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Pascoli, Vincent Jean; Turiault, Marc; Luescher, Christian

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In vivo reversal of cocaine-evoked synaptic potentiation resets drug-induced adaptive behavior

Vincent Pascoli[1], Marc Turiault[1] & Christian Lüscher[1,2]

[1]*Dept. of Basic Neurosciences, Medical Faculty, University of Geneva, CH–1211 Geneva, Switzerland*

[2]*Clinic of Neurology, Dept. of Clinical Neurosciences, Geneva University Hospital, CH–1211 Geneva, Switzerland.*

***Corresponding author*:** Christian Lüscher, Dept. of Basic Neurosciences, Medical Faculty, University of Geneva, CH–1211 Geneva, Switzerland. Tel: +41 22 379 5450. email: *christian.luscher@unige.ch*

Drug-evoked synaptic plasticity is observed at many synapses and may underlie behavioral adaptations in addiction. A causal link between drug-evoked plasticity at identified synapses and behavioral adaptations, however, is missing, and the benefits of restoring baseline transmission have yet to be demonstrated. Here, we found that cocaine potentiates excitatory transmission in D1R-expressing medium-sized spiny neurons (D1R-MSNs) with a time course that parallels locomotor sensitization. Depotentiation of cortical nucleus accumbens (NAc) 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, most likely by creating an imbalance between distinct populations of MSNs in the NAc. Our data also provide proof of principle that reversal of cocaine-evoked synaptic plasticity can treat behavioral alterations caused by addictive drugs and may inspire novel therapeutic approaches, involving deep brain stimulation or transcranial magnetic stimulation.

Mechanistic investigations of drug-evoked synaptic plasticity[1] start with the identification of the molecular drug targets. Cocaine, for example, exerts its reinforcing[2] and early neuroadaptive effects[3] by inhibiting the dopamine transporter (DAT), thus causing a strong increase of mesolimbic dopamine. Among the many signaling pathways subsequently engaged, type 1 dopamine receptor (D1R) dependent phosphorylation of the extracellular signal-regulated kinase (ERK) in the nucleus accumbens (NAc[4]) is of particular interest because it has been implicated in several behavioral adaptations[5-7]. Because cocaine-evoked ERK phosphorylation requires NMDAR and D1R[8], it may be associated with synaptic potentiation. This link is suggested by the observations that some forms of NMDA-dependent long-term potentiation (LTP) in the hippocampus and the dorsal striatum also depends on ERK activity[9,10] and/or on D1Rs[11].

Cocaine occludes LTP in the NAc

We therefore first tested whether a cocaine treatment interfered with activity-dependent LTP in the NAc[12,13]. 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 cut in half in slices from mice that had received a single injection of cocaine seven days prior to 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 NAc fall into two classes of about equal proportions defined by the type of dopamine receptor expressed, and a fraction of neurons (6-17%) that express both receptors[14,15]. 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 BAC transgenic mice expressing enhanced green fluorescent protein (eGFP) either in 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). Since 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 (Suppl. Fig. 1a-d). Moreover, a significant contribution from cells that express both types of receptor is very unlikely and the D2R overexpression recently reported in *drd2*-EGFP mice[16] interferes with neither the reported synaptic effects nor with the acute locomotor response to cocaine (Suppl. 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 NAc (Suppl. Fig. 3), but because MSNs in the shell are the immediate targets of DA neurons of the medial VTA that undergo the most significant changes in response to a single cocaine injection[17], we focused on NAc 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 NAc may underlie drug-related behavioral adaptations (reviewed in Refs [7,18]). For example, when 5 daily cocaine injections were followed by a 10-14 day withdrawal, an overall increase of the AMPA/NMDA receptor (AMPAR/NMDAR) ratio[19] or GluA1/2 surface expression[20] was observed, and both observations were reversed by a challenge injection of cocaine.

The failure of HFS to induce LTP selectively in D1R-MSNs following 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. 2a-d) and observed a significant increase in amplitude along with a modest change in frequency of unitary events in D1R-MSNs (while these parameters remained unchanged in D2R-MSNs). Given that the paired pulse ratio also remained unchanged (Fig. 2e), a postsynaptic mechanism underlying the increase of transmission and hence an occlusion scenario is the most likely explanation.

Cocaine-evoked potentiation depends on ERK

We first characterized the induction criteria for HFS LTP in MSNs *in vitro* and found that it depends on NMDAR activation (Fig. 3a). Applying the MEK (MAP ERK kinase) inhibitor U0126 for the duration of the induction protocol also led to a complete block (Fig. 3b), indicating that activation of the ERK pathway is essential for this form of LTP. 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, first in the dendrites and then in the nucleus[21] of D1R-MSNs, but not in D2R-MSNs[14]. In the nucleus ERK modulates gene expression, while in dendrites ERK is likely involved in the regulation of activity-dependent spine dynamics, synaptic glutamate receptor insertion, and local dendritic protein synthesis[22,23]. To provide *in vivo* evidence for ERK dependence of cocaine-evoked plasticity, we treated mice with SL327, a blood-brain barrier penetrant ERK pathway inhibitor, prior to the saline or cocaine injection. We found that this manipulation rescued HFS-LTP in D1R-MSNs one week later (Fig. 3c,d) without modification of the acute locomotor response to cocaine (Suppl. Fig. 4). 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 (Suppl. Fig. 1e,f).

Since inhibition of the ERK pathway blocks locomotor sensitization to cocaine[24-25], 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 behavioral adaptation. Moreover, ERK activation is correlated both with AMPAR expression at the cell surface throughout the NAc and with locomotor sensitization[20,26], but the synapses involved have not been identified, and no causal link has been established.

