LETTER

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Rescuing cocaine-induced prefrontal cortex hypoactivity prevents compulsive cocaine seeking

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Loss of control over harmful drug seeking is one of the most intractable aspects of addiction, as human substance abusers continue to pursue drugs despite incurring significant negative consequences[1]. Human studies have suggested that deficits in prefrontal cortical function and consequential loss of inhibitory control[2–4] could be crucial in promoting compulsive drug use. However, it remains unknown whether chronic drug use compromises cortical activity and, equally important, whether this deficit promotes compulsive cocaine seeking. Here we use a rat model of compulsive drug seeking[5–8] in which cocaine seeking persists in a subgroup of rats despite delivery of noxious foot shocks. We show that prolonged cocaine self-administration decreases *ex vivo* intrinsic excitability of deep-layer pyramidal neurons in the prelimbic cortex, which was significantly more pronounced in compulsive drug-seeking animals. Furthermore, compensating for hypoactive prelimbic cortex neurons with *in vivo* optogenetic prelimbic cortex stimulation significantly prevented compulsive cocaine seeking, whereas optogenetic prelimbic cortex inhibition significantly increased compulsive cocaine seeking. Our results show a marked reduction in prelimbic cortex excitability in compulsive cocaine-seeking rats, and that *in vivo* optogenetic prelimbic cortex stimulation decreased compulsive drug-seeking behaviours. Thus, targeted stimulation of the prefrontal cortex could serve as a promising therapy for treating compulsive drug use.

To investigate the neurobiological dysfunctions that underlie compulsive drug use, we used an animal model of addiction[5] previously shown to engender a subpopulation of rats with compulsive cocaine seeking. Rats underwent extended cocaine self-administration training (Fig. 1a) under a heterogeneous seeking-taking chain schedule[5] (details in the Supplementary Information). Daily self-administration sessions consisted of 30 trials that began with insertion of the seek lever. After one lever press and a variable interval (5 to 75 s), one additional press on the seek lever resulted in seek lever retraction and take lever insertion. One take lever press triggered a single intravenous cocaine infusion and retraction of the take lever. After >8 weeks of self-administration training, rats received four days of shock sessions in which 30% of the seek link ended with delivery of a noxious foot shock (0.4 mA, 0.5 s). Rats earning ≥10 cocaine infusions (14 of 46 rats, 30%, 24.1 ± 1.9 infusions) in the fourth shock session were considered shock-resistant, whereas rats earning <10 infusions (32 of 46 rats, 70%, 1.3 ± 0.3 infusions) were considered shock-sensitive (Supplementary Fig. 1). Previous works have observed a relatively similar proportion of shock-resistant rats[5–8] and suggested that these rats exhibit greater basal impulsivity but not higher reactivity to novelty[6]. Seek lever presses (Fig. 1b, c) and earned cocaine infusions (Fig. 1d, e) were significantly inhibited by foot shocks in shock-sensitive rats, whereas shock-resistant rats were much less affected (Fig. 1b–e). Baseline cocaine-seeking behaviours and earned cocaine infusions were similar in both groups (Fig. 1c, e), suggesting that divergent responses to contingent foot shocks did not reflect cocaine self-administration history[8].

Clinical evidence implicates prefrontal cortex (PFC) hypofunction[2–4] in the loss of inhibitory control over drug seeking, but a causal link between drug-induced neuroadaptations and compulsive seeking behaviour remains elusive. We focused on the prelimbic cortex, because a homologous region in the human PFC[9,10] regulates many cognitive functions, including decision making and inhibitory response control[11–15], which are compromised in human addicts[3]. We propose that chronic cocaine use induces prelimbic cortex hypoactivity, and that compromised prelimbic cortex function in turn impairs inhibitory

