Article

Neuron

Sufficiency of Mesolimbic Dopamine Neuron Stimulation for the Progression to Addiction

Highlights

- Dopamine neuron self-stimulation evokes synaptic plasticity in the NAc, driving relapse
- Dopamine is sufficient to trigger compulsive taking
- Neurons in the orbitofrontal cortex are hyperexcitable in mice resistant to punishment
- Chemogenetic inhibition of the OFC reduces compulsive self-stimulation

Authors

Vincent Pascoli, Jean Terrier, Agnès Hiver, Christian Lüscher

Correspondence

christian.luscher@unige.ch

In Brief

Pascoli, Terrier et al. demonstrate that DA neuron self-stimulation in mice is sufficient to induce key features of addiction, such as synaptic plasticity in the NAc, cue-associated relapse, and perseverance of consumption despite negative consequences. The orbitofrontal cortex drives the transition to compulsivity.

Pascoli et al., 2015, Neuron *88*, 1–13
December 2, 2015
http://dx.doi.org/10.1016/j.neuron.2015.10.017

CellPress

Please cite this article in press as: Pascoli et al., Sufficiency of Mesolimbic Dopamine Neuron Stimulation for the Progression to Addiction, Neuron (2015), http://dx.doi.org/10.1016/j.neuron.2015.10.017

Neuron
Article

CellPress

Sufficiency of Mesolimbic Dopamine Neuron Stimulation for the Progression to Addiction

Vincent Pascoli,[1,3] Jean Terrier,[1,3] Agnès Hiver,[1] and Christian Lüscher[1,2,*]
[1]Department of Basic Neurosciences, Medical Faculty, University of Geneva, 1211 Geneva, Switzerland
[2]Clinic of Neurology, Department of Clinical Neurosciences, Geneva University Hospital, 1211 Geneva, Switzerland
[3]Co-first author
*Correspondence: christian.luscher@unige.ch
http://dx.doi.org/10.1016/j.neuron.2015.10.017

SUMMARY

The factors causing the transition from recreational drug consumption to addiction remain largely unknown. It has not been tested whether dopamine (DA) is sufficient to trigger this process. Here we use optogenetic self-stimulation of DA neurons of the ventral tegmental area (VTA) to selectively mimic the defining commonality of addictive drugs. All mice readily acquired self-stimulation. After weeks of abstinence, cue-induced relapse was observed in parallel with a potentiation of excitatory afferents onto D1 receptor-expressing neurons of the nucleus accumbens (NAc). When the mice had to endure a mild electric foot shock to obtain a stimulation, some stopped while others persevered. The resistance to punishment was associated with enhanced neural activity in the orbitofrontal cortex (OFC) while chemogenetic inhibition of the OFC reduced compulsivity. Together, these results show that stimulating VTA DA neurons induces behavioral and cellular hallmarks of addiction, indicating sufficiency for the induction and progression of the disease.

INTRODUCTION

Addiction is a disease that evolves in several steps (Everitt et al., 2008; George et al., 2014). The diagnosis is made when recreational use becomes compulsive, persisting despite negative consequences. While a leading addiction hypothesis posits that drugs of abuse cause the disease because they excessively increase the concentration of dopamine (DA) in the brain, it is unclear whether triggering this system is sufficient to drive the transitions from recreational use to addiction (Di Chiara and Bassareo, 2007; Volkow and Morales, 2015). The supporting evidence for the DA hypothesis for drug reinforcement has accumulated over several decades and relies on the initial effect of drugs. For example, addictive drugs reduce the threshold for intracranial self-stimulation (ICSS) of the medial forebrain bundle, a fiber tract containing, among others, ascending DA projection from the midbrain (Stein, 1964; Crow, 1970; Kornetsky et al., 1979). Pharmacology and lesion studies then identified the mesocorticolimbic DA system as the origin of this circuit (Wise and Bozarth, 1982). In the late 1980s, a direct measure of the extracellular DA concentration with microdialysis confirmed that addictive drugs shared the property of evoking a DA surge in the NAc (Di Chiara and Imperato, 1988). This led to the proposal of a mechanistic classification of addictive drugs (Lüscher and Ungless, 2006).

Much less is known of how these initial effects of drug use facilitate the transition to addiction. DA-independent mechanisms have been considered because addictive drugs have other pharmacological targets. For instance, cocaine, in addition to inhibiting the DA transporter (DAT), also binds to SERT (serotonin transporter) and NET (norepinephrine transporter) to decrease serotonin and norepinephrine reuptake, respectively, thus increasing the concentration of all major monoamines (Han and Gu, 2006; Tassin, 2008). Similar concerns may apply to other psychostimulants. Moreover, there is a claim that opiates are, at least in the initial phase, DA independent (Badiani et al., 2011; Ting-A-Kee and van der Kooy, 2012). The DA hypothesis has also been challenged based on genetic mouse models, where, after interference with the DA system, some forms of drug-adaptive behavior were still apparent. For example, DAT knockout mice self-administer cocaine (Rocha et al., 1998), and abolishing DA synthesis either pharmacologically (Pettit et al., 1984) or genetically (Hnasko et al., 2007) failed to prevent drug self-administration or conditioned place preference. While better characterization of these transgenic mice and generation of double monoamine transporters knockouts have resolved some of these issues (Rocha, 2003; Thomsen et al., 2009), the sufficiency of DA to trigger cardinal features of addiction is unknown. To circumvent issues of non-specificity, we have therefore decided to allow mice to self-stimulate VTA DA neurons using an optogenetic approach.

Recent studies have shown that activation of DA neurons in the midbrain can induce place preference (Tsai et al., 2009) or reinforce instrumental behavior (Adamantidis et al., 2011; Witten et al., 2011; Kim et al., 2012; Rossi et al., 2013; McDevitt et al., 2014; Ilango et al., 2014). While this selective activation of DA pathways confirms intracranial self-stimulation (ICSS) studies carried out more than 30 years ago in delineating the reward system (Fouriezos et al., 1978), they fall short demonstrating the induction of late-stage adaptive behavior that defines addiction, nor did they identify the underlying neuronal adaptations. Here we used optogenetic manipulation not only to allow for direct testing of the sufficiency criterion for phasic DA signaling

in initiating reinforcement, but also to test for the transition to addiction.

A striking observation of the later stages of the disease is that even with the most addictive drugs, only a fraction of users becomes addicted (Warner et al., 1995; O'Brien, 1997). Human addicts will continue drug consumption despite negative consequences (see American Society for Addiction Medicine's "Definition of Addiction," DSM5, American Psychiatric Association, 2013), typically related to social and psychological defeats that are often delayed in time. Similarly, in rodents roughly one out of five animals that acquire self-administration of cocaine are eventually classified as addicted (Deroche-Gamonet et al., 2004; Kasanetz et al., 2010; but see George et al., 2014). Perseverance of drug intake despite negative consequences can also be modeled in rodents by introducing a simple aversive stimulus to the consumption schedule. While the human disease is more complex, associating punishment with consumption is a straightforward model of a core component of addiction.

Here, we used a mild foot shock to evaluate its consequence on self-administration of cocaine, sucrose, and optogenetic self-stimulation. We further investigate whether DA neuron self-stimulation can induce two addictive-related behaviors—cue-associated reward seeking and compulsivity associated with consumption despite negative consequences—and characterize the neural plasticity associated with these behaviors.

RESULTS

Acquisition of VTA DA Neuron Self-Stimulation

To control DA neuron activity, we injected a Cre-inducible adeno-associated virus (AAV) with a double-floxed inverted open reading frame (DIO) containing *ChR2* fused to enhanced yellow fluorescent protein (eYFP) (Atasoy et al., 2008; Brown et al., 2010) into the VTA of DAT-Cre mice. In addition, an optic fiber was placed to target the VTA (*ChR2*, see Experimental Procedures). Specificity of the *ChR2* expression was confirmed by the co-localization of eYFP with Tyrosine Hydroxylase (TH), an enzyme required for DA synthesis (Figure 1A).

First, to establish the laser stimulation protocol, mice were placed in an operant box where they could press an active lever, which triggered a number of laser stimulations that was varied (1, 2, 8, 32, 60, or 120 bursts) every two sessions. To emulate phasic firing pattern (Hyland et al., 2002; Mameli-Engvall et al., 2006; Zhang et al., 2009) typically induced by natural reward (Schultz, 1998), we used burst stimulation. One burst consisted of five laser pulses of 4 ms, at 20 Hz, and was repeated twice per second. We found that mice adapted their lever-pressing behavior as a function of bursts per laser stimulation, thus controlling the total number of bursts received (Figure 1B). This behavior was reminiscent of self-administration of addictive drugs, when the dose per infusion was varied (Piazza et al., 2000). For the subsequent experiments, we chose to administer 30 bursts per lever press, yielding a half-maximal number of bursts (Figure 1B). To mimic the delay in DA increase typically observed when drugs are administered intravenously (Aragona et al., 2008), we delayed the laser stimulation by 5 s and added a flashing cue light for 10 s (Figure 1C).

During 12 consecutive days, mice were allowed to self-stimulate a maximum of 80 times in 2 hr. Mice quickly increased the rate of laser stimulation, reaching 80 laser stimulations (LS) before the end of the first hour of a session (Figures 1D and 1E). The distinction between the active and inactive lever was rapidly acquired and the number of active lever presses increased accordingly with increasing fixed ratio (FR1, 2, 3) schedules (Figures 1F and 1G). In control experiments using DAT-Cre− mice or mice that expressed ChR2 in γ-aminobutyric acid (GABA) neurons (GAD-Cre+ mice, to target the inhibitory neurons of the VTA), rates of self-stimulation were low and continuously decreased across sessions. This also applied to two Cre+ animals where post hoc validation showed that the VTA was not infected with ChR2-eYFP (not shown). Moreover, no discrimination between the active and inactive lever was detected (Figures S1A and S1B).

We observed that DAT-Cre+ mice pressed more often on the active lever than required for the laser stimulation. In fact such "futile" active lever presses accounted for more than 30% of all active lever presses (Figure S2A) and occurred—as sessions progressed—mostly between cue and laser stimulation onset (Figures S2B and S2C). This singular behavior developed during acquisition and may reflect impulsive responses.

Taken together, burst activity in VTA DA neurons strongly reinforces lever responding.

Occlusion of VTA DA Neuron Self-Stimulation by Cocaine

To test whether VTA DA neuron self-stimulation hinges on the same brain circuits that are targeted by addictive drugs to reinforce behavior, we injected cocaine intraperitoneally (i.p.) immediately prior to self-stimulation sessions (free access to laser for 45 min, Figure 2A). At baseline, well-trained animals pressed about 400 times to obtain 85 LS in 45 min under the FR3 schedule. After cocaine injection, the performance decreased significantly in a dose-dependent fashion to about 30 LS for 100 lever presses with the highest dose (Figure 2B). This occlusion was most pronounced during the first 30 min of the session, reflecting the pharmacokinetics of the drug (Figure 2C). This experiment indicates that reinforcement by optogenetic self-stimulation and reinforcement by cocaine share underlying neural circuits.