Reversal of behavioral sensitization

We first confirmed that a single injection of cocaine was sufficient to cause locomotor sensitization to a second injection of the same dose[27]. This was the case when challenged a week but not a month after the initial cocaine injection (Suppl. Fig. 5). The behavioral 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 behavioral alterations. To test this prediction experimentally, we injected channelrhodopsin (ChR2) expressing adeno-associated virus into the infralimbic cortex and implanted light guides into the NAc to be able to selectively activate *in vivo* the terminals of this major excitatory input. The histological verification of the injection site did confirm robust expression in the infralimbic cortex, along with sparser expression in the prelimbic cortex (Fig. 4a, Suppl. Fig. 6). To further validate this approach, we recorded photocurrents from infected cortical neurons and prepared slices of the NAc shell in which wide-field light exposure led to robust AMPAR-mediated EPCSs (Fig. 4b). We next applied light pulses at 1 Hz for 10 minutes (an established LTD protocol to reduce synaptic transmission at this synapse[28]). We found that this protocol strongly depressed transmission in NAc slices from both saline and cocaine treated mice

(Fig. 4c). Interestingly, the magnitude of the depression was significantly larger in the latter, in line with an efficient depotentiation on top of the LTD. Since the LTD/depotentiation was NMDAR-dependent and without change of the paired pulse ratio, it is likely expressed by a postsynaptic mechanism and therefore constitutes an actual reversal of cocaine-evoked potentiation (Suppl. Fig. 7).

We next applied the above-validated protocol *in vivo* with the goal of establishing a causal link between cocaine-evoked plasticity and behavioral sensitization. We placed light guides into the ventral striatum, past the NAc core, thus preferentially aiming at the principal cortical input onto MSNs of the NAc shell, i.e. axons that have their origin in the infralimbic cortex (Suppl. Fig. 6). When freely moving mice were treated with the optogenetic depotentiation protocol 45 minutes prior to 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. 4d). 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. 4e) to a level comparable to baseline transmission (Fig. 2) without any effect on paired pulse ratio (Suppl. Fig. 8). In contrast, the mEPSC amplitude and frequency were not modified in D2R-MSNs after cocaine (Fig. 4f). Finally, after cocaine treatment and optogenetic depotentiation the HFS protocol resulted in LTP *ex vivo* (Fig. 4g), confirming the restoration of baseline transmission in D1R-MSNs.

Light stimulation after chronic cocaine

Behavioral 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 7 days later (Fig. 4, Ref. 27). While certainly not sufficient to induce addiction, such early forms of drug-induced adaptations are considered permissive building blocks for more definite behavioral 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 d withdrawal. To confirm this hypothesis we recorded mEPSCs at the end of this treatment, as well as 1 h and 24 h after a challenge injection (Fig. 5a-c). This experiment confirmed that excitatory synapses onto D1R-MSNs were selectively potentiated at the end of the withdrawal period.

One hour after the challenge injection, transmission in D1R-MSNs was back to baseline, but 24h after the injection the synaptic potentiation reappeared. We also observed a non-significant trend for an increase in the frequency of the events in both the D1R- and D2R-MSNs. Our results are in line with previous reports in the literature[29,30], but contrast with studies that argue against a potentiated synaptic transmission based on a normalized AMPAR/NMDR ratio 24h after a challenge injection[19]. Similarly, using a protein crosslinking assay to quantify AMPAR at the cell surface yielded data that failed to correlate with behavioral sensitization[31]. However these studies did not claim to focus on a specific synapse, which makes a direct comparison difficult.

We then tested whether, after this chronic exposure to cocaine, the light stimulation was still effective in reversing locomotor sensitization. After the 5 days of injection we imposed ten days of withdrawal, and treated the mice 45 minutes with the light stimulation before injecting a challenge dose. This completely reversed the locomotor sensitization (Fig. 5d). Since the first challenge injection yielded a locomotor behavior similar to the response to the second challenge injection 24 h later (Suppl. Fig. 9), we concluded that the former was not sufficient to cause a re-priming of the sensitization. As a control, we did not observe an acute effect during the light stimulation on the locomotor behavior (Suppl. Fig. 10).

Finally, to estimate how long the effect of the light stimulation lasted, we tested for behavioral sensitization five days after the intervention (Fig. 5 e). No significant locomotor sensitization was observed even when the mice were challenged after this extended time following the light stimulation.

Discussion

Our results identify NMDAR- and ERK-dependent LTP in D1R-MSNs of the NAc as a form of synaptic plasticity required for locomotor sensitization to cocaine. ERK activation most likely constitutes a general feature of addictive drugs, because in all brain regions that receive DA inputs tetrahydrocannabinol (THC), amphetamines, morphine and nicotine also activate ERK signaling[64]. 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 regulation[32].

Here 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

behavior. We chose an optogenetic depotientiaton of inputs from the infralimbic cortex to the NAc shell because of the strong anatomical connection and the functional implication of this projection in cocaine-seeking behavior[3334,35]. Indeed, when applied *in vivo*, this protocol efficiently depotentiated the synaptic transmission as demonstrated *ex vivo* and led to an abolishment of behavioral sensitization, even when it was induced by repetitive cocaine exposure. We cannot exclude the possibility that the optogenetic manipulation may also have transiently affected inputs onto D2R-MSNs to contribute to the effects on the behavior. However, mEPSCs in D2R-MSNs remained unaffected by the optogenetic depotentiation protocol in cocaine-treated animals. The idea is appealing that the behavioral adaptation, which closely reflects the potentiation of excitatory transmission onto D1R-MSNs is due to an imbalance of the two classes of MSNs[36]. However, unlike the dorsal striatum where two strictly segregated classes of neurons innervate distinct projection targets[37], the situation in the NAc is less established[38]. Future studies will have to determine how circuit function is affected by the potentiation of D1R-MSNs.

