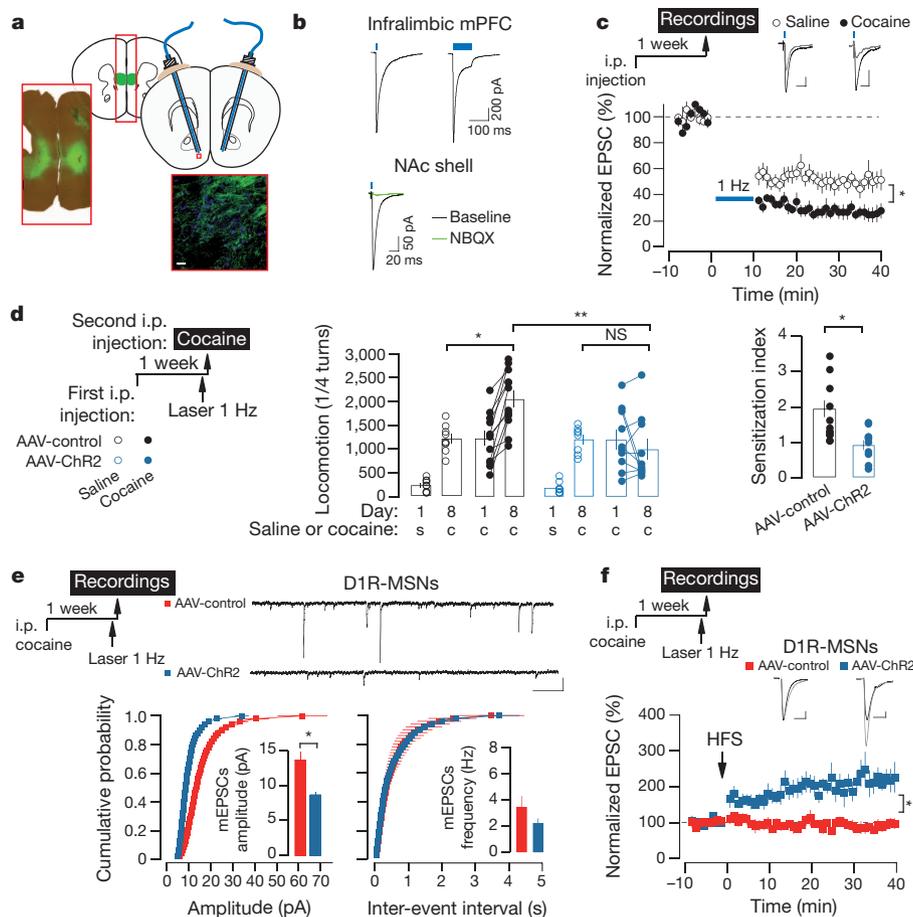


Figure 3 | Reversal of cocaine-evoked potentiation abolishes locomotor sensitization to cocaine. **a**, Schematic illustration of the site infected with AAV coding for eGFP-ChR2 and of the bilateral cannula implantation with optic fibres inserted in the nucleus accumbens shell. Insets show AAV-eGFP-ChR2 expression in the infralimbic cortex and high-resolution image of axonal inputs onto nucleus accumbens shell neurons (blue stains nuclei, $\times 60$ magnification). **b**, Averaged current traces in response to light pulses (470 nm) of 4 ms and 100 ms in infected cells in slices of the infralimbic medial prefrontal cortex (mPFC; top). In nucleus accumbens (NAc) slices, light pulses (4 ms) evoked AMPAR EPSCs (blocked by NBQX 20 μ M, green trace, bottom). **c**, Low-frequency stimulation (light pulses of 4 ms at 1 Hz for 10 min) induced depression of AMPAR EPSCs evoked with light in nucleus accumbens slices from mice injected with saline or cocaine one week previously (48 \pm 5.7% and 27 \pm 4.5%, respectively, $t_{14} = 2.851$). $n = 8$, $*P < 0.05$. **d**, Quarter turns done by mice in the circular corridor for 60 min after injection represent locomotion (scatter plots of individual score and bars of mean \pm s.e.m. are shown). Multiple-way repeated-measures analysis of variance for matching data yielded: interaction between day, virus and treatment $F_{(1,35)} = 13.41$, $P < 0.001$; effect of day $F_{(1,35)} = 78.16$, $P < 0.001$; effect of virus $F_{(1,35)} = 17.78$, $P < 0.001$; effect of treatment $F_{(1,35)} = 88.65$, $P < 0.001$. Wilcoxon or t -test: $*P < 0.001$ for