Synaptic Plasticity Associated with Seeking after Withdrawal

To further compare optogenetic self-stimulation to addictive drugs, we next asked whether mice would relapse to self-stimulation of VTA DA neurons following several weeks of withdrawal. Since cue-associated drug seeking is an established model of relapse (Epstein et al., 2006; Soria et al., 2008; Bossert et al., 2013), we placed mice back into the operant chamber 30 days after the last self-stimulation session, where active lever pressing now triggered the cue light *without* laser stimulation (Figure 3A). Robust cue-associated seeking behavior, demonstrated by a high rate of active lever presses, was only apparent in mice with expression of eYFP-*ChR2* in VTA DA neurons (DAT-Cre+ but not DAT-Cre− mice, Figure 3B).

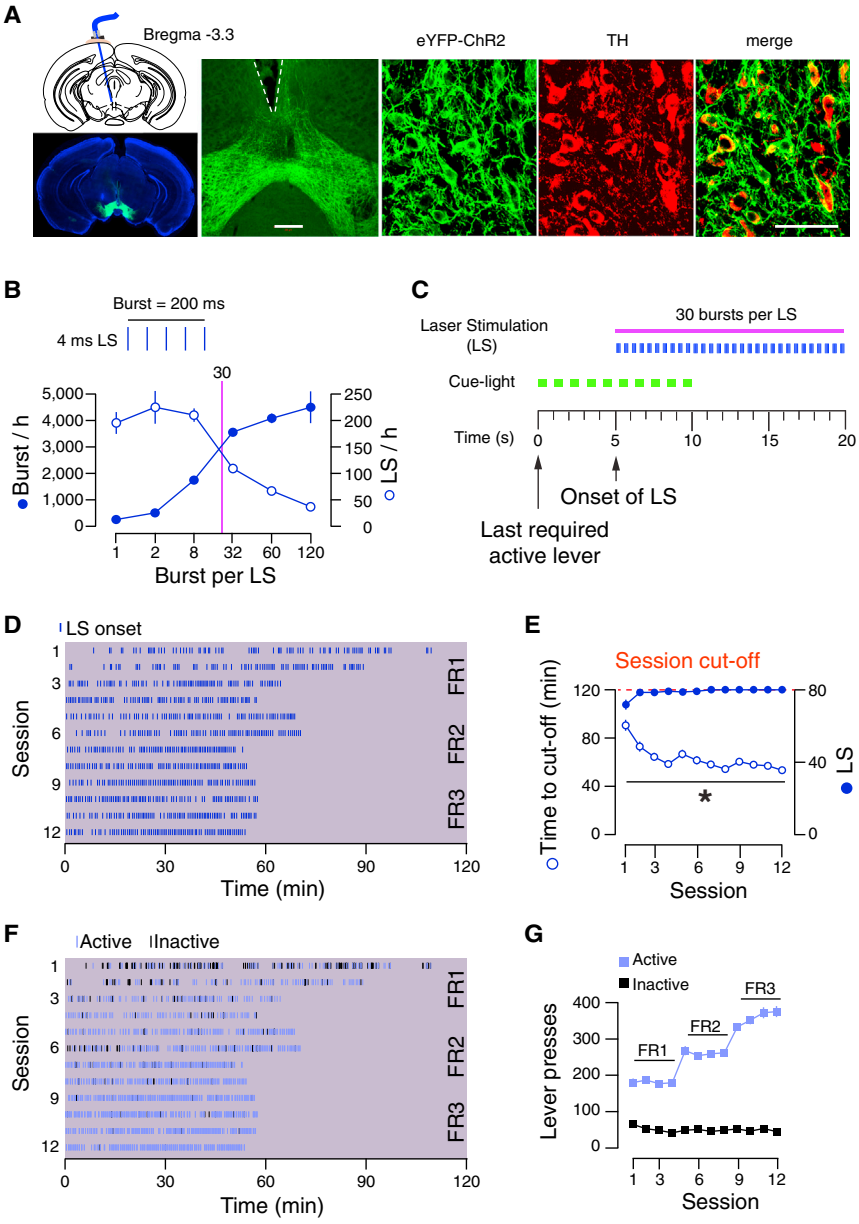

Figure 1. Optogenetic Self-Stimulation of VTA DA Neurons in Mice

(A) Schematic of optic fiber placement above the VTA (top, left), with coronal image of VTA from a DAT-Cre+ mouse infected with AAV5-DIO-ChR2-eYFP (bottom, left). Zoom of infected VTA with optic fiber tract (center; scale bar, 100 μm) and co-localization of ChR2-eYFP with tyrosine hydroxylase (TH) expressing neurons (right). Scale bar, 50 μm.

(B) Mean number of bursts (one burst = 5 pulses, 4 ms width, 20 Hz) and laser stimulations (LS) received as a function of bursts per laser stimulation during 60 min sessions (open and closed circles, respectively; n = 7). Number of bursts per laser stimulation was fixed at 30 for subsequent experiments (purple line).

(C) Schematic of laser stimulation schedule.

(D) Raster plot for laser stimulation (blue dots represent laser stimulation onset) during the 12 daily acquisition sessions of 80 LS or 2 hr maximum for a DAT-Cre+ mouse. Fixed ratio (FR) was increased by one every 4 days.

(E) Time (min) to reach 80 LS (open circle) or number of LS (closed circle) for each acquisition session (n = 43 mice). Elapsed time decreased over sessions, ANOVA for repeated measures: $F_{11,462}$ = 22.65, *p < 0.001.

(F) Raster plot for active and inactive lever presses (example mouse).

(G) Number of active and inactive lever presses (n = 43 mice).

Data are mean ± SEM.

Previous studies have shown the causal link between cue-associated relapse and synaptic plasticity evoked by cocaine in a subtype of NAc neurons expressing the DA D1R (Pascoli, Terrier et al., 2014). Therefore, to evaluate this synaptic plasticity, we generated DAT-Cre mice crossed with *Drd1a-tdTomato* mice to identify the medium-sized spiny neurons (MSNs) subtype in the NAc. Instead of the seeking test, slices of the NAc were prepared where D1R-MSNs were red, contrasting with green fibers from VTA DA neurons infected with flox-ChR2-eYFP (Figure 3C). Whole-cell patch-clamp recordings ex vivo revealed a rectifying current voltage relationship for AMPAR-evoked postsynaptic currents (AMPAR-EPSCs) and an increased AMPAR/NMDAR ratio (Figures 3D and 3E), in the D1R-MSNs but not in the D2R-MSNs. Similar findings previously obtained after withdrawal from cocaine self-administration were shown to indicate the combined insertion of GluA2 lacking and GluA2 containing AMPARs, at separate inputs onto D1R-MSNs (Pascoli, Terrier et al., 2014).

Self-Stimulation despite Punishment

Substance use despite negative consequences is another crucial defining feature of addiction (see DSM5 definition, American Psychiatric Association, 2013). Rat models have been established (Deroche-Gamonet et al., 2004; Pelloux et al., 2007, 2015; Chen et al., 2013) where an electric shock introduced in the cocaine self-administration schedule suppresses cocaine consumption in some animals. Following 12 days of initial exposure (acquisition), mice were allowed to have three additional sessions at FR3 but with a reduced session cut-off (60 min or 40 rewards maximum). These three sessions served as a baseline for the subsequent four sessions, where every third laser stimulation was paired with a foot shock (500 ms; 0.2 mA) predicted by a novel cue (Figure 4A). The intensity and duration of the foot shock were adjusted to completely suppress lever pressing for sucrose reward (see also data below). The punishment schedule led to two opposite behavioral responses (Figure 4B). Some mice rapidly stopped responding

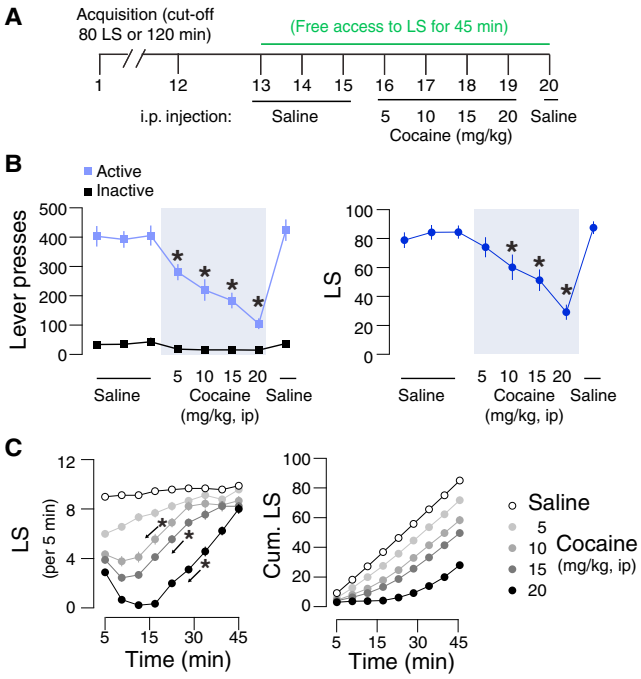

Figure 2. Effect of Cocaine Injection on VTA DA Neuron Self-Stimulation

(A) Schedule of the experiment. After acquisition of VTA DA neuron self-stimulation, mice underwent daily sessions of 45 min with free access to LS just after i.p. injections of saline or increasing doses of cocaine.

(B) Active and inactive lever presses during each session. Cocaine dose-dependently reduced active lever pressing and the number of LS (n = 9 mice). One-way ANOVA for repeated measures: $F_{1,32} = 23.69$, $p < 0.001$ and $F_{1,32} = 21.80$, $p < 0.001$, for active lever press and LS, respectively; Bonferroni post hoc analysis: *$p < 0.05$.

(C) Number of LS per 5 min bin and cumulative values (n = 9 mice). Mixed two-way ANOVA for repeated measures: treatment, $F_{4,10} = 10.86$, $p < 0.001$; time, $F_{8,40} = 41.83$, $p < 0.001$; interaction, $F_{32,40} = 4.16$, $p < 0.001$ for LS per 5 min bin. Bonferroni post hoc analysis: *$p < 0.05$.

when the punishment was introduced (called "sensitive"), whereas others continued responding to obtain the maximum number of laser stimulations and can be considered as "resistant" to punishment. The two clusters of animals fully emerged at the end of the four punishment sessions (Figure 4C). "Resistant mice" maintained the number of laser stimulations (less than 20% reduction) while "sensitive mice" decreased self-stimulation by more than 80%. With these criteria, only one animal (gray dots) could not be assigned. This observation demonstrates that forced burst activity evoked by self-stimulation of VTA DA neurons is sufficient to induce perseverance of consumption despite negative consequences in a fraction of mice. As a control, in an independent group of mice that had established resistance or sensitivity to the punishment associated with self-stimulation, nociception was evaluated using the tail-flick assay. No difference in the latency to withdraw the tail immersed in hot water between sensitive and resistant was detected (Figure S3).