Several studies have already reported that pharmacological and molecular manipulations of key players of synaptic plasticity in the NAc can affect adaptive behaviors associated with addictive drug exposure. For example, the inhibition of calcium-permeable AMPARs, a hallmark of late stage cocaine-evoked synaptic plasticity in the NAc[39] and the viral expression of a peptide that impairs GluA1 trafficking[40], both reduce cue-induced cocaine seeking.

In conclusion, we provide proof of principle that optogenetic manipulations can be used to reverse cocaine-evoked synaptic plasticity and thus abolish locomotor sensitization. While the light stimulation fully resets locomotor behavior, 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, since chronic cocaine exposure also induces a number of additional adaptive changes including structural remodeling (e.g. increase in spines, Ref.41-43) and alterations of gene expression[32]. It will also be of interest to know whether the reported anaplasticity in addicted rats after many sessions of self-administration[44] can be overcome by the optogenetic depotentiation protocol used here.

Sensitization to cocaine-associated stimuli has been linked with incentive saliency[45] 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 withdrawal[45]. 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[46] or transcranial magnetic stimulation may induce forms of synaptic plasticity that reverse drug-evoked adaptations, thus curbing the risk of relapse.

Methods summary

C57BL/6 or heterozygous BAC transgenic mice, in which enhanced green fluorescent protein (EGFP) expression was driven by either D1R (drd1a-EGFP) or D2R (drd2-EGFP) gene regulatory elements were injected i.p. with saline or cocaine and placed immediately in the locomotor recording apparatus. In some experiments SL327 was injected 1h prior to saline or cocaine. Standard surgical procedures[3] were used to infect mice with ChR2-AAV or a control-AAV (0,5 µl) in the infralimbic medial prefrontal cortex while the light-guides were aimed at both NAc (shell). 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 described[27]. Acute brain slices from the various groups were prepared for electrophysiological recordings as previously described[28]. 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 d before behavioral testing or *ex vivo* electrophysiology recordings, respectively.

Full methods are available online.

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Figure legends

Figure 1: Cocaine disrupts HFS-induced LTP in D1R-MSNs of the NAc. AMPAR EPSCs evoked by electrical stimulation of glutamatergic inputs were recorded from medium spiny neurons in NAc shell under whole-cell voltage-clamp mode at a holding potential of –70 mV before and after high frequency stimulation (HFS, 100 pulses at 100 Hz repeated 4 times at 0.1 Hz paired with depolarization at 0 mV). A week or a month after wild type mice were injected intraperitoneally with saline or cocaine, acute NAc slices were prepared and HFS was applied after a 5-10 min baseline. Normalized EPSC (%) as a function of time and overlay of averaged (20 trials) traces of AMPAR EPSCs before (black line) and after (gray line) HFS are represented. Symbols represent average of 6 trials. **(a)** A week after cocaine injection, LTP was cut in half (cocaine, n = 30, 149 ± 14.7 % versus saline, n = 15, 224 ± 17.9 %, $P = 0.003$). **(b)** After a month, no difference between treatments was detected (cocaine, n = 13, 206 ± 21.3 % versus saline, n = 10, 204 ± 26.1 %, $P = 0.942$). **(c)** Using *drd1a*- and *drd2*-EGFP mice treated with saline a week before *ex vivo* electrophysiology, no difference was detected in the amplitude of LTP induced by HFS when D1R-MSNs, n = 14, 206 ± 16.0 % versus D2R-MSNs, n = 15, 229 ± 24.1 %, $P = 0.441$. **(d)** Cocaine abolished LTP in D1R-MSNs, n = 20, 115 ± 8.5 % versus D2R-MSNs, n = 21, 239 ± 12.7 %, $P \leq 0.001$. Scale bars: 10 ms and 20 pA. Error bars: s.e.m.