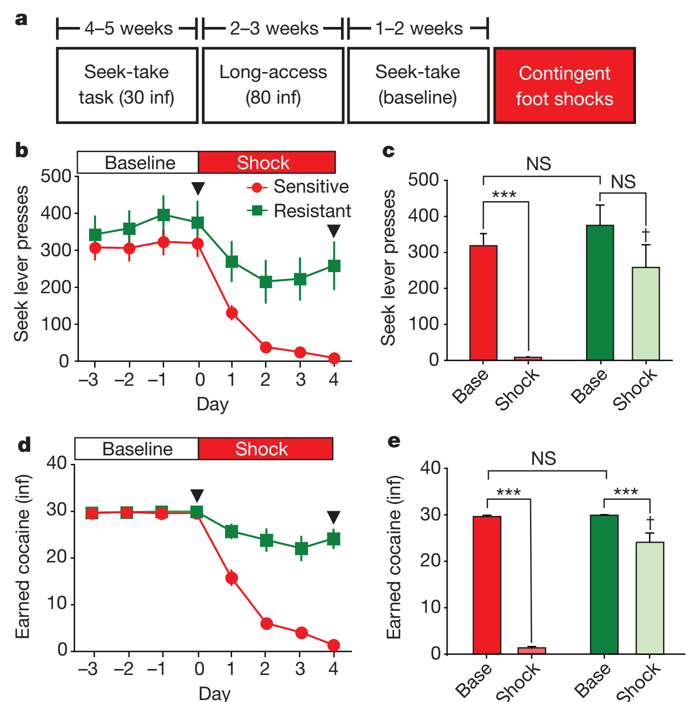

Figure 1 | Prolonged cocaine self-administration induces compulsive cocaine seeking in a subpopulation of rats. a, Experimental timeline showing the seek-take task and long-access sessions before the beginning of the foot shock sessions. After approximately two months of cocaine self-administration, rats received four days of shock sessions, in which a foot shock was administered in 30% of trials. **b**, Foot shocks decreased seek-lever presses in shock-sensitive rats ($n = 32$) but not shock-resistant rats ($n = 14$). **c**, Seek-lever presses on last day of baseline and last day of shock (arrowheads in **b**) ($F_{(3,68)} = 27.89$, $P < 0.001$; Tukey's post-hoc: ***$P < 0.001$ baseline versus shock sessions; †$P < 0.001$ shock-sensitive versus shock-resistant shock session). **d**, Foot shocks greatly reduced cocaine infusions in shock-sensitive rats. **e**, Earned cocaine infusions (inf) on last day of baseline and last day of shock ($F_{(3,68)} = 246.8$, $P < 0.001$; Tukey's post-hoc: ***$P < 0.001$ baseline versus shock session; †$P < 0.001$ shock-sensitive versus shock-resistant shock sessions). NS, not significant. Error bars indicate s.e.m.

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control over compulsive drug seeking. Thus, we performed *ex vivo* electrophysiology to determine whether cocaine self-administration reduced prelimbic cortex intrinsic excitability, because action potentials are the main mechanism of neuronal communication and altered spike firing contributes to neuronal dysfunction[16] and central nervous system disorders[17].

Intrinsic excitability of deep-layer, morphologically confirmed (Fig. 2a)[18] pyramidal prelimbic cortex neurons from shock-sensitive and shock-resistant rats was evaluated 24 h after the fourth shock session, and was compared with naive rats. We targeted deep-layer pyramidal prelimbic cortex neurons because they project to brain structures implicated in drug-seeking behaviours, including the nucleus accumbens, dorsal striatum and amygdala[15,19,20]. We first measured the amount of current required to induce an action potential in response to brief depolarization (2 ms, 0–2,500 pA, 10 pA steps). Relative to naive and shock-sensitive neurons, shock-resistant neurons required almost twice as much current to elicit an action potential