We next applied the above-validated protocol *in vivo* with the goal of establishing a causal link between cocaine-evoked plasticity and behavioural sensitization. We placed light guides into the ventral striatum, past the nucleus accumbens core, thus preferentially aiming at the principal cortical input onto MSNs of the nucleus accumbens shell, that is, axons that have their origin in the infralimbic cortex (Supplementary Fig. 8). When freely moving mice were treated with the optogenetic depotentiation protocol 45 min before the injection of the cocaine challenge at day 8, locomotor sensitization was completely erased. In control experiments, light stimulation did not affect the locomotor response at day 8 when the first injection was saline instead of cocaine or when a control virus was used (Fig. 3d). To ensure that the light stimulation restored normal transmission, we recorded *ex vivo* mEPSCs and found that an effective light treatment significantly

reduced their mean amplitude in D1R-MSNs (Fig. 3e) to a level comparable to baseline transmission (Fig. 1e) without any effect on paired pulse ratio (Supplementary Fig. 10). In contrast, the mEPSC amplitude and frequency were not modified in D2R-MSNs after cocaine (Supplementary Fig. 11). Lastly, after cocaine treatment and optogenetic depotentiation the HFS protocol resulted in LTP *ex vivo* (Fig. 3f), confirming the restoration of baseline transmission in D1R-MSNs. Behavioural sensitization typically refers to the observation of increased locomotor responses with repeated injections of cocaine. However, sensitization becomes apparent already after the second injection and is best observed about a week later²² (Fig. 3). Although certainly not sufficient to induce addiction, such early forms of drug-induced adaptations are considered permissive building blocks for more definite behavioural alterations. If this is the case, the synaptic

potentiation onto D1R-MSNs should still be observed after chronic cocaine exposure, for example after 5 daily injections followed by a 10-day withdrawal. To confirm this hypothesis we recorded mEPSCs at the end of this protocol, as well as 1 h and 24 h after a challenge injection (Supplementary Fig. 12). This experiment confirmed that excitatory synapses onto D1R-MSNs were selectively potentiated at the end of the withdrawal period.

We then tested whether, after this chronic exposure to cocaine, light stimulation was still effective in reversing locomotor sensitization. After the 5 days of injection we imposed 10 days of withdrawal, and treated the mice with light stimulation 45 min before injecting a challenge dose. This completely reversed locomotor sensitization (Fig. 4a). When a second challenge injection was administered 24 h later, sensitized responses were again only observed in control mice. As a control, we did not observe an acute effect on locomotor behaviour during light stimulation (Supplementary Fig. 14).

Lastly, to estimate how long the effect of the light stimulation lasted, we tested for behavioural sensitization 5 days after the intervention (Fig. 4b). No significant locomotor sensitization was observed even when the mice were challenged at this extended time after light stimulation.

Our results identify NMDAR- and ERK-dependent LTP in D1R-MSNs of the nucleus accumbens as a form of synaptic plasticity required for locomotor sensitization to cocaine. ERK activation probably constitutes a general feature of addictive drugs, because in all brain regions that receive dopamine inputs tetrahydrocannabinol, amphetamines, morphine and nicotine also activate ERK signalling⁴. Through cocaine-driven ERK phosphorylation, potentiation is induced selectively in D1R-MSNs, which leads to the occlusion of HFS-driven LTP. These findings are in line with observations that ERK activation may control AMPAR trafficking directly, an effect that may also be maintained over days through activation of ERK nuclear targets leading to gene regulation²³.