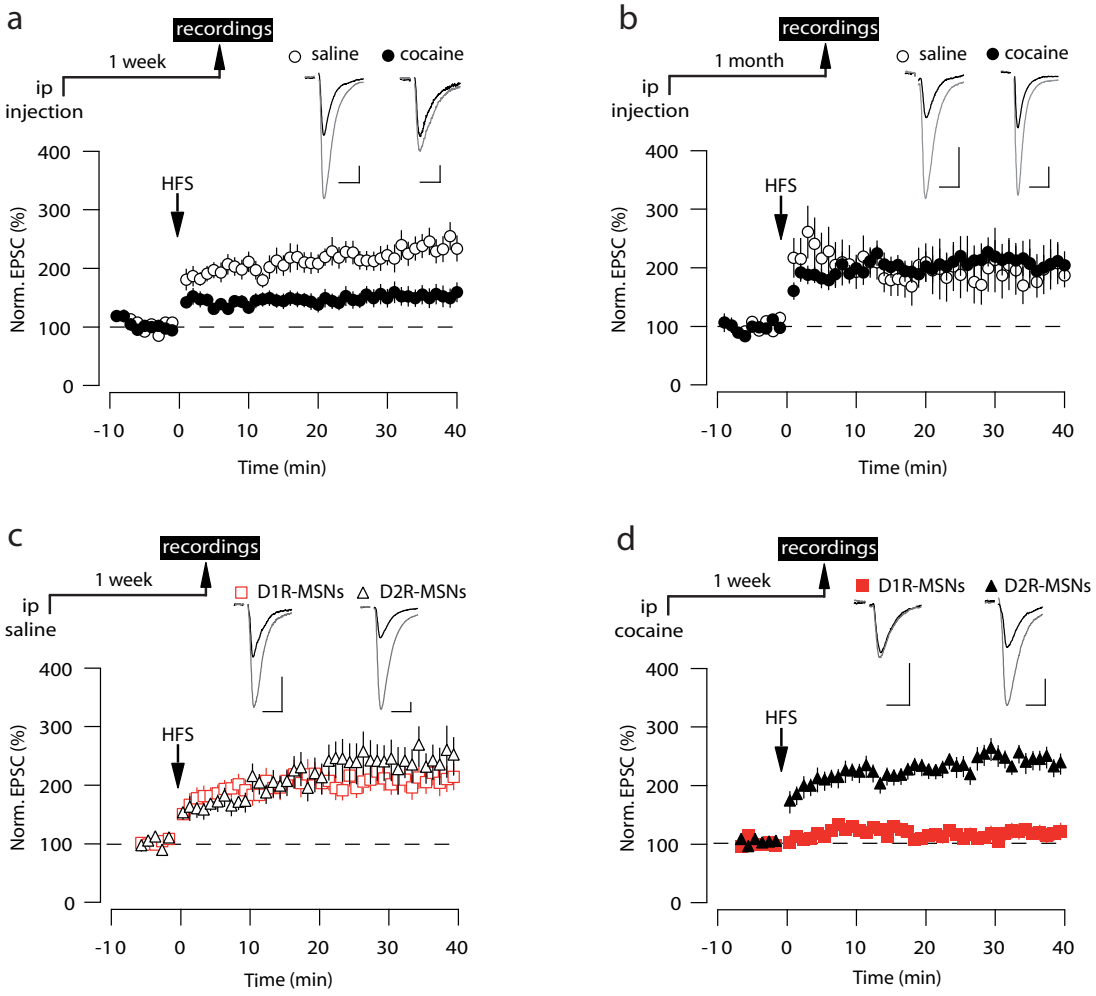
Figure 2: Cocaine-evoked potentiation is expressed by a postsynaptic mechanism. **(a, c)** Sample traces of miniature EPSCs (mEPSCs) recorded from D1R-MSNs or from D2R-MSNs a week after injection of saline or cocaine. Scale bars: 200 ms and 20 pA. **(b)** Cumulative probability and mean values of amplitude and frequency for each experimental group. Cocaine (n=8) significantly increases mean amplitude of mEPSCs in D1R-MSNs (**P* = 0.001 compared to saline, n=8), which is also shown by the significant change in the cumulative amplitude distribution [*P* < 0.01, Kolmogorov-Smirnov (K-S) test]. No significant changes in mEPSCs frequency (*P* = 0.124) or in cumulative inter-event interval distribution (K-S, *P* > 0.05) of D1R-MSNs was detected. **(d)** In D2R-MSNs neither amplitude nor frequency was modified by cocaine (n = 8) versus saline (n = 8) (amplitude *P* = 0.38, K-S P > 0.05; frequency *P* = 0.56, K-S *P* > 0.05). **(e)** Paired-pulse ratio (PPR) measured with a 50 ms inter-stimulus interval is not modified by cocaine treatment either in D1R- or D2R-MSNs (cocaine, n = 23, 1.4 ± 0.1 versus saline, n = 13, 1.7 ± 0.11, *P* = 0.092; cocaine, n = 26, 1.8 ± 0.1 versus saline, n = 15, 1.6 ± 0.1, *P* = 0.339, respectively). Scale bars: 20 ms and 20 pA. Error bars: s.e.m.

Figure 3: HFS-LTP and cocaine-evoked potentiation both depend on ERK activation. (a) Bath application of an NMDAR antagonist (AP5, 100 μM) during (15 min before and 10 min after) the HFS protocol blocked LTP of AMPAR EPSCs in MSNs of the NAc shell (control, n = 7, 209 ± 15 % versus AP5, n = 9, 93 ± 9 %, P ≤ 0.001). **(b)** Bath application of the MEK inhibitor (U0126, 5 μM, 15 min before until 10 min after HFS protocol) also blocked LTP (control, n = 6, 234 ± 17.8 % versus U0126, n = 9, 109 ± 16.2 %, *P* ≤ 0.001). **(c)** Intraperitoneal administration of the MEK inhibitor (SL237, 40 mg/kg) in *drd1a*-EGFP mice or *drd2*-EGFP mice 1 h before saline did not modify HFS-LTP in D1R- or D2R-MSNs when assessed one week later. In these conditions, no significant change in LTP magnitude was detected in D1R-MSNs (n = 6, 224 ± 31 % versus D2R-MSNs, n = 6, 221 ± 27 %, *P* = 0.928). **(d)** Intraperitoneal administration of the MEK inhibitor (SL237, 40 mg/kg) in *drd1a*-EGFP mice or *drd2*-EGFP mice 1 hour before cocaine restored HFS-LTP in D1R-MSNs when assessed one week later. In these conditions, no significant change in LTP magnitude was detected in D1R-MSNs (n = 13, 200 ± 22 % versus D2R-MSNs, n = 11, 232 ± 27 %, *P* = 0.363). Scale bars: 10 ms and 20 pA. Error bars: s.e.m.