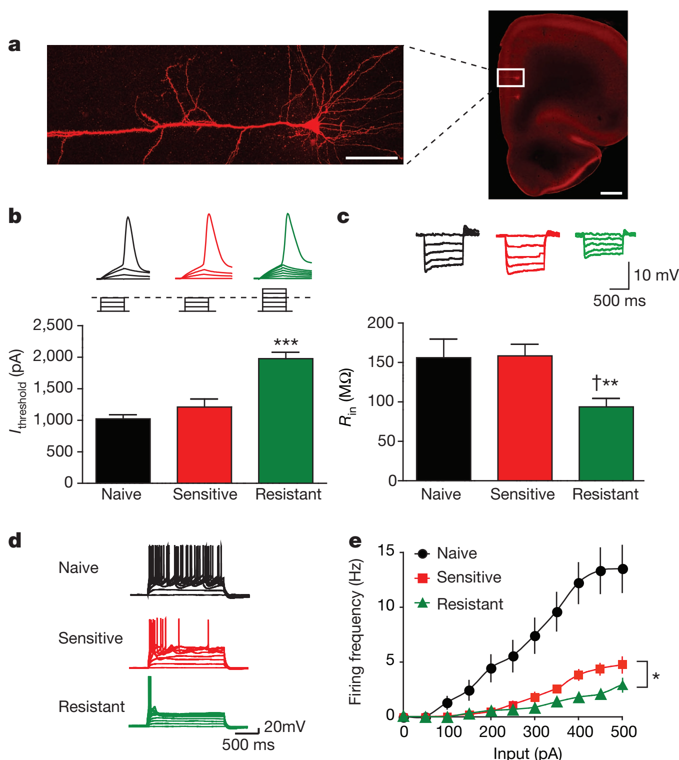

Figure 2 | Profoundly hypoactive prelimbic cortex neurons in shock-resistant rats. **a**, Morphological features of a recorded prelimbic cortex pyramidal neuron. Scale bars represent 0.1 mm and 1 mm in the left and right images, respectively. **b**, Shock-resistant prelimbic cortex neurons required more current to elicit firing with a 2-ms current step. Top, examples of sub-threshold membrane depolarization and the first action potential elicited by current injection. Square pulses illustrate increasing current injection. Bottom, current required for firing was higher in shock-resistant rats ($F_{(3,45)} = 18.53$, $P < 0.001$; Tukey's post-hoc: ***$P < 0.001$ naive or shock-sensitive versus shock-resistant) (naive, $n = 10$ cells; shock-sensitive, $n = 13$ cells; shock-resistant, $n = 16$ cells). **c**, Top, neuronal responses to hyperpolarizing steps. Bottom, input resistance was decreased only in shock-resistant rats ($F_{(3,60)} = 3.986$, $P = 0.011$; Tukey's post-hoc: †$P < 0.01$ naive versus shock-resistant, **$P < 0.01$ shock-sensitive versus shock-resistant) (naive, $n = 12$ cells; shock-sensitive, $n = 24$ cells; shock-resistant, $n = 16$ cells). **d**, Prelimbic cortex firing in response to current injection. **e**, Firing frequency versus current injection (1 s duration, 0 to 500 pA, 50 pA steps) (two-way analysis of variance (ANOVA) among all groups: treatment, $F_{(3,59)} = 17.45$, $P < 0.001$; interaction, $F_{(30,590)} = 11.95$, $P < 0.001$; two-way ANOVA between shock-resistant and shock-sensitive: treatment, $F_{(1,33)} = 6.440$, *$P = 0.016$; interaction group × input, $F_{(10,330)} = 6.201$, $P < 0.001$) (naive, $n = 15$ cells; shock-sensitive, $n = 19$ cells; shock-resistant, $n = 16$ cells). Statistical analyses include neurons from rats in Supplementary Fig. 5. Error bars indicate s.e.m.

(Fig. 2b) and exhibited significantly smaller input resistance (Fig. 2c). Thus, the ability of prelimbic cortex neurons to rapidly generate an action potential was compromised in shock-resistant rats. In addition, prelimbic cortex neurons from shock-resistant and shock-sensitive rats showed impairments in repetitive firing induced by a more moderate, sustained depolarizing currents (1 s, 0–500 pA, 50 pA steps), with a significantly greater impairment in shock-resistant versus shock-sensitive rats (Fig. 2d, e). Also, cocaine intake under shock correlated with decreased input resistance and increased current required for firing across all rats tested (Supplementary Fig. 2). Analysis of the action potential shape during whole-cell recordings and nucleated patch electrophysiological recordings revealed no differences in Na^+ channel activation, or in the composition of somatic K^+ currents or barium-sensitive K^+ conductances (Supplementary Table 1 and Supplementary Figs 3 and 4) among naive, shock-sensitive and shock-resistant rats. Control experiments in which shock-sensitive rats self-administered additional cocaine after each shock session suggested that the quantity of cocaine per se was not responsible for firing adaptations in shock-resistant rats (Supplementary Fig. 5).