We next asked, post hoc, whether any particular feature during the acquisition phase of self-stimulation could have predicted the resistance to punishment. Sensitive and resistant mice made an identical number of active and inactive lever presses during baseline sessions, and all mice reached the maximum of 80 LS (Figures S4A and S4B), in a similar amount of time (Figures S4A and S4C). While the fraction of futile active lever press was again not different in the two sub-populations (Figures 4D and S4D), the number of futile lever presses before the onset of the laser stimulation became significantly higher in resistant mice by the end of the acquisition sessions (Figures 4E and S4E). As this behavior developed during acquisition, it may contribute, along with innate impulsivity (Economidou et al., 2009; Broos et al., 2012; Jentsch et al., 2014), in establishing the resistance to punishment. In addition, a progressive ratio trial was performed at day 11 to quantify the motivation for the optogenetic stimulation (Richardson and Roberts, 1996). Resistant mice exhibited a breakpoint not statistically different to sensitive mice (Figure S4F).

Resistance to Punishment for Cocaine but Not for Sucrose

To test whether the paradigm of consumption despite harmful consequences along with impulse lever pressing could also predict compulsive intake of an addictive drug, a new cohort of mice underwent 12 days of cocaine self-administration. Experimental parameters for cocaine self-administration acquisition were set to a maximum of 80 infusions of cocaine within 4 hr during acquisition and to 40 infusions within 2 hr during the three baseline sessions preceding the four punishment sessions (Figures 5A and S5A). Again, two groups emerged after pairing cocaine reward with electric shocks. Indeed, 5 out of 22 mice were classified as resistant (less than 20% decrease from baseline), while 17 qualified as sensitive (more than 80% decrease) and one animal fell in between (13 infusions on day 19) (Figure 5B). We then looked for behavioral predictors of resistance to punishment. Between the two groups, the number of infusions, the rate of infusion, and the number of active or inactive lever presses were not different (Figures S5B–S5D), and the breaking points were similar (Figure S5E). What differed was the evolution of the distribution in time of the futile presses on the active lever. In the first four sessions, futile lever presses regularly decreased during the time-out periods in both resistant and sensitive mice, while at the end of the acquisition, only sensitive mice maintained this behavior (Figures 5C and 5D and S5F). By contrast, resistant mice tended to increase their total number of futile lever presses (Figures 5C and S5D), especially in the last quarter of the time-out period (Figure 5D). While qualitatively similar to the observation previously made with the optogenetic stimulation of DA neurons (see above), the clustering of the futile presses during the early time-out period was not seen with cocaine, most likely owing to the slower kinetics with which the drug increased DA levels. Nevertheless, similar conclusions could be drawn based on this singular evolution of futile lever press distribution during the short period of time preceding "the internal detection of the DA surge." Our observations thus suggest that the distribution of the futile active lever presses predicts drug use despite negative consequences.

Finally we repeated the experiment with ad libitum-fed mice that could lever press for a sucrose reward. Once punishment

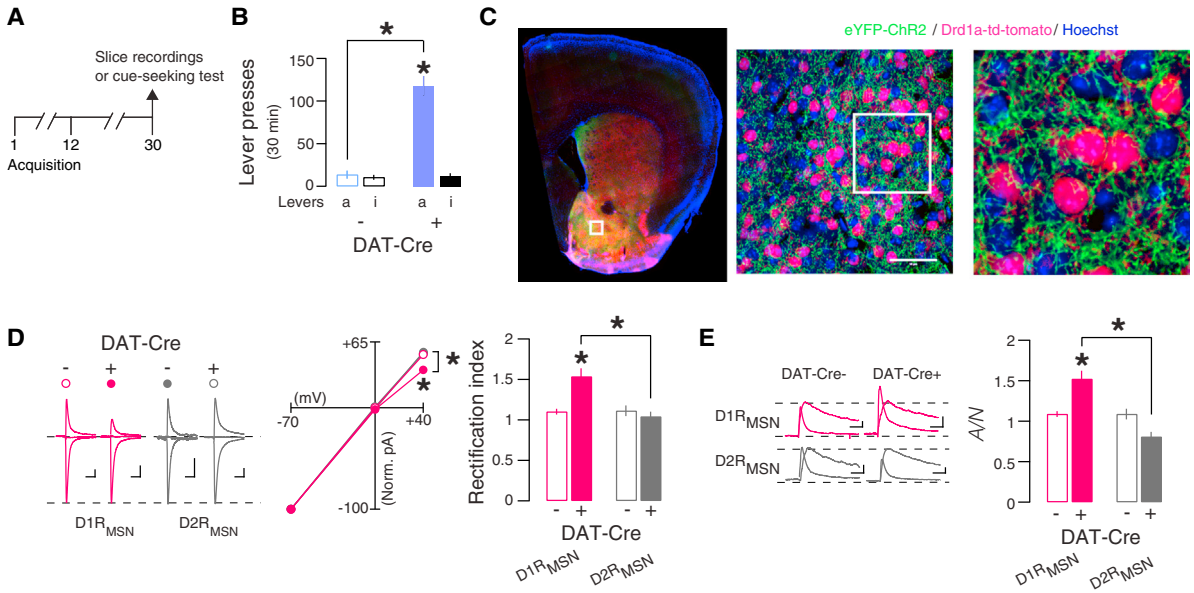

Figure 3. Cue-Associated Seeking and Synaptic Plasticity Evoked by Withdrawal from VTA DA Neuron Self-Stimulation

(A) Experiment schedule. One month after acquisition, mice underwent a cue-associated seeking session or were used for slice electrophysiology recordings.

(B) Active (a) and inactive (i) lever presses during a 30 min cue-associated seeking test for DAT-Cre+ and DAT-Cre− (n = 7 and 5 mice, respectively). Mixed two-way ANOVA: lever, $F_{1,10} = 180.70$, $p < 0.001$; genotype, $F_{1,10} = 46.16$, $p < 0.001$; interaction, $F_{1,10} = 150.80$, $p < 0.001$. Bonferroni post hoc analysis: *$p < 0.05$.

(C) Image of NAc shell (right; nuclear staining with Hoescht in blue) from a double transgenic mouse (DAT-Cre+/ *Drd1a-tdTomato*+) infected with AAV5-DIO-ChR2-eYFP in the VTA. Scale bar, 100 μm.

(D) Example traces of AMPAR-EPSCs recorded at −70, 0, and +40 mV from D1R- and D2R-MSNs of a DAT-Cre+ or DAT-Cre− mouse (left) and grouped data for I/V (current in function of voltage) relationship (middle) and rectification index (right; n = 10–16 cells). Bonferroni post hoc analysis following significant mixed two-way ANOVA: *$p < 0.05$ (see Table S1 for F values).

(E) Example traces of AMPAR and NMDAR-EPSCs recorded at +40 mV from D1R and D2R-MSNs of a DAT-Cre+ or DAT-Cre− mouse (left) and grouped data for A/N ratio (AMPA-EPSC amplitude divided by NMDA-EPSC amplitude) (n = 10–15 cells). Bonferroni post hoc analysis following significant mixed two-way ANOVA: *$p < 0.05$ (see Table S1 for F values).

Data are mean ± SEM.

was introduced, all mice stopped self-administering the sucrose (Figure 5E), demonstrating that this schedule suppressed the intake of a non-essential natural reward, but allowed the detection of compulsive intake of an addictive drug or strong DA neuron stimulation.

Taken together, these results demonstrate that VTA DA self-stimulation is sufficient to induce compulsivity, as shown by the resistance to punishment in a subset of mice (68%). Similarly, after cocaine SA, some mice became resistant to punishment (23%), which was never the case after sucrose SA (Figure 5F).

A Cellular Correlate of Resistance to Punishment

To pinpoint the brain area that may control the decision to persevere in self-administration despite negative consequences, we first monitored generic "neuronal activity" by counting the number of neurons in which the punishment session triggered the expression of the immediate early gene cFos in 15 different regions. Mice were intracardially perfused with PFA 90 min after the end of the last punishment session. The control groups included naive animals, as well as mice yoked to sensitive or resistant mice in order to control the possibly confounding effect of the number of shocks received.

While in most of the chosen regions, the number of cFos-positive neurons was highest in slices from resistant mice compared to naive mice slices, two types of responses emerged, of which the prelimbic cortex (PL) and the lateral OFC are examples. In the PL we found a similar increase of cFos-positive cells in resistant mice and their yoked controls, while in the OFC this increase was only apparent in the resistant and not the corresponding yoked mice (Figures 6A and 6B). To quantify this difference, all data were first normalized to expression levels in naive animals. Then, the ratio was calculated between the resistant over sensitive divided by the yoked to resistant over yoked to sensitive ($Ratio_{cfos}$ = (R/S) / (YR/YS), Figure 6B). This procedure identified the cingulate cortex, the OFC, and VTA as the regions that are activated in resistant but not in sensitive mice and where there was little difference in both groups of yoked controls (similar low cFos-positive neurons in yoked, in fact). Finding the VTA is not surprising, as it is the region of laser-stimulated neurons. This is in line with a previous report showing that ChR2 stimulation triggers cFos activation (Lobo et al., 2010; Van den Oever et al., 2013). A low $ratio_{cfos}$ was found in regions where the activation was similar in sensitive and resistant (such as CeA and PAG). The $ratio_{cfos}$ was also low when the activation was paralleled by a high difference in the yoked controls (such as the PL, Figure 6C for summarized $ratio_{cfos}$ data). A similar cFos expression in resistant and yoked resistant mice was therefore most likely driven by the number of foot shocks and had little

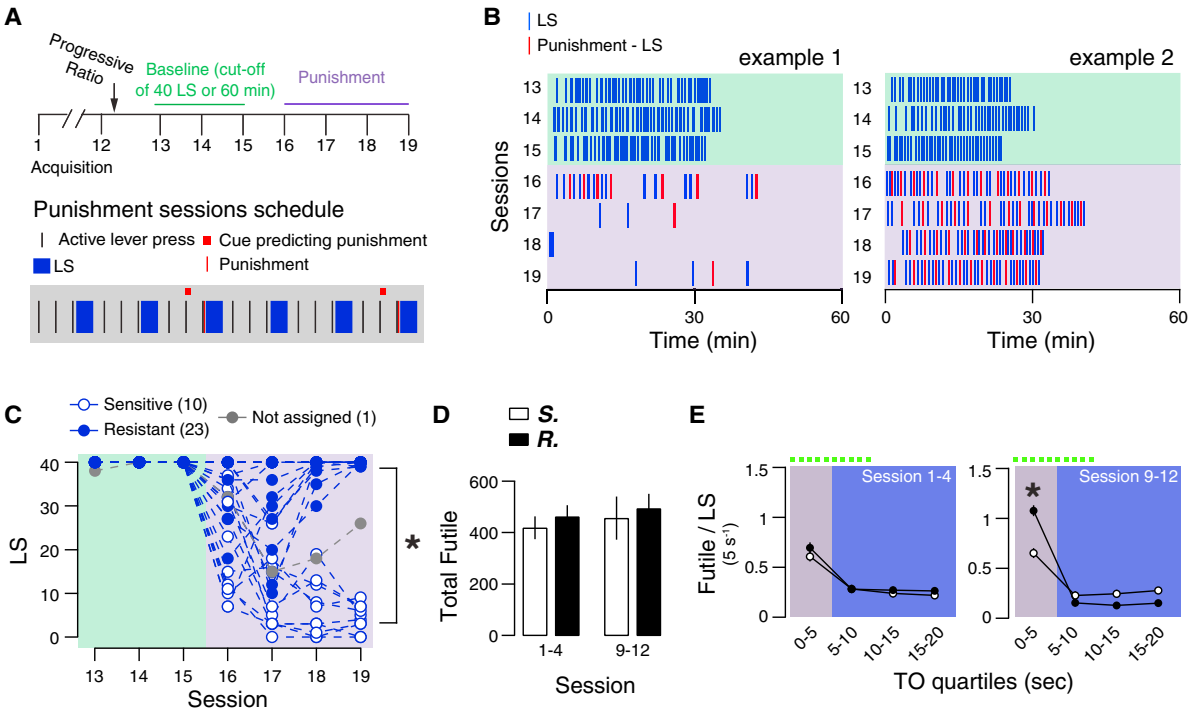

Figure 4. Self-Stimulation despite Foot Shock in a Subset of Mice

(A) Experiment schedule is shown at top. After acquisition sessions, mice underwent a progressive ratio session, 3 days of additional training with a reduced cut-off (maximum of 40 LS or 60 min) and four punishment sessions (cut-off maintained at 40 LS or 60 min). Schematic of the punishment sessions schedule is shown at bottom. Every third LS was paired with a foot shock (0.2 mA, 500 ms) and preceded by a new cue predicting punishment, following the second active lever press of the FR3 schedule.