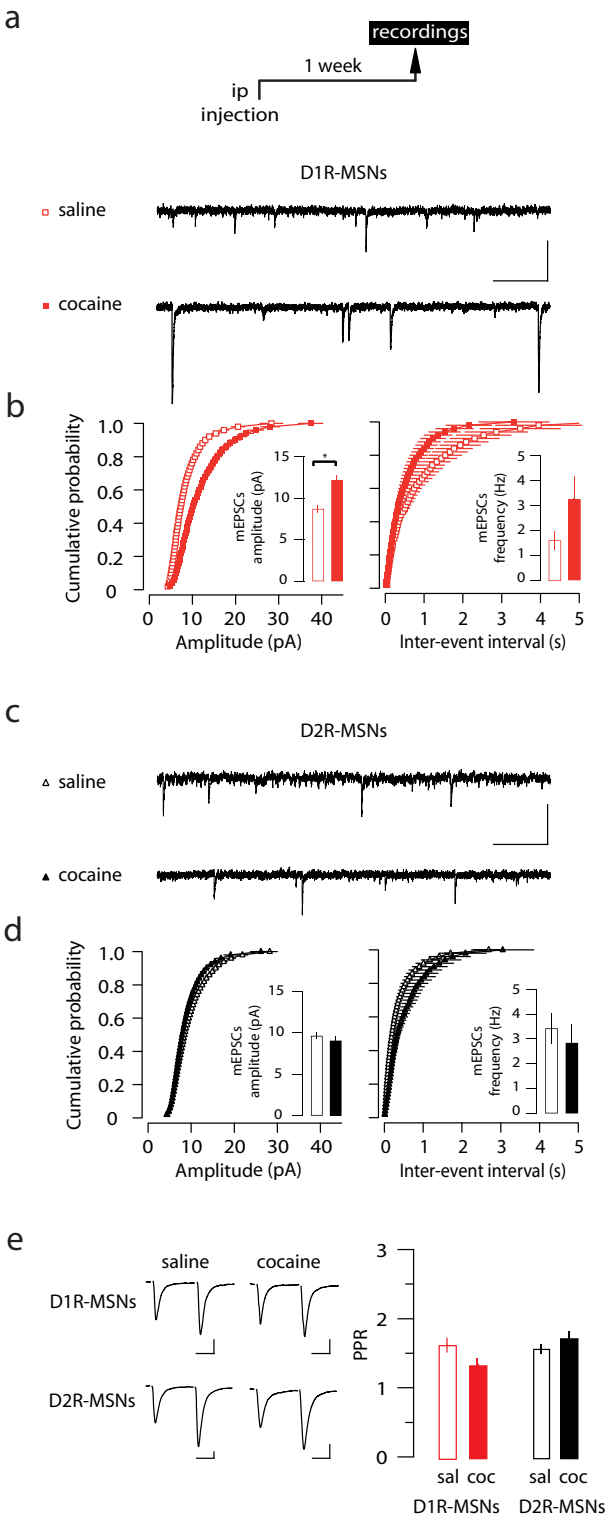
Figure 4: Reversal of cocaine-evoked potentiation erases locomotor sensitization to cocaine. **(a)** Schematic illustration of the infected site with AAV coding for eGFP-ChR2 and of the bilateral cannula implantation with optic fibers inserted in NAc shell. (Left inset) Picture showing eGFP cortical expression of a wild type mouse injected with AAV-eGFP-ChR2. (Right inset) Confocal picture of axonal inputs (green) onto NAc shell neurons (blue nuclear staining, Hoechst, 60 fold magnification). **(b)** Overlay of averaged traces (20 trials) in response to light pulses (470 nm) of 4 ms and 100 ms in infected cells in infralimbic cortex slices (top). In NAc slices, light pulses (4 ms) evoked AMPAR EPSCs (blocked by NBQX 20 µM, green trace, bottom). **(c)** Low Frequency Stimulation (light pulses of 4 ms at 1 Hz for 10 min) induced a stronger depression of AMPAR EPSCs evoked with light in NAc slices from mice injected with cocaine 1 week prior than in slices from saline injected mice (cocaine, n = 8, 27 ± 4.5 % compared to saline, n = 8, 48 ± 5.7 %, P = 0.013). **(d)** Locomotor sensitization induced by cocaine in mice infected with AAV-ChR2 or AAV-control. Scatter plots of individual score and bars represent mean ± s.e.m. of quarter-turns in the circular corridor for 60 min after injection. The sensitization index (scatter plots of individual index and bars represent mean ± s.e.m.) for each group was calculated. Illumination of the NAc through optic fibers with laser-evoked light pulses (4 ms pulse, 1 Hz, 10 min) 45 min prior to injection of cocaine on day 8 erased locomotor sensitization in mice infected with ChR2 but not in control virus injected mice. Differences were analyzed with a multiple-way repeated-measures analysis of variance for matching data [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 analysis yielded: $*P < 0.001$ for cocaine (n = 12) versus saline (n = 9) pretreatment on day 8 in AAV-control infected mice and $P = 0.203$ cocaine (n = 10) versus saline (n = 8) pretreatment on day 8 in AAV-ChR2 infected mice. $\# P = 0.0015$ for locomotor response to cocaine on day 8 for AAV-ChR2 infected mice (n = 10) versus AAV-control infected mice (n = 12). No effect of light protocol on locomotor response to cocaine on day 8 when mice receive saline on day 1 was detected ($P > 0.05$ ChR2 virus, n = 8, versus control virus, n = 9). Sensitization index $P = 0.002$ for AAV-ChR2 compared to control-AAV. (E and F) Instead of testing locomotor sensitization to cocaine, acute slices of cocaine- and laser-treated mice were prepared on day 8 (45 min after light stimulation), and mEPSCs were recorded. Recordings from AAV-ChR2 infected mice were compared to AAV-control infected mice. Sample traces of mEPSCs recorded from D1R-MSNs **(e)** or from D2R-MSNs **(f)**. Scale bars: 200 ms and 20 pA. Cumulative probability and mean values of amplitude and frequency for each experimental group are shown. All mice received cocaine on day 1. In D1R-MSNs, mEPSCs amplitude significantly decreased with no change in frequency (n = 10 in ChR2 ; n = 9 in control, mean amplitude, $P \leq 0.001$ and cumulative amplitude probability, K-S, $P < 0.001$; mean frequency, $P = 0.165$ and cumulative inter-event interval probability, K-S, $P > 0.05$). No difference was detected after the light illumination protocol from D2R-MSNs of AAV-ChR2 infected mice (n = 8) versus AAV-control infected mice (n = 8) (K-S > 0.05 for cumulative probability amplitude and inter-event interval and $P = 0.57$ and 0.52 for mean amplitude and mean frequency, respectively). **(g)** HFS-LTP in D1R-MSNs of mice infected with ChR2 virus or with a control virus and treated with cocaine (20 mg/kg) one week before light stimulation (4 ms light pulses, 1 Hz, 10 min). Slices were prepared 45 min before light stimulation. Scale bars: 10 ms and 20 pA. light stimulation restored the ability of HFS to induce LTP (AAV-ChR2 (n = 7) 191 ± 21.2 % versus AAV-control (n = 7) 93 ± 9.1 %, $P = 0.001$). Error bars: s.e.m.