Thus, our results demonstrate that long-term cocaine self-administration reduced prelimbic cortex excitability, with a much more pronounced effect in compulsive rats. If compromised spike firing was causally related to compulsive cocaine seeking, then increasing prelimbic cortex neuronal activity could decrease compulsive behaviour. One way to compensate for reduced excitability is to take advantage of *in vivo* optogenetic stimulation[21,22], in which prelimbic cortex neurons can be activated in a temporally precise manner during cocaine self-administration trials. Deep-layer prelimbic cortex neurons expressing channelrhodopsin-2 (ChR2) (Supplementary Fig. 6a) showed minimal loss of spike fidelity at photostimulation frequencies up to 20 Hz both *ex vivo* and *in vivo* (Supplementary Fig. 6b, c). Also, 1 Hz prelimbic cortex photoactivation evoked field potentials in the nucleus accumbens core, a downstream prelimbic cortex target[23], without loss of fidelity or generation of long-term depression (LTD) of glutamatergic function (Supplementary Fig. 7), although other frequencies can induce prelimbic cortex LTD[24]. Thus, photoactivation of ChR2-expressing prelimbic cortex neurons can be reliably achieved at physiologically relevant timescales.

To test whether enhancing activity in a hypoactive prelimbic cortex would decrease compulsive cocaine seeking, adeno-associated virus (AAV) encoding ChR2 fused to enhanced yellow fluorescent protein (ChR2–eYFP) was bilaterally injected into the prelimbic cortex, with bilateral implantation of chronic optic fibres[22] targeting the prelimbic cortex (Supplementary Fig. 8). The effect of prelimbic cortex stimulation on cocaine seeking was assessed in two separate sessions (Fig. 3A), and was only determined in the compulsive cocaine-seeking shock-resistant rats. The first prelimbic cortex ChR2 stimulation was administered during the last baseline session to determine the effect on control cocaine seeking. The second ChR2 stimulation session was given 24 h after the fourth shock session to determine whether prelimbic cortex activation could alter compulsive cocaine seeking; this session was identical to shock sessions except with the addition of photostimulation. In both ChR2 stimulation sessions, 1 Hz light pulses (10 ms duration, 473 nm) were delivered to the prelimbic cortex starting at the seek lever extension and terminating at the conclusion of the seek link. Prelimbic cortex stimulation had no effect on control, baseline cocaine-seeking behaviours (Fig. 3B, a–e). Similarly, *in vivo* prelimbic cortex photoactivation did not promote motivated behavioural responding (Supplementary Fig. 9).

In stark contrast to the baseline ChR2 stimulation session, prelimbic cortex photoactivation by ChR2 significantly attenuated foot-shock-resistant cocaine seeking. Photostimulation decreased earned cocaine infusions and increased the latency to seek-lever press (Fig. 3C, a and b), with a trend for decreased seek-lever presses and increased inter-press interval (Fig. 3C, c–e). Thus, restoring prelimbic cortex

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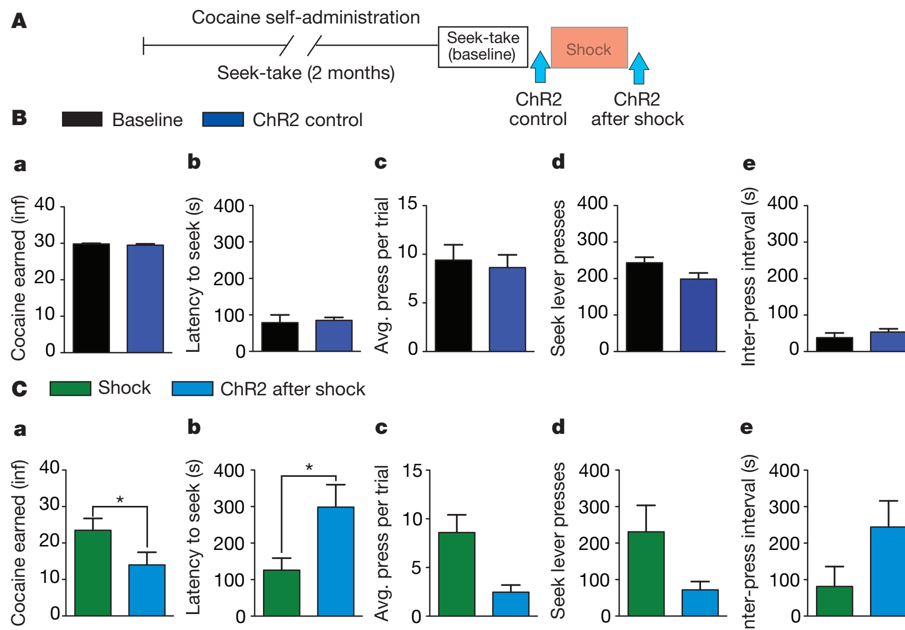