(B) Raster plots for two example mice behaving differently during the punishment sessions while having similar response during training sessions.

(C) Of 34 mice, those maintaining high LS consumption were categorized as resistant to punishment (closed blue circles), while those that stopped responding were categorized as sensitive (open blue circles). One animal (gray circles) was not categorized. Mixed two-way ANOVA for session: $F_{6,186} = 102.7$, $p < 0.001$, R-S $F_{1,186} = 316.4$, $p < 0.001$, interaction $F_{6,186} = 78.48$, $p < 0.001$). Bonferroni post hoc analysis for R versus S during session 19: *$p < 0.05$.

(D) Analysis of total futile active lever presses during acquisition sessions 1–4 and 9–12 in sensitive and resistant mice (n = 10 and 23, respectively).

(E) Analysis of futile active lever press distribution during the 20 s time-out period in acquisition sessions 1–4 and 9–12, in sensitive and resistant mice (n = 10 and 23, respectively). Two-way ANOVA for quartile: $F_{3,93} = 101.7$, $p < 0.001$ and $F_{3,93} = 72.05$, $p < 0.001$; R versus S: $F_{1,93} = 0.53$ (ns, not statistically significant) and $F_{1,93} = 0.10$ (ns); interaction: $F_{3,93} = 0.93$ (ns) and $F_{3,93} = 11.36$, $p < 0.001$, for sessions 1–4 and 9–12, respectively. Bonferroni post hoc analysis for R versus S: *$p < 0.05$. Difference between sessions 1–4 and 9–12 was only significant in resistant mice for the first quartile (mixed two-way ANOVA: quartile: $F_{3,132} = 161.2$, $p < 0.001$; session: $F_{1,132} = 0.04$ [ns]; interaction: $F_{3,132} = 22.73$, $p < 0.001$).

to do with the resistance to punishment. Taken together, the high $ratio_{cfos}$ in the OFC suggests that neural activity in this region is associated with resistance to punishment and may thus favor the transition to addiction.

Plasticity for Resistance to Punishment

To identify the substrate of the increased neuronal activity in the OFC in the mice resisting punishment, we prepared slices of the PL and L-OFC 24 hr after the last punishment session to test for intrinsic excitability. The two regions were chosen because of their very distinct pattern of c-Fos expression in the previous experiments. The neuronal excitability was quantified by counting the number of action potentials (APs) elicited by the injection of increasing amounts of current (from 0 to 600 pA) in whole-cell recordings. These recordings revealed a sustained hypo-excitability in pyramidal neurons of the PL of resistant mice (and their yoked control) when compared to sensitive or naive mice (Figure 7A). The resting membrane potential (RMP) of recorded neurons was not different between the experimental groups (Figure 7B). These results strongly suggest that the excitability of neurons in the PL directly correlates with the number of shocks received, and maybe not with the decision to resist punishment. This most likely reflects a negative feedback adaptation triggered by neuronal excitation elicited by the foot shocks the day before. By contrast, neurons from L-OFC were more excitable only in resistant mice. Excitability of neurons from yoked mice was not different than excitability of neurons from naive mice, ruling out an effect of the foot shock itself (Figures 7C and 7D). This increased activity of OFC neurons likely underlies the cFos expression and may drive the resistance to punishment.

Reduction of Compulsivity with Chemogenetic Inhibition of OFC

To test for causality between enhanced OFC neuron excitability and resistance to punishment, we expressed the inhibitory

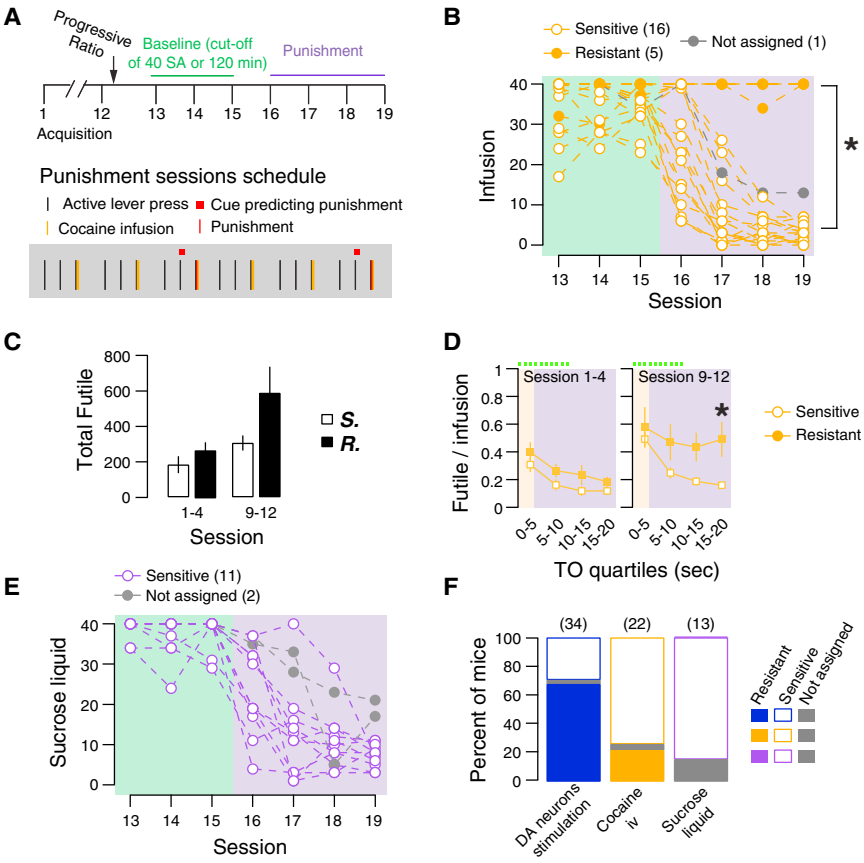

Figure 5. Resistance to Punishment for Cocaine Self-Administration but Not for Sucrose

(A) Representation of punishment session schedule. Every third cocaine infusion (0.5 mg/kg) was paired with a foot shock (0.2 mA, 500 ms) and preceded by a new cue predicting punishment following the second active lever press of the FR3 schedule. Punishment sessions lasted for a maximum of 2 hr or 40 infusions (see Figure S3).

(B) Of 22 mice, 5 resistant mice maintaining high cocaine consumption (closed orange circles), 16 sensitive mice stopping intake (open orange circles), and one intermediate responder (gray circles) were detected. Bonferroni post hoc analysis for R versus S during session 19: *p < 0.05 following a significant mixed two-way ANOVA (see Table S1).

(C) Futile active lever presses during acquisition sessions 1–4 and 9–12 in sensitive and resistant mice (n = 5 and 16, respectively).

(D) Futile active lever press distribution during the 20 s time-out period in acquisition sessions 1–4 and 9–12 in sensitive and resistant mice (n = 5 and 16, respectively). Mixed two-way ANOVA (quartile: $F_{3,57} = 30.48$, $p < 0.001$ and $F_{3,57} = 18.60$, $p < 0.001$; R versus S: $F_{1,57} = 1.57$ [ns] and $F_{1,57} = 6.304$, $p = 0.021$; interaction: $F_{3,57} = 0.44$ [ns] and $F_{3,57} = 4.55$, $p = 0.006$, for sessions 1–4 and 9–12, respectively). Bonferroni post hoc analysis for R versus S: *p < 0.05. Persistence of futile lever presses during the entire time-out period was observed only in resistant mice during sessions 9–12. Bonferroni post hoc analysis for quartile 0–5 versus 15–20: for 1–4 $t_{15} = 8.44$, $p < 0.001$ and $t_4 = 5.31$, $p < 0.001$, for S and R, respectively; for 9–12 $t_{15} = 10.05$, $p < 0.001$ and $t_4 = 1.45$ (ns), for S and R, respectively.

(E) Punishment session in mice trained for sucrose reward. Of 13 mice tested, 11 animals stopped responding (open purple circles) and two were intermediate responders (gray circles).

(F) Percentage of resistant and sensitive responders revealed by punishment associated with VTA DA neuron self-stimulation and cocaine or sucrose self-administration.

DREADD (designer receptors exclusively activated by designer drugs: CamKIIα-hM4D) in pyramidal neurons of the OFC of DAT-Cre+ mice (Figure 8A). In acute slices from the OFC, bath application of CNO (clozapine-N-oxide) induced a slow outward current, most likely mediated by GIRK channels, that was reversed by barium (Ba^{2+}), a non-specific blocker of potassium channels (Figure 8B). The CNO also shifted the input/output curve to the right (Figure 8C). The DAT-Cre+ mice infected with AAV1/CamKIIα-hM4D-mCherry in the OFC (Figure 8D) acquired DA neuron self-stimulation paradigm followed by two successive blocks with the punishment schedule, the first in the presence of CNO and the second without CNO. The two blocks were interrupted by 6 days without punishment (Figure 8E). At the end of the first punishment block, in the presence of CNO, only 5 of 16 mice were resistant (Figure 8F, left panel). In contrast, without OFC inhibition, during the second punishment period, 14 out of 16 were classified as "resistant" (Figures 8F, right panel, and 8G). In other words the fraction of resistant mice was significantly lower in the presence of CNO compared to the first cohort of 34 mice previously tested in the same conditions (between-group comparison, Figure 8H) and became similar to the first cohort without CNO (within-group comparison). Finally, for the nine mice that changed from sensitive to resistant, CNO did not modify the tail-flick latency upon immersion into hot water (Figure 8I).

Taken together, this experiment demonstrates that the activity of pyramidal neurons of the OFC drives the decision to continue self-stimulation despite negative consequences that represents a key feature of the transition to addiction in rodents.