Figure 5: Optogenetic depotentiation resets behavioral sensitization induced by chronic cocaine injections. (a) Schematic representation of treatment protocol. **(b)** Top, sample traces of mEPSCs recorded from D1R- or D2R-MSNs on withdrawal day 10, and 1 h and 24 h after a challenge injection. Scale bars: 200 ms and 20 pA. Bottom panels**,** cumulative probability of amplitude and frequency for each experimental group. Cumulative amplitude distributions are different between D1R- and D2R-MSNs on day 15 and 24 h after the challenge injection of cocaine [K-S = 0.009, $P < 0.01$ and K-S < 0.0001, $P < 0.001$, respectively] but not 1 h after the challenge [K-S = 0.95, $P > 0.05$]. No significant change between D1R- and D2R-MSNs in mEPSCs cumulative inter-event interval distribution at any time point (K-S = 0.84, 0.24 and 0.056, $P > 0.05$) was detected. **(c)** Mean values of amplitude and frequency of each experimental group were compared to values obtained in saline treated mice (continuous and dotted gray lines represent mean value and s.e.m. respectively taken from Fig. 2). Note that mean amplitude of mEPSCs in D1R-MSNs is increased on day 15 and 24 h after the challenge injection (*$P < 0.001$ and *$P = 0.002$, respectively), but not 1 h after ($P = 0.49$). In D2R-MSNs, amplitude of mEPSCs on day 15 or after the challenge is not different from saline ($P = 0.72$, $P = 0.60$ and $P = 0.27$, for the 3 time points respectively). A non-significant increase of mEPSCs frequency compared to saline treated mice was observed in D1R-MSNs and D2R-MSNs (D1R-MSNs : $P = 0.065$, $P = 0.057$ and $P = 0.057$; D2R-MSNs : $P = 0.15$, $P = 0.089$ and $P = 0.068$, respectively on day 15, 1 h and 24 h after the challenge injection of cocaine). The analyses were done in D1R-MSNs (n = 10, 9 and 9 for before, 1 h and 24 h, respectively) D2R-MSNs (n = 11, 8 and 10 for before, 1 h and 24 h, respectively). **(d)** Locomotor sensitization induced by 5 daily injections of cocaine in mice infected with AAV-ChR2 or AAV-control. Graph represents mean ± s.e.m. of quarter-turns in the circular corridor for 30 min after injection. Illumination of the NAc through optic fibers with laser-evoked light pulses (4 ms pulses at 1 Hz for 10 min) 45 min prior to a challenge injection of cocaine on day 15 erased locomotor sensitization in mice infected with ChR2 but not in control virus injected mice. Differences between groups were analyzed with a multiple-way repeated-measures analysis of variance for matching data during the 5 days of cocaine [interaction between day and treatment $F_{(7,41)} = 2,13$, $P = 0.035$; effect of day $F_{(4,41)} = 7.23$, $P < 0.001$; effect of treatment $F_{(2,41)} = 99.64$, $P < 0.001$]. Bonferroni analysis yielded: *$P < 0.001$ and *$P = 0.013$ for day 5 versus day 1 in AAV-control infected mice (n = 13) and AAV-ChR2 infected mice (n = 15), respectively. No difference between AAV-control infected mice and AAV-ChR2 infected mice was detected from day 1 to day 5. Cocaine significantly increases locomotor activity when compared to saline (n= 13, not indicated on graph). On day 15, the 3 groups received a challenge injection of cocaine after the light stimulation, only the AAV-control infected mice pretreated with 5 injections of cocaine showed a sensitized locomotor response (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 pretreatment in AAV-control infected mice, # $P < 0.001$ for AAV-ChR2 infected mice versus AAV-control infected mice, pretreated with cocaine. **(e)** Same experiment as (d) except that optogenetic treatment was given 5 days before the challenge. Locomotor sensitization in mice infected with ChR2 is reduced compared to control virus-injected mice. Multiple-way repeated-measures analysis of variance for matching data during the 5 days of cocaine [interaction between day and treatment $F_{(7,45)} = 2,64$, $P = 0.009$; effect of day $F_{(4,45)} = 10.79$, $P < 0.001$; effect of treatment $F_{(2,45)} = 136.21$, $P < 0.001$]. Bonferroni: *$P < 0.005$ for day 2, 3, 4 or 5 versus day 1 in AAV-control infected mice (n = 21) and AAV-ChR2 infected mice (n = 13). No difference between AAV-control infected mice and AAV-ChR2 infected mice was detected from day 1 to day 5. On