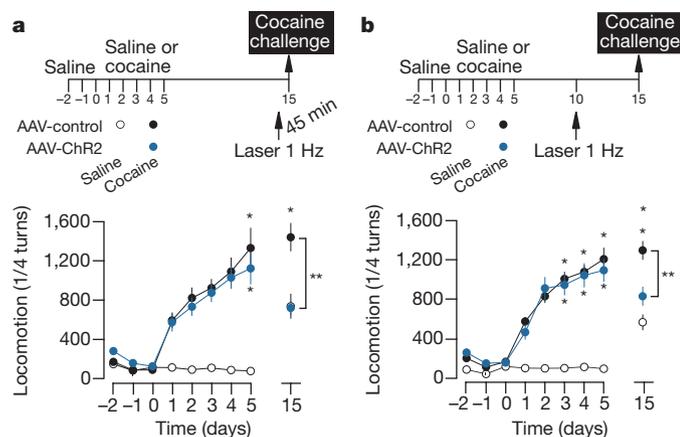


Figure 4 | Optogenetic depotentiation resets behavioural sensitization induced by chronic cocaine injections. **a**, After 5 daily cocaine injections a robust locomotor sensitization is measured in AAV-control and AAV-ChR2 infected mice. The light stimulation protocol (45 min before a challenge injection of cocaine on day 15) abolished the locomotor sensitized response in AAV-ChR2 infected mice (one-way analysis of variance: effect of treatment $F_{(2,41)} = 88.65$, $P < 0.001$. Post-hoc comparison by Bonferroni test yielded: $*P < 0.001$ for cocaine versus saline pre-treatment in AAV control; $**P < 0.001$ for AAV-ChR2 versus AAV-control infected mice, pre-treated with cocaine ($n = 13-15$). **b**, Light stimulation protocol applied on day 10 still reduced the locomotor sensitized response to a cocaine challenge injection on day 15 (one-way analysis of variance: effect of treatment $F_{(2,45)} = 15.31$, $P < 0.001$. Post-hoc comparison by Bonferroni test yielded: $*P < 0.001$ for cocaine versus saline pre-treatment in AAV-control infected mice; $**P = 0.003$ for AAV-ChR2 versus AAV-control infected mice, pre-treated with cocaine. No sensitization in AAV-ChR2 ($P = 0.27$, compared to saline pre-treatment; $n = 11-21$). Error bars show s.e.m.

We have explored and gained insight into the molecular mechanisms of synaptic adaptations to develop a strategy for reversal of cocaine-evoked potentiation with the goal of normalizing behaviour. We chose an optogenetic depotentiation of inputs from the infralimbic cortex to the nucleus accumbens shell because of the strong anatomical connection and the functional implication of this projection in cocaine-seeking behaviour^{24,25}. It is an appealing idea that behavioural adaptation, which closely reflects the potentiation of excitatory transmission onto D1R-MSNs, is due to an imbalance of the two classes of MSNs²⁶.

Several studies have already reported that pharmacological and molecular manipulations of key players in synaptic plasticity in the nucleus accumbens can affect adaptive behaviours associated with addictive drug exposure. For example, the inhibition of calcium-permeable AMPARs, a hallmark of late-stage cocaine-evoked synaptic plasticity in the nucleus accumbens²⁷ and the viral expression of a peptide that impairs GluA1 trafficking²⁸, reduce cue-induced cocaine seeking and cocaine-primed reinstatement, respectively.

We provide proof of principle that optogenetic manipulations can be used to reverse cocaine-evoked synaptic plasticity and thus abolish locomotor sensitization. Although light stimulation fully resets locomotor behaviour, sensitization begins to reappear after a few days, suggesting that several treatment sessions may be required to obtain long-lasting effects. This is not surprising, as chronic cocaine exposure also induces a number of additional adaptive changes including structural remodelling (for example, increase in spines²⁹) and alterations of gene expression²³.

Sensitization to cocaine-associated stimuli has been linked with incentive saliency³⁰ and may explain the exceptionally strong motivation of addicts to obtain the drug. With chronic use, early adaptive changes such as those described here may build up to enhance craving during cocaine withdrawal³⁰. Successful interventions that reverse these changes in animal models could inspire novel treatments for human addiction, a disease with a high social burden. Indeed, novel protocols of deep brain stimulation or transcranial magnetic stimulation may induce forms of synaptic plasticity that reverse drug-evoked adaptations, thus curbing the risk of relapse.