DISCUSSION

A recently proposed addiction model distinguishes three steps in the progression of the disease: sporadic recreational drug use, followed by intensified, sustained, escalated drug use, and eventually compulsive use associated with loss of control (Piazza and Deroche-Gamonet, 2013; but see George et al., 2014). Our study demonstrates that stimulation of VTA DA neurons is sufficient to drive this progression with a relatively rapid time course.

By mimicking a naturally occurring burst-firing pattern, an efficient release of DA is evoked in target regions of the VTA, such as the NAc (Bass et al., 2010). DA levels in the NAc therefore likely govern the self-stimulation, just as rodents self-administer the next infusion of cocaine or heroin once the DA concentration

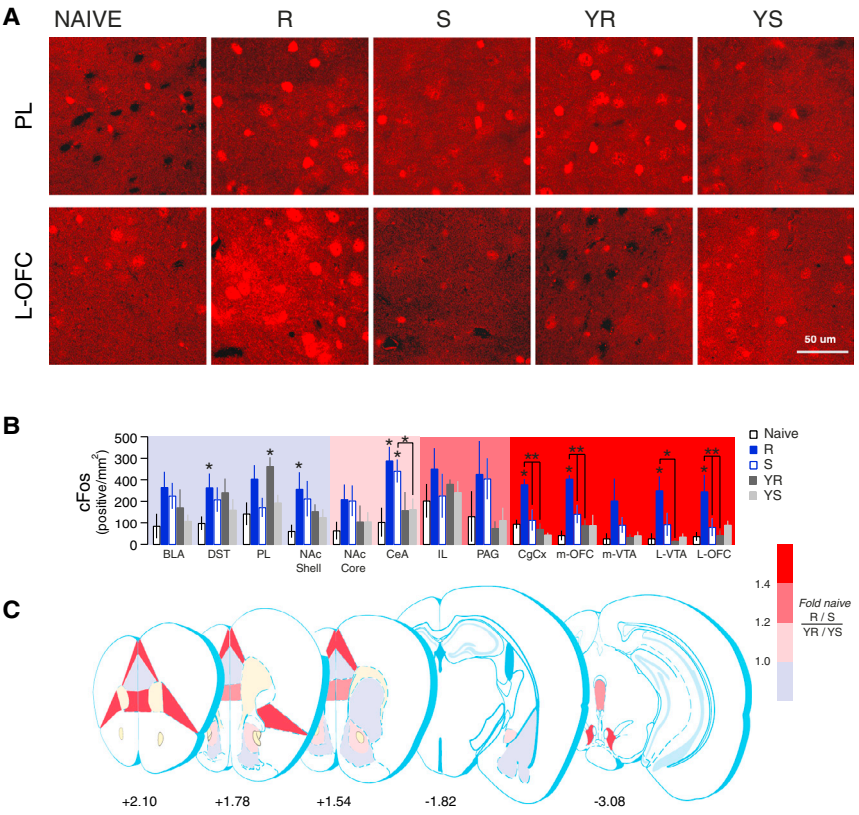

Figure 6. Correlation between Behavioral Response and Brain Region Activity following Punishment

(A) Representative images of cFos staining in the prelimbic medial prefrontal cortex (PL) and the lateral orbitofrontal cortex (L-OFC) from naive, resistant (R), sensitive (S), yoked to resistant (YR), and yoked to sensitive (YS) mice, perfused 90 min after the last punishment session. Scale bar, 50 μm.

(B) Quantification of cFos-positive cells in selected brain areas (n = 3–5 mice/group). *p < 0.05 for significant difference between two groups (Student's t test). Data show mean ± SEM. Background colors represent the ratio of cFos activation among the different experimental groups ([R/S] / [YR/YS] normalized to naive).

(C) Schematic representation of the ratio of cFos activation in examined brain regions. Red signifies regions enriched in cFos specifically in resistant mice; blue marks brain structures enriched in R and S or in R and YR mice.

drops below threshold (Wise et al., 1995). This is also supported by our observation that cocaine, injected i.p., can occlude self-stimulation. Thus, DA neuron self-stimulation closely resembles drug self-administration, even though its kinetics is certainly faster than any pharmacological substance, including cocaine, as suggested by the different rate of responses observed in the present study.

While we selectively targeted DA neurons of the VTA, their optogenetic self-stimulation may have activated groups of cells with different physiological functions. For example, it has recently been suggested that some DA neurons code for aversive stimuli (Lammel et al., 2012; Gunaydin et al., 2014). These cells project to mPFC, while VTA DA neurons projecting to lateral NAc shell mediate positive reinforcement (Lammel et al., 2012). It would be interesting to assess self-stimulation and progression with selective targeting (Gunaydin et al., 2014). Since our manipulation activated all VTA DA neurons, just as cocaine acts on all DAT-expressing neurons, it is conceivable that some DA neurons would drive reinforcement learning while other DA neurons would drive aversion learning. The net effect would still be a reinforcement of the behavior; however, the "aversion neurons" could contribute to the induction of an opponent process (Koob, 2013; Wise and Koob, 2014).

After forced abstinence, re-exposition to the context induced seeking of the self-stimulation, an established rodent model of drug relapse. Remarkably the underlying neural plasticity is indistinguishable from the one observed after withdrawal from cocaine self-administration (Pascoli, Terrier et al., 2014). This adds to a study that previously reported identical synaptic plasticity in VTA DA neurons evoked by a single session of optical stimulation or a first injection of an addictive drug (Brown et al., 2010). A pattern of synaptic adaptations is emerging that c adaptive behavior common to all addictive drugs.

A striking feature of our study is the dichotomy in the response to an aversive stimulus that is strong enough to disrupt consumption of non-essential natural reward in all animals. In our setting, resistant mice did not show a significantly higher motivation for the reward self-delivery, which contrasts with a study with cocaine in rats (Pelloux et al., 2007). The behavioral predictor for resistance to punishment in mice, however, was futile lever pressing during the 5 s preceding the onset of the DA neuron stimulation. The inability to wait until reward delivery can therefore be seen as a marker of impulsivity (Dalley et al., 2011; Olmstead, 2006; Everitt et al., 2008; Winstanley, 2011; Leyton and Vezina, 2014). We were intrigued by the observation that impulsive taking only developed after several sessions of self-stimulation. This raises the possibility that resistance to punishment (and by extension vulnerability to addiction) may not be fully innate, but develops during the initial phases toward addiction. If this is the case, then the dichotomy observed by us and others (Deroche-Gamonet et al., 2004) may not be solely determined by genetic factors. This would also explain that a similar fraction of individuals becomes addicted in genetically relatively homogeneous mouse strains and genetically certainly more diverse human populations.

If resistance to punishment reveals the individual vulnerability for addiction, estimated to top 20% in humans even with cocaine (Warner et al., 1995; O'Brien, 1997; George et al., 2014), then the much higher proportion found here could reflect the power of the direct and selective DA neuron stimulation. In other words, selective DA neuron stimulation may be much more addictive than any drug. This may be explained by the non-selective action of pharmacological substances. In the case of cocaine, for

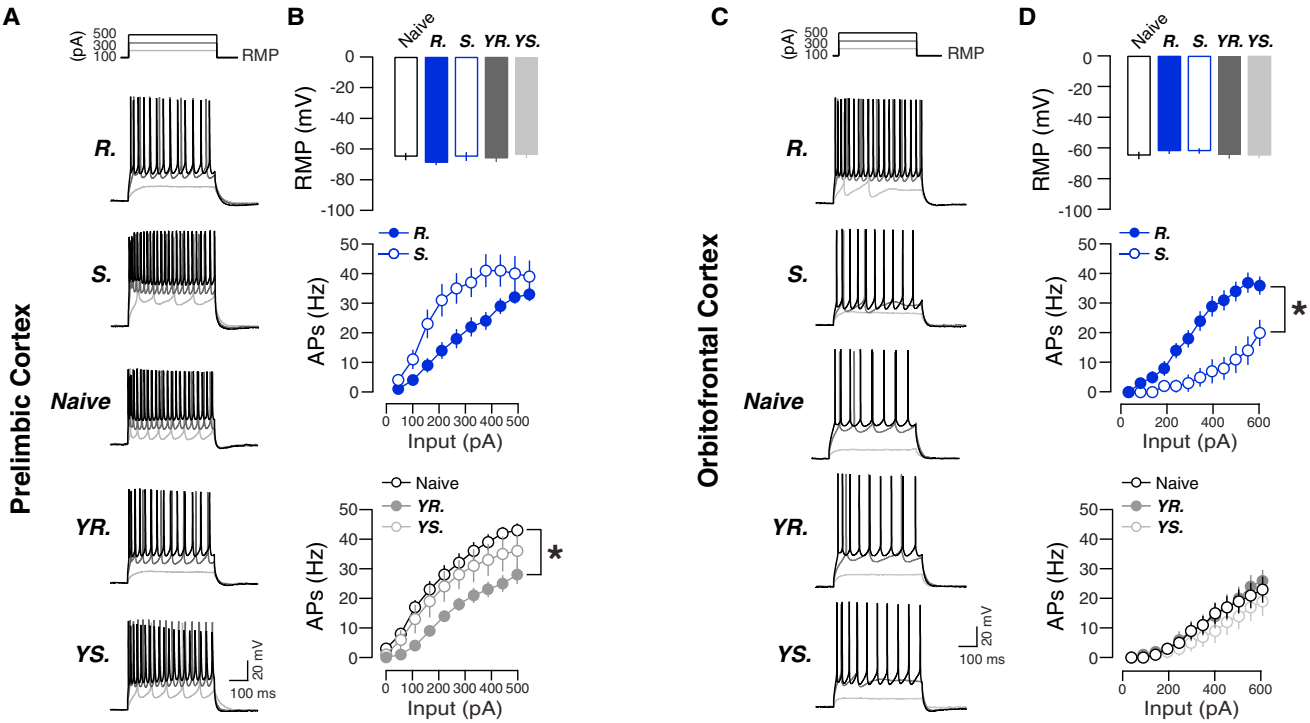

Figure 7. Alterations of Neuronal Excitability in the Prelimbic and Orbitofrontal Cortex

(A) Example traces of electrophysiological response (spikes) to current injection (100, 300, and 500 pA) in pyramidal neurons of the prelimbic cortex (PL) from naive, resistant (R), sensitive (S), yoked to resistant (YR), and yoked to sensitive (YS) mice recorded 1–2 days after the last punishment session.

(B) Resting membrane potential (RMP) was not altered (top). Number of action potentials (APs) as a function of current injection (50–500 pA) in PL (n = 9–16 cells from 2–3 mice/group). ANOVA performed on AUC ($F_{4,56}$ = 9.18, p < 0.001). Bonferroni post hoc: *p < 0.05 for R versus S and YR versus naive.

(C) As for (A), but with recordings in the lateral orbitofrontal cortex (L-OFC).

(D) RMP was not altered (top). Number of APs as a function of current injection (50–600 pA) in the L-OFC (n = 10–14 cells from two mice/group). ANOVA performed on AUC ($F_{4,53}$ = 4.90, p = 0.002). Bonferroni post hoc: *p < 0.05 for R versus S.