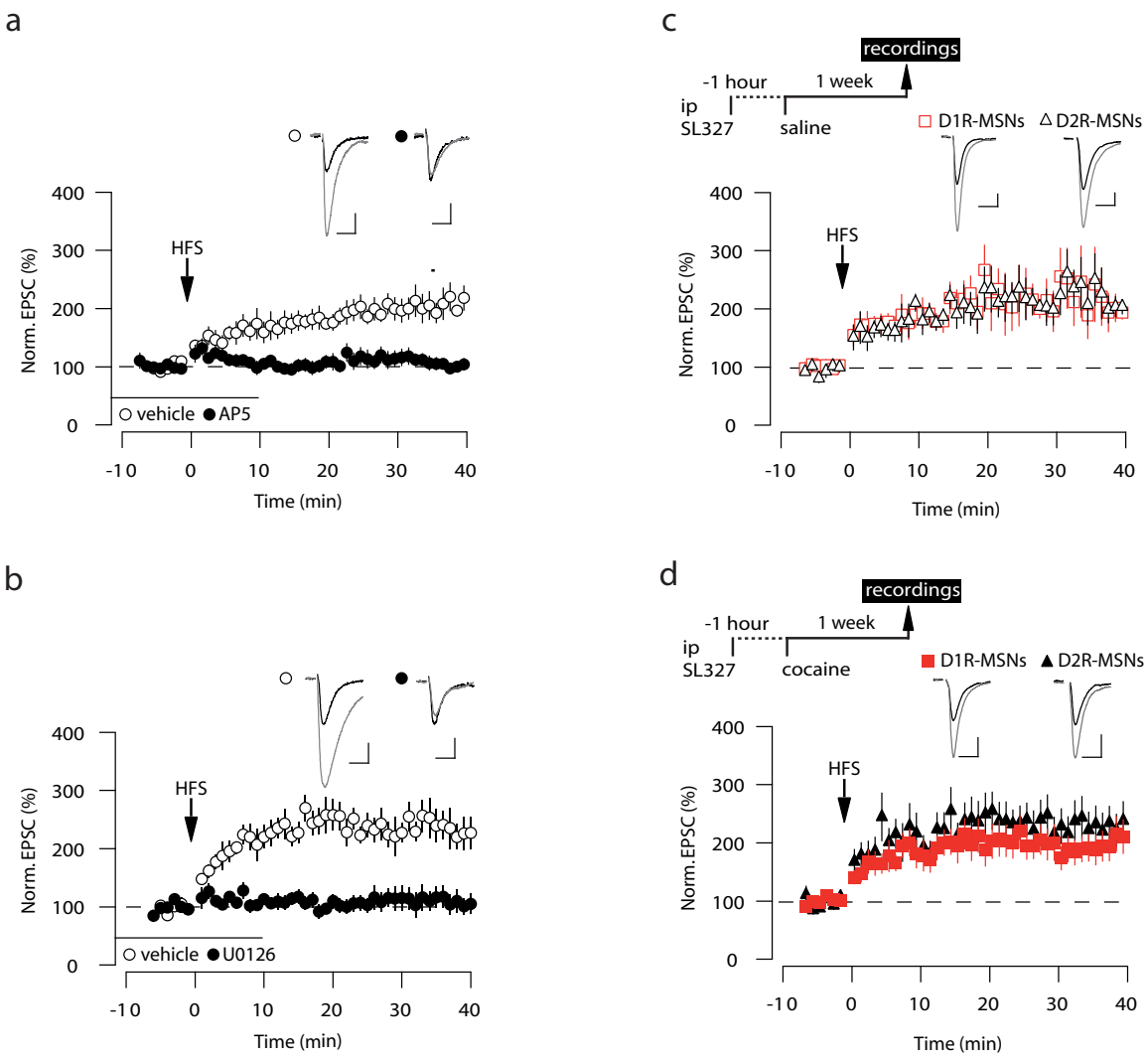
day 15, only the AAV-control infected mice pretreated with 5 injections of cocaine showed a sensitized locomotor response to a challenge injection (one-way analysis of variance: effect of treatment $F_{(2,45)} = 15.31$, $P < 0.001$, Post hoc comparison by Bonferroni test yielded: $*P < 0.001$ for cocaine versus saline pretreatment in AAV-control infected mice, $\# P = 0.003$ for AAV-ChR2 infected mice versus AAV-control infected mice, pretreated with cocaine. No sensitization in AAV-ChR2 ($P = 0.27$, compared to saline pretreatment).