Figure 3 | *In vivo* optogenetic stimulation of prelimbic cortex suppresses compulsive cocaine seeking. **A**, Timeline illustrating when cocaine-seeking behaviours were analysed in shock-resistant rats. **B**, **C**, Comparisons were made between the last baseline session and ChR2 control session (**B**), and between the fourth shock session and the ChR2 plus shock session 24 h later (**C**). **B**, **a–e**, Prelimbic cortex photoactivation did not alter baseline cocaine self-administration. **C**, Light stimulation suppressed compulsive cocaine-seeking behaviours, with decreased cocaine rewards earned (**a**, baseline/shock $F_{(1,5)} = 6.447$, $P = 0.052$; ChR2/no ChR2: $F_{(1,5)} = 13.998$, $P = 0.013$; interaction: $F_{(1,5)} = 17.699$, $P = 0.008$; Tukey's post-hoc: $*P < 0.05$) and increased latency to press the seek lever (**b**, baseline/shock $F_{(1,5)} = 5.756$, $P = 0.062$; ChR2/no ChR2: $F_{(1,5)} = 13.297$, $P = 0.015$; interaction: $F_{(1,5)} = 30.304$, $P = 0.003$; Tukey's post-hoc: $*P < 0.05$). A trend was observed in seek-lever presses (**c**, **d**) and inter-press interval (**e**). $n = 6$ rats. Error bars indicate s.e.m.

activity decreased compulsive cocaine seeking only after rats had experienced foot shocks. In addition, ChR2-mediated activation of prelimbic cortex reduced compulsive responding starting from the beginning of the cocaine-intake session (Supplementary Fig. 10), suggesting that reduced intake may not require learning or other processes which might develop across the cocaine-intake session.

On the basis of these results, we proposed that prelimbic cortex inhibition might promote compulsive intake in shock-sensitive rats.