Data are mean ± SEM.

example, monoamines other than DA may actually delay the induction of addiction. Indeed, serotonin may oppose DA-dependent adaptive behaviors such as responding for conditioned reward, self-stimulation, and conditioned place preference (Wang et al., 1995; Fletcher and Korth, 1999; Fletcher et al., 2002) by facilitating the association of cues to aversive stimuli (Bauer, 2015; Hindi Attar et al., 2012). Alternatively, the difference may reside in the difference of kinetics between optogenetic self-stimulation and pharmacological induction of extracellular DA increase. Such addictive-potency variation may also exist among different drugs of abuse (George et al., 2014).

While we cannot formally exclude differences in DA release and/or relative signaling to contribute to the establishment of punishment resistance, this scenario is unlikely because the histological validation of the infection of animals included in the study showed *eYFP-ChR2* expression in the entire VTA. Moreover, the optogenetic stimulation protocol designed to saturate DA release led to self-stimulation that culminated in unimodally distributed values for the breaking point, reflecting the incentive motivation.

Another surprising result is that the number of electric foot shocks correlated with the excitability of the neurons in the PL. Decreased excitability of pyramidal neurons and increased AMPAR/NMDAR ratio in pyramidal neurons of the same cells has been observed in "addicted rats," yet these studies did not control for the effect of electric shocks per se (Kasanetz et al., 2010, 2013; Chen et al., 2013). The non-dissociation may therefore be explained by the dual role of the mPFC in both decision processes and fear integration (Peters et al., 2009). For the converse, change in excitability of pyramidal neurons in the infralimbic cortex correlates with foot shocks (Santini et al., 2008). This evidence does not exclude the possibility that mPFC plays a prominent role for the decision of intake pursuit. However, our cFos analysis and observations of intrinsic excitability point to the OFC and cingulate cortex. Furthermore, inhibition of neuronal excitability in the OFC with DREADD prevented resistance to punishment. This causal link represents an important step in understanding the cellular mechanisms responsible for the transition to addiction. Future studies will be needed to test whether this also applies to the whole range of addictive drugs.

Our findings are in line with observations that a dysfunction of the OFC can impair cost-benefit decision making (Seo and Lee, 2010; Walton et al., 2010; Fellows, 2011) and may drive compulsive behaviors (Burguière et al., 2013). In humans, drug abuse has been linked to impaired decision-making and altered OFC

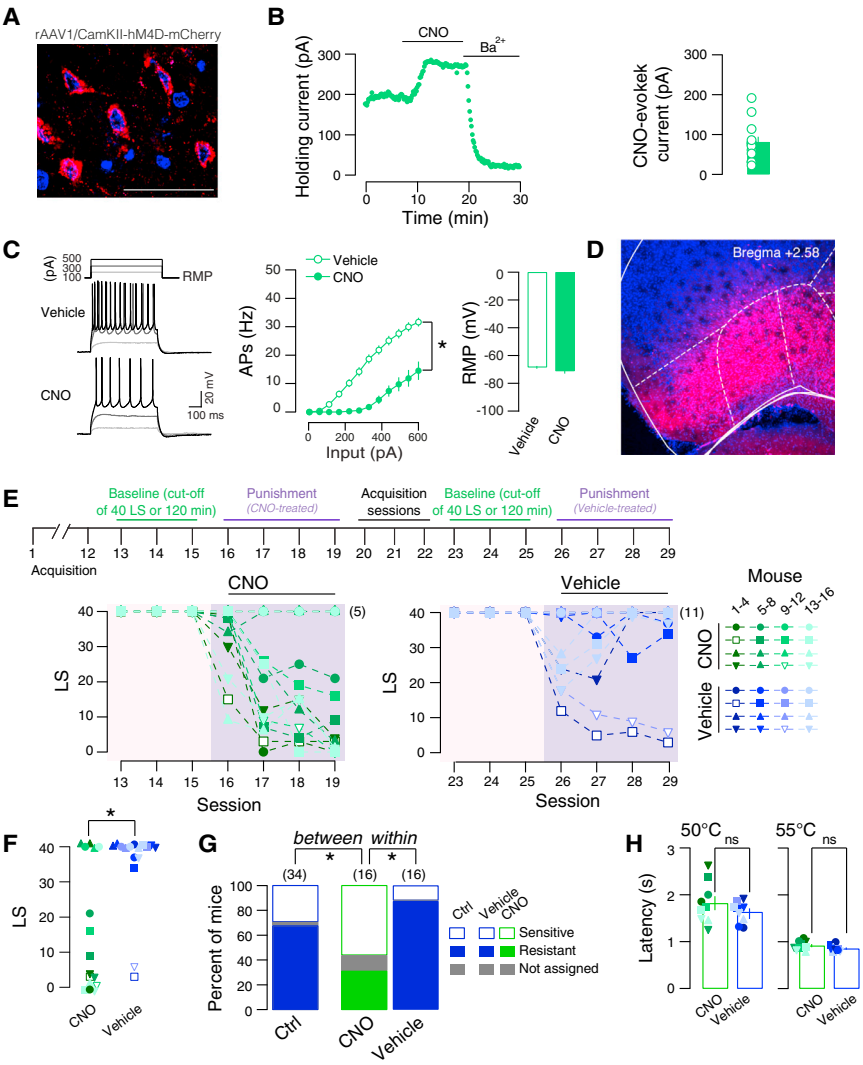

Figure 8. Reduction of Compulsive Self-Stimulation by Chemogenetic Inhibition of the OFC

(A) Image of coronal section of the OFC infected with AAV1/CamKII-hMD4-mCherry together with nuclear staining (blue) at high magnification. Scale bar, 50 μm.

(B) Patch clamp in OFC pyramidal neurons infected with hMD4-mCherry. Bath application of clozapine-N-oxide (CNO, 10 μM) evoked an outward current recorded at −50 mV and plotted as a function of time, which is reversed by barium (Ba^{2+}, 1 mM). Example cell (left panel). Quantification of the CNO-evoked outward current (n = 13, right panel).

(C) Example traces of APs evoked by current injection (100, 300, and 500 pA) in pyramidal neurons of the OFC in the presence or absence of CNO (10 μM). Group data for firing frequency plotted as a function of current injection. CNO decreased neuronal excitability (Student's t test: t_{49} = 4.99, *p < 0.05 for CNO versus vehicle). Resting membrane potential (RMP) was not significantly altered by CNO.

(D) Confocal picture of a coronal section from a mouse infected with AAV1/CamKII-hMD4-mCherry together with nuclear staining (blue) at low magnification, showing OFC. White lines are adapted from Paxinos brain atlas.

(E) Two blocks of four punishment sessions were given in mice receiving CNO (i.p., 2 mg/kg) or vehicle 1 hr before. Top panel, schematic representation of the experimental schedule. Under CNO, five mice resisted punishment, nine were sensitive, and two could not be assigned (left panel). After washout and re-acquisition, the same animals were assigned as follows: 14 resistant and two sensitive mice (right panel). Data for each mouse are shown.

(F) Focus on the last punishment session of the two blocks (CNO or vehicle) to allow direct comparison for each animal (n = 16). CNO decreased LS (t_{15} = 4.27, *p < 0.01).

(G) Percentage of resistant, sensitive, and intermediate responders detected when punishment was associated with VTA DA neuron self-stimulation, under CNO or vehicle (n = 16), and comparison to the first batch of animals previously tested in the same conditions (Ctrl, n = 34). Inhibition of the OFC significantly increased the proportion of sensitive mice (Fisher exact test: p = 0.039 for proportion of resistant mice when comparing CNO versus Ctrl; exact binomial test: p < 0.001, for resistant proportion in presence of CNO versus vehicle; Fisher exact test: p = 0.35 for resistant proportion when comparing vehicle versus Ctrl).

(H) Latency to remove the tail from the hot water (50°C and 55°C) 1 hr after injection of CNO or vehicle in mice responsive to CNO during the punishment task (n = 9).

Data are mean ± SEM.

function (Lucantonio et al., 2012; Gowin et al., 2013). Taken together, the activity of OFC neurons emerges as a key determinant for the transition to compulsive drug use (Everitt et al., 2007). This does not preclude a role for drug-evoked plasticity at excitatory afferents onto MSNs observed here and in other studies (Kasanetz et al., 2010). It will be interesting to evaluate whether manipulations aiming at controlling the excitability of the OFC affect motivation in addicts.

Here we propose DA neuron self-stimulation as a powerful model to study the stages leading to addiction. We reproduce core components of drug addiction, such as relapse, synaptic plasticity, and perseverance of consumption despite negative consequences. While the model is certainly not suited to study effects specific for a given drug (e.g., compare opioid to psychostimulants), it has several advantages. It allows for a precise temporal control of the reward delivery, it is very specifically activating only the VTA DA neurons, and last but not least, it gives the possibility of studying mice for a much longer time than with drug self-administration. By focusing on the defining commonality of addictive drugs, the hope is to unravel the neural mechanisms underlying also non-substance-dependent forms of addiction (Alavi et al., 2012; Robbins and Clark, 2015) and thus contribute to a general theory of the disease. Optogenetic disease models thus allow a decisive step for a thorough understanding of the

neuronal dysfunction involved in late stages of addiction and will guide novel, rational treatments for a disease currently without a cure.

EXPERIMENTAL PROCEDURES

Animals

Mice were heterozygous BAC transgenic mice in which the Cre recombinase expression was under the control of the regulatory elements of the DA transporter gene (DAT-Cre+ mice; Turiault et al., 2007). DAT-Cre mice were originally provided by Günther Schutz. DAT-Cre+ mice crossed with mice in which tomato expression was driven by D1R (*Drd1a-tdTomato* from Jackson Laboratories) gene regulatory element were also used. Control mice were DAT-Cre− and DAT-Cre−/Drd1a-tdTomato+ and GAD-Cre+ (Gad65Cre non-inducible; Kätzel et al., 2011). Weights and genders were homogeneously distributed among the groups. Transgenic mice had been backcrossed in the C57BL/6 line for a minimum of four generations. Mice were single housed after surgery. All animals were kept in a temperature- and humidity-controlled environment with a 12 hr light/12 hr dark cycle (lights on at 7:00). All procedures were approved by the Institutional Animal Care and Use Committee of the University of Geneva.

DA Neuron Self-Stimulation Acquisition and Progressive Ratio

Each of the 12 acquisition sessions lasts 120 min or until the mouse reaches 80 LS, whatever comes first. During the first four sessions, a single press on the active lever (termed fixed ratio one, or FR1) resulted in a 10 s illumination of a cue light (pulses of 1 s at 1 Hz). After a delay of 5 s, onset of a 15 s laser stimulation (473 nm) decomposed of 30 bursts separated by 250 ms (each burst consisted of 5 laser pulses of 4 ms pulse width at 20 Hz; Brown et al., 2010). A 20 s timeout followed the rewarded lever press, during which lever presses had no consequence but were recorded. Next, a FR2 (sessions 5–8) and a FR3 (sessions 9–12) were introduced, respectively.