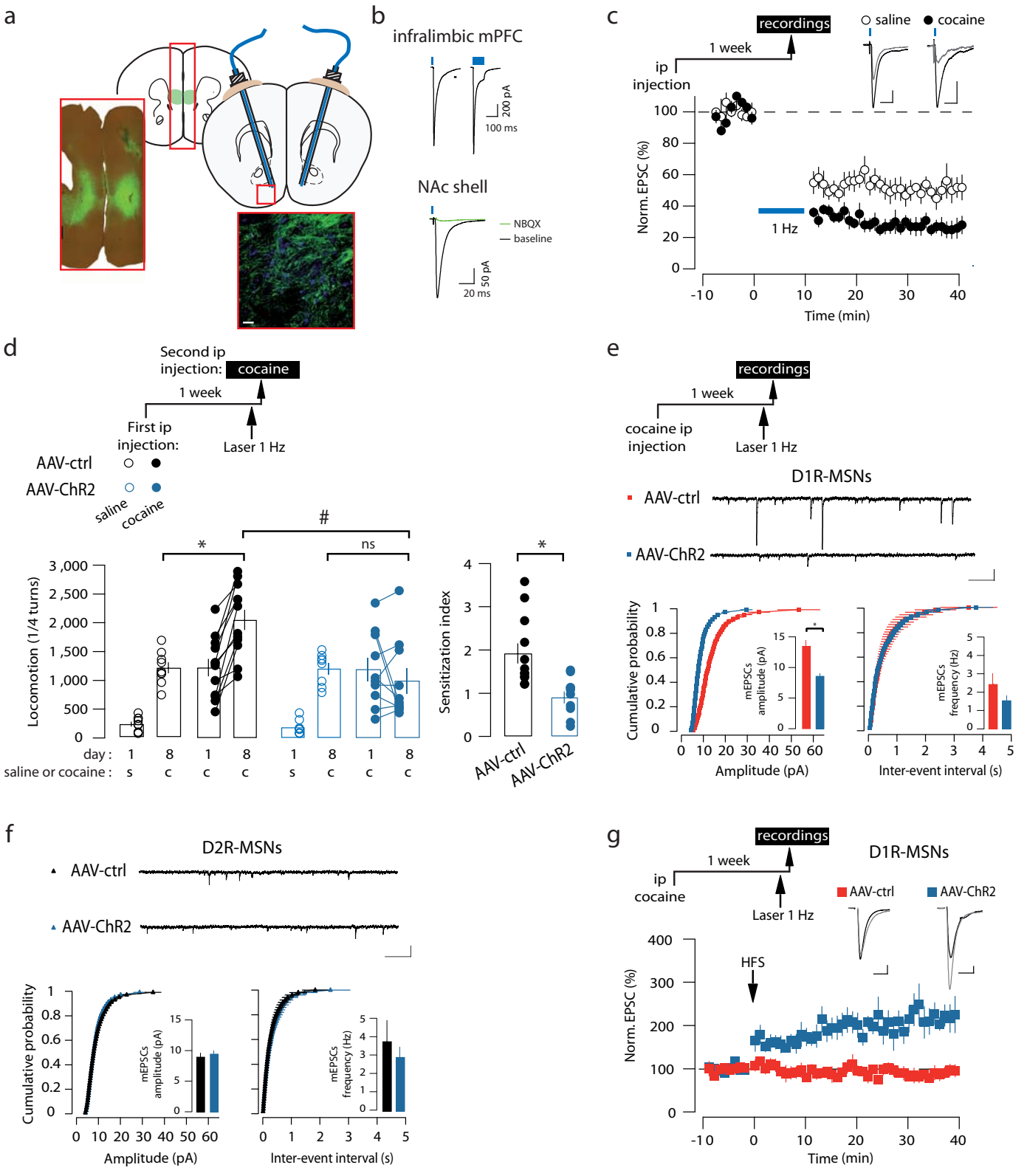
Pascoli et al., Figure 1



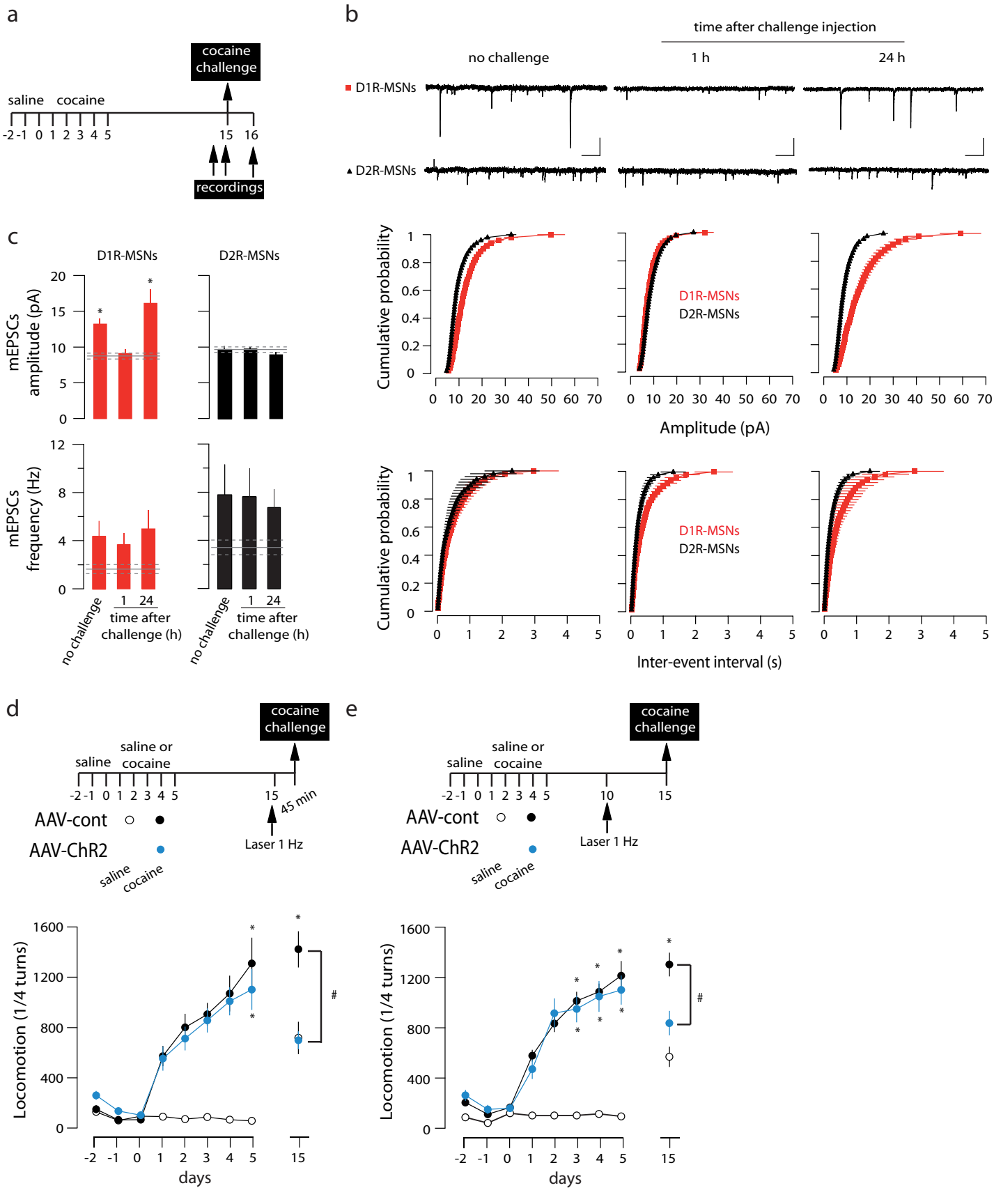
Pascoli et al., Figure 2



Pascoli et al., Figure 3



Pascoli et al., Figure 4



Pascoli et al., Figure 5