METHODS SUMMARY

All experiments were reviewed by the institutional ethics committee and approved by the relevant authorities of the Canton of Geneva. C57BL/6 or heterozygous BAC transgenic female and male mice, in which eGFP expression was driven by either D1R (*drd1a-eGFP*) or D2R (*drd2-eGFP*) gene regulatory elements were injected intraperitoneally with saline or cocaine. We then prepared brain slices for electrophysiological recordings as previously described³ or placed the mice in an apparatus to quantify locomotor behaviour. Locomotor sensitization or synaptic plasticity was monitored at the various time points after the cocaine injection. A two-injection or five-injection protocol to induce locomotor sensitization was used as previously described²². Standard surgical procedures³ were used to infect mice with ChR2-AAV or a control AAV (0.5 μ l) in the infralimbic medial prefrontal cortex while the light guides were aimed at both nucleus accumbens (shell). In depotentiation experiments, a 473-nm solid-state laser was used to carry out the *in vivo* light stimulation protocol in awake mice (600 pulses of 4 ms duration at 1 Hz, 10–20 mW), 45 min or 5 days before behavioural testing or *ex vivo* electrophysiology recordings, respectively.

Full Methods and any associated references are available in the online version of the paper at www.nature.com/nature.

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Supplementary Information is linked to the online version of the paper at www.nature.com/nature.

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Author Contributions V.P. carried out all electrophysiology experiments and was helped by M.T. with the behavioural experiments. C.L. designed the study and wrote the manuscript together with V.P. and M.T.

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METHODS

Mouse. C57BL/6 or heterozygous BAC transgenic mice, in which eGFP expression was driven by either D1R (*drd1a-eGFP*) or D2R (*drd2-eGFP*) gene regulatory elements were backcrossed³¹ in C57BL/6 mice for three to four generations, were used. Mice were housed in groups of 3–4 except for those implanted with guide cannulae, in which case animals were housed separately. All animals were kept in a temperature- and hygrometry-controlled environment with a 12 h light/12 h dark cycle. Mice were injected intraperitoneally with 20 mg kg⁻¹ cocaine, 40 mg kg⁻¹ SL327 (dissolved in 25% DMSO) or 0.9% saline (injection volume 10 ml kg⁻¹). Immediately after injection, mice were placed in the locomotor recording apparatus for 1 h. All procedures were approved by the Institutional Animal Care and Use Committee of the University of Geneva.

Locomotor sensitization. Locomotor activity was measured as the number of quarter turns entirely crossed by a mouse in a circular corridor. Locomotor chamber apparatus was placed under a video tracking system (Any-maze, Stoelting) and measurements were made automatically by the software. After 3 days of habituation to the test apparatus, mice underwent the experimental procedure, which consisted of two sessions of 60 min separated by 1 week (or 1 month), called day 1 and day 8 (or day 30). During the day 1 session, mice received saline or cocaine and were placed immediately in the corridor for 60 min. One week or 1 month later (day 8 or day 30), a second session was performed during which all mice were injected with cocaine before being placed in the circular corridor for 60 min. The light stimulation protocol (600 pulses of 4 ms at 1 Hz) was done 45 min before the second cocaine injection. To compare the effects of various times after the first injections or various virus infections, locomotor activity in response to the second cocaine injection was normalized to the mean locomotor activity of saline-pre-treated mice and the sensitization index was calculated by dividing the normalized locomotor response to the second injection by the normalized response to the first injection. Locomotor sensitization was also evaluated during challenge sessions that followed a chronic treatment (5 days of cocaine 15 mg kg⁻¹, 10 days withdrawal). The light stimulation protocol was done 45 min or 5 days before the challenge injection of cocaine.

Virus stereotaxic injection of ChR2-AAV or control AAV. AAV1 viruses produced at the University of North Carolina (Vector Core Facility) were injected into the infralimbic cortex of 15–20 g wild-type or BAC transgenic mice. Anaesthesia was induced and maintained with isoflurane (Baxter AG). The animal was placed in a stereotaxic frame (Angle One) and craniotomies were performed using stereotaxic coordinates (anterio-posterior, +1.9; medio-lateral, ±0.3; dorso-ventral, 2.4–2.6). Injections of AAV1 viruses (0.5 µl) were carried out using graduated pipettes (Drummond Scientific Company), broken back to a tip diameter of 10–15 µm, at a rate of ~0.05 µl min⁻¹. In all experiments the viruses were allowed a minimum of 3 weeks to incubate before any other procedures were carried out. As a control, some mice were injected with an AAV containing only GFP.