Punishment Sessions

After acquisition, mice underwent three additional sessions with a reduced cut-off (maximum 40 LS or 60 min, the session ending whatever comes first). These sessions served as a baseline before starting the punishment session. Punishment sessions occur exactly in the same conditions as for baseline sessions except that every third LS is paired with a foot shock (500 ms, 0.2 mA) starting immediately after the rewarded active lever press (5 s before the onset of the laser stimulation). In addition, a new cue (house light) predicting the oncoming shock was paired with the second lever press of the FR3 schedule.

Cocaine Self-Administration

DAT-Cre+ mice were used for this experiment. The procedure used here has already been detailed elsewhere (Pascoli, Terrier et al., 2014). Briefly, mice were implanted in the right jugular vein with mouse-designed catheters (CamCaths, model MIVSA). After 5–7 days of recovery, mice underwent 12 hr of food deprivation and started acquisition in the exact same conditions as detailed above for the optogenetic stimulation, except that a rewarded active lever press resulted in an infusion of 0.5 mg/kg of cocaine (cocaine hydrochloride, provided by the pharmacy of Geneva University Hospital, dissolved in 0.9% saline at 0.50 mg/ml and delivered at 0.0177 ml/s as a unit dose depending on the weight of the mouse). Mice were allowed to self-administer 80 infusions within 240 min. After 12 days of acquisition and a progressive increase to FR3, mice underwent three baseline sessions (40 infusions maximum, or 120 min) and four punishment sessions.

Sucrose Self-Administration

Mice (DAT-Cre+) underwent 12 hr of food deprivation before the first acquisition session during which the active lever press resulted in elevation of a cup containing 0.1 ml of a 20% sucrose solution available for 5 s. Each daily session stopped after 240 min or when mice obtained 80 rewards. After 12 days of acquisition with a progressive increase to FR3, mice underwent three baseline sessions (40 rewards maximum or 120 min) and four punishment sessions similarly to the mice that self-administered blue light or cocaine.

Tail-Flick Assay

The tail of the mouse was immersed in water (at 50°C or 55°C). The latency to withdraw the tail was determined with three repeated measures for each water temperature.

Stereotaxic injections, immunohistochemistry, slice electrophysiology, and statistics were performed as previously described (Pascoli et al., 2011, 2014). All experimental procedures are described in detail in the Supplemental Information.

SUPPLEMENTAL INFORMATION

Supplemental Information includes Supplemental Experimental Procedures, six figures, and one table and can be found with this article online at http://dx.doi.org/10.1016/j.neuron.2015.10.017.

AUTHOR CONTRIBUTIONS

V.P., J.T., and A.H. carried out the behavioral experiments while V.P. did the electrophysiological recordings and coordinated the analysis. The study was designed and written by all authors.

ACKNOWLEDGMENTS

The work was supported by grants from the Swiss National Foundation and the ERC advanced grant (MeSSI), Carigest SA, the Academic Society of Geneva, and the Fondation Privée des Hopitaux Universitaires de Genève. J.T. is a MD-PhD student paid by the Swiss Confederation.

Received: March 2, 2015
Revised: August 31, 2015
Accepted: October 8, 2015
Published: November 12, 2015

REFERENCES

Adamantidis, A.R., Tsai, H.C., Boutrel, B., Zhang, F., Stuber, G.D., Budygin, E.A., Touriño, C., Bonci, A., Deisseroth, K., and de Lecea, L. (2011). Optogenetic interrogation of dopaminergic modulation of the multiple phases of reward-seeking behavior. J. Neurosci. *31*, 10829–10835.

Alavi, S.S., Ferdosi, M., Jannatifard, F., Eslami, M., Alaghemandan, H., and Setare, M. (2012). Behavioral Addiction versus Substance Addiction: Correspondence of Psychiatric and Psychological Views. Int. J. Prev. Med. *3*, 290–294.

American Psychiatric Association (2013). Diagnostic and Statistical Manual of Mental Disorders, Fifth Edition (American Psychiatric Association).

Aragona, B.J., Cleaveland, N.A., Stuber, G.D., Day, J.J., Carelli, R.M., and Wightman, R.M. (2008). Preferential enhancement of dopamine transmission within the nucleus accumbens shell by cocaine is attributable to a direct increase in phasic dopamine release events. J. Neurosci. *28*, 8821–8831.

Atasoy, D., Aponte, Y., Su, H.H., and Sternson, S.M. (2008). A FLEX switch targets Channelrhodopsin-2 to multiple cell types for imaging and long-range circuit mapping. J. Neurosci. *28*, 7025–7030.

Badiani, A., Belin, D., Epstein, D., Calu, D., and Shaham, Y. (2011). Opiate versus psychostimulant addiction: the differences do matter. Nat. Rev. Neurosci. *12*, 685–700.

Bass, C.E., Grinevich, V.P., Vance, Z.B., Sullivan, R.P., Bonin, K.D., and Budygin, E.A. (2010). Optogenetic control of striatal dopamine release in rats. J. Neurochem. *114*, 1344–1352.

Bauer, E.P. (2015). Serotonin in fear conditioning processes. Behav. Brain Res. *277*, 68–77.

Bossert, J.M., Marchant, N.J., Calu, D.J., and Shaham, Y. (2013). The reinstatement model of drug relapse: recent neurobiological findings, emerging research topics, and translational research. Psychopharmacology (Berl.) *229*, 453–476.

Broos, N., Diergaarde, L., Schoffelmeer, A.N., Pattij, T., and De Vries, T.J. (2012). Trait impulsive choice predicts resistance to extinction and propensity to relapse to cocaine seeking: a bidirectional investigation. Neuropsychopharmacology *37*, 1377–1386.

Brown, M.T.C., Bellone, C., Mameli, M., Labouèbe, G., Bocklisch, C., Balland, B., Dahan, L., Luján, R., Deisseroth, K., and Lüscher, C. (2010). Drug-driven AMPA receptor redistribution mimicked by selective dopamine neuron stimulation. PLoS ONE *5*, e15870.

Burguière, E., Monteiro, P., Feng, G., and Graybiel, A.M. (2013). Optogenetic stimulation of lateral orbitofronto-striatal pathway suppresses compulsive behaviors. Science *340*, 1243–1246.

Chen, B.T., Yau, H.-J., Hatch, C., Kusumoto-Yoshida, I., Cho, S.L., Hopf, F.W., and Bonci, A. (2013). Rescuing cocaine-induced prefrontal cortex hypoactivity prevents compulsive cocaine seeking. Nature *496*, 359–362.

Crow, T.J. (1970). Enhancement of coaine of intra-cranial self-stimulation in the rat. Life Sci. *9*, 375–381.

Dalley, J.W., Everitt, B.J., and Robbins, T.W. (2011). Impulsivity, compulsivity, and top-down cognitive control. Neuron *69*, 680–694.

Deroche-Gamonet, V., Belin, D., and Piazza, P.V. (2004). Evidence for addiction-like behavior in the rat. Science *305*, 1014–1017.

Di Chiara, G., and Bassareo, V. (2007). Reward system and addiction: what dopamine does and doesn't do. Curr. Opin. Pharmacol. *7*, 69–76.

Di Chiara, G., and Imperato, A. (1988). Drugs abused by humans preferentially increase synaptic dopamine concentrations in the mesolimbic system of freely moving rats. Proc. Natl. Acad. Sci. USA *85*, 5274–5278.

Economidou, D., Pelloux, Y., Robbins, T.W., Dalley, J.W., and Everitt, B.J. (2009). High impulsivity predicts relapse to cocaine-seeking after punishment-induced abstinence. Biol. Psychiatry *65*, 851–856.

Epstein, D.H., Preston, K.L., Stewart, J., and Shaham, Y. (2006). Toward a model of drug relapse: an assessment of the validity of the reinstatement procedure. Psychopharmacology (Berl.) *189*, 1–16.

Everitt, B.J., Hutcheson, D.M., Ersche, K.D., Pelloux, Y., Dalley, J.W., and Robbins, T.W. (2007). The orbital prefrontal cortex and drug addiction in laboratory animals and humans. Ann. N Y Acad. Sci. *1121*, 576–597.

Everitt, B.J., Belin, D., Economidou, D., Pelloux, Y., Dalley, J.W., and Robbins, T.W. (2008). Review. Neural mechanisms underlying the vulnerability to develop compulsive drug-seeking habits and addiction. Philos. Trans. R. Soc. Lond. B Biol. Sci. *363*, 3125–3135.

Fellows, L.K. (2011). Orbitofrontal contributions to value-based decision making: evidence from humans with frontal lobe damage. Ann. N Y Acad. Sci. *1239*, 51–58.

Fletcher, P.J., and Korth, K.M. (1999). RU-24969 disrupts d-amphetamine self-administration and responding for conditioned reward via stimulation of 5-HT1B receptors. Behav. Pharmacol. *10*, 183–193.

Fletcher, P.J., Azampanah, A., and Korth, K.M. (2002). Activation of 5-HT(1B) receptors in the nucleus accumbens reduces self-administration of amphetamine on a progressive ratio schedule. Pharmacol. Biochem. Behav. *71*, 717–725.

Fouriezos, G., Hansson, P., and Wise, R.A. (1978). Neuroleptic-induced attenuation of brain stimulation reward in rats. J. Comp. Physiol. Psychol. *92*, 661–671.

George, O., Koob, G.F., and Vendruscolo, L.F. (2014). Negative reinforcement via motivational withdrawal is the driving force behind the transition to addiction. Psychopharmacology (Berl.) *231*, 3911–3917.

Gowin, J.L., Mackey, S., and Paulus, M.P. (2013). Altered risk-related processing in substance users: imbalance of pain and gain. Drug Alcohol Depend. *132*, 13–21.

Gunaydin, L.A., Grosenick, L., Finkelstein, J.C., Kauvar, I.V., Fenno, L.E., Adhikari, A., Lammel, S., Mirzabekov, J.J., Airan, R.D., Zalocusky, K.A., et al. (2014). Natural neural projection dynamics underlying social behavior. Cell *157*, 1535–1551.

Han, D.D., and Gu, H.H. (2006). Comparison of the monoamine transporters from human and mouse in their sensitivities to psychostimulant drugs. BMC Pharmacol. *6*, 6.

Hindi Attar, C., Finckh, B., and Büchel, C. (2012). The influence of serotonin on fear learning. PLoS ONE *7*, e42397.

Hnasko, T.S., Sotak, B.N., and Palmiter, R.D. (2007). Cocaine-conditioned place preference by dopamine-deficient mice is mediated by serotonin. J. Neurosci. *27*, 12484–12488.

Hyland, B.I., Reynolds, J.N.J., Hay, J., Perk, C.G., and Miller, R. (2002). Firing modes of midbrain dopamine cells in the freely moving rat. Neuroscience *114*, 475–492.

Ilango, A., Kesner, A.J., Keller, K.L., Stuber, G.D., Bonci, A., and Ikemoto, S. (2014). Similar roles of substantia nigra and ventral tegmental dopamine neurons in reward and aversion. J. Neurosci. *34*, 817–822.

Jentsch, J.D., Ashenhurst, J.R., Cervantes, M.C., Groman, S.M., James, A.S., and Pennington, Z.T. (2014). Dissecting impulsivity and its relationships to drug addictions. Ann. N Y Acad. Sci. *1327*, 1–26.

Kasanetz, F., Deroche-Gamonet, V., Berson, N., Balado, E., Lafourcade, M., Manzoni, O., and Piazza, P.V. (2010). Transition to addiction is associated with a persistent impairment in synaptic plasticity. Science *328*, 1709–1712.

Kasanetz, F., Lafourcade, M., Deroche-Gamonet, V., Revest, J.-M., Berson, N., Balado, E., Fiancette, J.-F., Renault, P., Piazza, P.V., and Manzoni, O.J. (2013). Prefrontal synaptic markers of cocaine addiction-like behavior in rats. Mol. Psychiatry *18*, 729–737.

Kätzel, D., Zemelman, B.V., Buetfering, C., Wölfel, M., and Miesenböck, G. (2011). The columnar and laminar organization of inhibitory connections to neocortical excitatory cells. Nat. Neurosci. *14*, 100–107.

Kim, K.M., Baratta, M.V., Yang, A., Lee, D., Boyden, E.S., and Fiorillo, C.D. (2012). Optogenetic mimicry of the transient activation of dopamine neurons by natural reward is sufficient for operant reinforcement. PLoS ONE *7*, e33612.

Koob, G.F. (2013). Negative reinforcement in drug addiction: the darkness within. Curr. Opin. Neurobiol. *23*, 559–563.

Kornetsky, C., Esposito, R.U., McLean, S., and Jacobson, J.O. (1979). Intracranial self-stimulation thresholds: a model for the hedonic effects of drugs of abuse. Arch. Gen. Psychiatry *36*, 289–292.

Lammel, S., Lim, B.K., Ran, C., Huang, K.W., Betley, M.J., Tye, K.M., Deisseroth, K., and Malenka, R.C. (2012). Input-specific control of reward and aversion in the ventral tegmental area. Nature *491*, 212–217.

Leyton, M., and Vezina, P. (2014). Dopamine ups and downs in vulnerability to addictions: a neurodevelopmental model. Trends Pharmacol. Sci. *35*, 268–276.

Lobo, M.K., Covington, H.E., 3rd, Chaudhury, D., Friedman, A.K., Sun, H., Damez-Werno, D., Dietz, D.M., Zaman, S., Koo, J.W., Kennedy, P.J., et al. (2010). Cell type-specific loss of BDNF signaling mimics optogenetic control of cocaine reward. Science *330*, 385–390.

Lucantonio, F., Stalnaker, T.A., Shaham, Y., Niv, Y., and Schoenbaum, G. (2012). The impact of orbitofrontal dysfunction on cocaine addiction. Nat. Neurosci. *15*, 358–366.

Lüscher, C., and Ungless, M.A. (2006). The mechanistic classification of addictive drugs. PLoS Med. *3*, e437.

Mameli-Engvall, M., Evrard, A., Pons, S., Maskos, U., Svensson, T.H., Changeux, J.-P., and Faure, P. (2006). Hierarchical control of dopamine neuron-firing patterns by nicotinic receptors. Neuron *50*, 911–921.

McDevitt, R.A., Tiran-Cappello, A., Shen, H., Balderas, I., Britt, J.P., Marino, R.A.M., Chung, S.L., Richie, C.T., Harvey, B.K., and Bonci, A. (2014). Serotonergic versus nonserotonergic dorsal raphe projection neurons: differential participation in reward circuitry. Cell Rep. *8*, 1857–1869.

O'Brien, C.P. (1997). A range of research-based pharmacotherapies for addiction. Science *278*, 66–70.

Olmstead, M.C. (2006). Animal models of drug addiction: Where do we go from here? Q J Exp Psychol (Hove) *59*, 625–653.

Pascoli, V., Turiault, M., and Lüscher, C. (2011). Reversal of cocaine-evoked synaptic potentiation resets drug-induced adaptive behaviour. Nature *481*, 71–75.

Pascoli, V., Terrier, J., Espallergues, J., Valjent, E., O'Connor, E.C., and Lüscher, C. (2014). Contrasting forms of cocaine-evoked plasticity control components of relapse. Nature *509*, 459–464.

Pelloux, Y., Everitt, B.J., and Dickinson, A. (2007). Compulsive drug seeking by rats under punishment: effects of drug taking history. Psychopharmacology (Berl.) *194*, 127–137.

Pelloux, Y., Murray, J.E., and Everitt, B.J. (2015). Differential vulnerability to the punishment of cocaine related behaviours: effects of locus of punishment, cocaine taking history and alternative reinforcer availability. Psychopharmacology (Berl.) *232*, 125–134.

Peters, J., Kalivas, P.W., and Quirk, G.J. (2009). Extinction circuits for fear and addiction overlap in prefrontal cortex. Learn. Mem. *16*, 279–288.

Pettit, H.O., Ettenberg, A., Bloom, F.E., and Koob, G.F. (1984). Destruction of dopamine in the nucleus accumbens selectively attenuates cocaine but not heroin self-administration in rats. Psychopharmacology (Berl.) *84*, 167–173.

Piazza, P.V., and Deroche-Gamonet, V. (2013). A multistep general theory of transition to addiction. Psychopharmacology (Berl.) *229*, 387–413.

Piazza, P.V., Deroche-Gamonent, V., Rouge-Pont, F., and Le Moal, M. (2000). Vertical shifts in self-administration dose-response functions predict a drug-vulnerable phenotype predisposed to addiction. J. Neurosci. *20*, 4226–4232.

Richardson, N.R., and Roberts, D.C. (1996). Progressive ratio schedules in drug self-administration studies in rats: a method to evaluate reinforcing efficacy. J. Neurosci. Methods *66*, 1–11.

Robbins, T.W., and Clark, L. (2015). Behavioral addictions. Curr. Opin. Neurobiol. *30*, 66–72.

Rocha, B.A. (2003). Stimulant and reinforcing effects of cocaine in monoamine transporter knockout mice. Eur. J. Pharmacol. *479*, 107–115.

Rocha, B.A., Fumagalli, F., Gainetdinov, R.R., Jones, S.R., Ator, R., Giros, B., Miller, G.W., and Caron, M.G. (1998). Cocaine self-administration in dopamine-transporter knockout mice. Nat. Neurosci. *1*, 132–137.

Rossi, M.A., Sukharnikova, T., Hayrapetyan, V.Y., Yang, L., and Yin, H.H. (2013). Operant self-stimulation of dopamine neurons in the substantia nigra. PLoS ONE *8*, e65799.

Santini, E., Quirk, G.J., and Porter, J.T. (2008). Fear conditioning and extinction differentially modify the intrinsic excitability of infralimbic neurons. J. Neurosci. *28*, 4028–4036.

Schultz, W. (1998). The phasic reward signal of primate dopamine neurons. Adv. Pharmacol. *42*, 686–690.

Seo, H., and Lee, D. (2010). Orbitofrontal cortex assigns credit wisely. Neuron *65*, 736–738.

Soria, G., Barbano, M.F., Maldonado, R., and Valverde, O. (2008). A reliable method to study cue-, priming-, and stress-induced reinstatement of cocaine self-administration in mice. Psychopharmacology (Berl.) *199*, 593–603.

Stein, L. (1964). Self-stimulation of the brain and the central stimulant action of amphetamine. Fed. Proc. *23*, 836–850.

Tassin, J.P. (2008). Uncoupling between noradrenergic and serotonergic neurons as a molecular basis of stable changes in behavior induced by repeated drugs of abuse. Biochem. Pharmacol. *75*, 85–97.

Thomsen, M., Hall, F.S., Uhl, G.R., and Caine, S.B. (2009). Dramatically decreased cocaine self-administration in dopamine but not serotonin transporter knock-out mice. J. Neurosci. *29*, 1087–1092.

Ting-A-Kee, R., and van der Kooy, D. (2012). The neurobiology of opiate motivation. Cold Spring Harb. Perspect. Med. *2*, a012096–a012096.

Tsai, H.C., Zhang, F., Adamantidis, A., Stuber, G.D., Bonci, A., de Lecea, L., and Deisseroth, K. (2009). Phasic firing in dopaminergic neurons is sufficient for behavioral conditioning. Science *324*, 1080–1084.

Turiault, M., Parnaudeau, S., Milet, A., Parlato, R., Rouzeau, J.-D., Lazar, M., and Tronche, F. (2007). Analysis of dopamine transporter gene expression pattern – generation of DAT-iCre transgenic mice. FEBS J. *274*, 3568–3577.

Van den Oever, M.C., Rotaru, D.C., Heinsbroek, J.A., Gouwenberg, Y., Deisseroth, K., Stuber, G.D., Mansvelder, H.D., and Smit, A.B. (2013). Ventromedial prefrontal cortex pyramidal cells have a temporal dynamic role in recall and extinction of cocaine-associated memory. J. Neurosci. *33*, 18225–18233.

Volkow, N.D., and Morales, M. (2015). The Brain on Drugs: From Reward to Addiction. Cell *162*, 712–725.

Walton, M.E., Behrens, T.E.J., Buckley, M.J., Rudebeck, P.H., and Rushworth, M.F.S. (2010). Separable learning systems in the macaque brain and the role of orbitofrontal cortex in contingent learning. Neuron *65*, 927–939.

Wang, Y., Joharchi, N., Fletcher, P.J., Sellers, E.M., and Higgins, G.A. (1995). Further studies to examine the nature of dexfenfluramine-induced suppression of heroin self-administration. Psychopharmacology (Berl.) *120*, 134–141.

Warner, L.A., Kessler, R.C., Hughes, M., Anthony, J.C., and Nelson, C.B. (1995). Prevalence and correlates of drug use and dependence in the United States. Results from the National Comorbidity Survey. Arch. Gen. Psychiatry *52*, 219–229.

Winstanley, C.A. (2011). The utility of rat models of impulsivity in developing pharmacotherapies for impulse control disorders. Br. J. Pharmacol. *164*, 1301–1321.

Wise, R.A., and Bozarth, M.A. (1982). Action of drugs of abuse on brain reward systems: an update with specific attention to opiates. Pharmacol. Biochem. Behav. *17*, 239–243.

Wise, R.A., and Koob, G.F. (2014). The development and maintenance of drug addiction. Neuropsychopharmacology *39*, 254–262.

Wise, R.A., Newton, P., Leeb, K., Burnette, B., Pocock, D., and Justice, J.B., Jr. (1995). Fluctuations in nucleus accumbens dopamine concentration during intravenous cocaine self-administration in rats. Psychopharmacology (Berl.) *120*, 10–20.

Witten, I.B., Steinberg, E.E., Lee, S.Y., Davidson, T.J., Zalocusky, K.A., Brodsky, M., Yizhar, O., Cho, S.L., Gong, S., Ramakrishnan, C., et al. (2011). Recombinase-driver rat lines: tools, techniques, and optogenetic application to dopamine-mediated reinforcement. Neuron *72*, 721–733.

Zhang, L., Doyon, W.M., Clark, J.J., Phillips, P.E.M., and Dani, J.A. (2009). Controls of tonic and phasic dopamine transmission in the dorsal and ventral striatum. Mol. Pharmacol. *76*, 396–404.