Cannula implantation. Following anaesthesia and craniotomy over the nucleus accumbens, two holes were drilled around the craniotomy and screws were placed in the holes. Two weeks after viral injections, guide cannulae (Plastics One) were lowered slowly into position using stereotaxic coordinates (bilaterally antero-posterior, +1.5; medio-lateral, ±1.6; dorso-ventral 4.1; 15° angle) and cemented in place using dental cement (Lang Dental MFG Company) to encase the base of the guide cannulae and the screws. Once the cement had dried, a dummy cannula (Plastics One) was placed inside each guide cannula to prevent infection.

Slice electrophysiology. Coronal 200–250-µm slices of mouse forebrain were prepared in cooled artificial cerebrospinal fluid (ACSF) containing (in mM): NaCl 119, KCl 2.5, MgCl 1.3, CaCl₂ 2.5, Na₂HPO₄ 1.0, NaHCO₃ 26.2 and glucose 11, bubbled with 95% O₂ and 5% CO₂. Slices were kept at 32–34 °C in a recording chamber superfused with 2.5 ml min⁻¹ ACSF. Visualized whole-cell voltage-clamp recording techniques were used to measure holding and synaptic responses of MSNs of the nucleus accumbens shell, identified in some experiments by the

presence of the GFP of BAC transgenic mice by using a fluorescent microscope (Olympus BX50WI, fluorescent light U-RFL-T). The holding potential was -70 mV, and the access resistance was monitored by a hyperpolarizing step of -14 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 (50–100 µs) at 0.1 Hz through bipolar stainless steel electrodes placed at the cortex-nucleus accumbens border. The internal solution contained (in mM): 140 k-glucuronate, 5 KCl, 130 CsCl, 10 HEPES, 0.2 EGTA, 2 MgCl₂, 4 Na₂ATP, 0.3 Na₃GTP and 10 sodium creatine-phosphate. Currents were amplified (Multiclamp 700B, Axon Instruments), filtered at 5 kHz and digitized at 20 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). LTP was induced by using the following HFS protocol: 100 pulses at 100 Hz repeated 4 times at 0.1 Hz paired with depolarization at 0 mV^{32,33}.

Miniature EPSCs were recorded in the presence of tetrodotoxin (0.5 µM). The frequency, amplitudes and kinetic properties of these currents were then analysed using the Mini Analysis software package (v.4.3, Synaptosoft). Cocaine or light illumination of ChR2-induced changes in cumulative miniature EPSC amplitude and inter-event interval distribution were analysed for statistical significance using the nonparametric two-sample Kolmogorov-Smirnov test (KyPlot) with a conservative critical probability level of $P < 0.05$.

Paired pulse ratio (PPR) was calculated by dividing the second evoked EPSC by the first with a 50-ms interval in between.

AMPA EPSCs evoked with ChR2 stimulation by 4-ms light pulses (LED, Thorlabs) were recorded in the same conditions as electrically evoked synaptic currents. Low-frequency stimulation (LFS; 1 Hz for 10 min) was applied with light pulses and the magnitude of LTD was determined by comparing average EPSCs that were recorded 20–30 min after induction to EPSCs recorded immediately before induction.

In vivo stimulation of infralimbic cortex projections in the nucleus accumbens shell. Virus injected and cannulated animals were allowed a minimum of 1 week to recover and to express the virus. 473-nm solid-state lasers (GMP, CH) were used to carry out the *in vivo* stimulation protocol in awake mice. A fibre optic (Thorlabs) was customized to enable the mouse to move freely during stimulation. Briefly, the plastic cap of a dummy cannula (Plastics One) was hollowed out and a hole of sufficient diameter for the fibre optic to pass through made in the top. This was threaded onto the fibre optic, one end of which was stripped to leave a 200 µm external diameter. The fibre was then lowered into the guide cannula on the mouse and the hollowed-out dummy cannula cap screwed onto the guide cannula. An FC/PC rotative fibre-optic rotary joint (Doric lenses) was used to release torsion in the fibre caused by the animal's rotation. The fibre was connected to the laser, which delivered 4 ms pulses at 1 Hz for 10 min, an established LTD protocol at excitatory synapses in the nucleus accumbens³⁴. All stimulations were carried out in the mouse home cage (except in the experiment shown in Supplementary Fig. 10, in which stimulation was performed during locomotor recordings in the circular corridor) 45 min or 5 days before behavioural testing or *ex vivo* electrophysiology recordings.

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