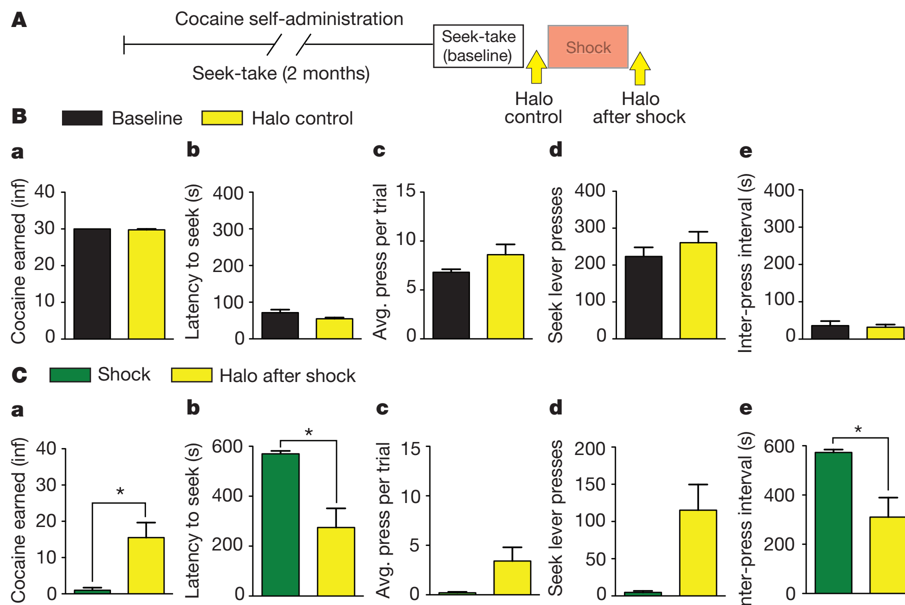

Figure 4 | *In vivo* optogenetic inhibition of prelimbic cortex enhances compulsive cocaine seeking. **A**, Timeline illustrating when cocaine-seeking behaviours were analysed. **B**, **C**, Comparisons were made between the last baseline session and eNpHR3.0 control session (**B**), and between the fourth shock session and the eNpHR3.0 plus shock session 24 h later (**C**). **B**, **a–e**, Prelimbic cortex photoactivation did not alter baseline cocaine self-administration. **C**, Light stimulation promoted cocaine-seeking behaviours in shock-sensitive rats, including increased cocaine rewards earned (**a**, baseline/shock $F_{(1,3)} = 126.99$, $P = 0.001$; eNpHR3.0/no eNpHR3.0: $F_{(1,3)} = 10.038$, $P = 0.051$; interaction: $F_{(1,3)} = 12.271$, $P = 0.039$), decreased latency to press seek lever (**b**, baseline/shock $F_{(1,3)} = 118.59$, $P = 0.002$; eNpHR3.0/no eNpHR3.0: $F_{(1,3)} = 17.269$, $P = 0.025$; interaction: $F_{(1,3)} = 10.751$, $P = 0.046$) and decreased inter-press interval (**e**, baseline/shock $F_{(1,3)} = 124.12$, $P = 0.002$; eNpHR3.0/no eNpHR3.0: $F_{(1,3)} = 12.941$, $P = 0.037$; interaction: $F_{(1,3)} = 10.642$, $P = 0.047$). A trend was observed in seek-lever presses (**c**, **d**). $n = 4$ rats. NS, not significant. $*P < 0.05$ Tukey's post-hoc. Error bars indicate s.e.m. Halo, halorhodopsin (optogenetic inhibitor).

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Thus, AAV encoding halorhodopsin–eYFP (eNpHR3.0–eYFP)[21,22] was bilaterally injected into the prelimbic cortex, with bilateral chronic optic fibres targeting the prelimbic cortex (Supplementary Fig. 11). Prelimbic cortex inhibition by eNpHR3.0 was verified *ex vivo* (Supplementary Fig. 12). The effect of prelimbic cortex inhibition was assessed in two sessions with identical schedules as in the ChR2 experiments (Fig. 4A), except that neurons were inhibited by a continuous light pulse (532 nm) during the seek part of the seek-take chain. Prelimbic cortex inhibition had no effect on control, baseline cocaine-seeking behaviours (Fig. 4B, a–e), but significantly increased cocaine seeking during foot shock sessions in these shock-sensitive rats, with greater earned cocaine infusions (Fig. 4C, a), decreased latency to seek-lever press (Fig. 4C, b), decreased inter-press interval (Fig. 4C, e) and a trend for increased seek-lever presses (Fig. 4C, c and d). Thus, eNpHR3.0 inhibition of prelimbic cortex neurons increased propensity for compulsive cocaine seeking.

Our results suggest that prolonged cocaine use depressed prelimbic cortex excitability, and that, in a select population of rats, a profound prelimbic cortex hypoactivity drove compulsive cocaine seeking. We speculate that crossing a critical threshold of prelimbic cortex hypoactivity promotes compulsive behaviours. Notably, prelimbic cortex-mediated inhibitory control was only evident after self-administration was paired with foot shock, but not during baseline sessions before foot shock. In agreement, other groups have proposed that cortical areas promote compulsive intake in the presence of a challenge, for example, in the face of aversive consequences, in contrast to striatal areas that would sustain habitual intake in the absence of such challenges[2,25]. Thus, in the absence of conflict, optogenetic manipulation of a brain region that mediates inhibitory control would have no effects. However, when confronted with the possibility of punishment, prelimbic cortex hypoactivity would bias behaviour towards compulsive seeking. Our results also suggest that prelimbic cortex stimulation might be clinically efficacious against compulsive seeking, with few side effects on non-compulsive reward-related behaviours in addicts; whether prelimbic cortex ChR2 stimulation reduces cocaine seeking through effects on emotional conditioning or cognitive processing[11–15] remains to be resolved. In conclusion, we provide an effective method to decrease compulsive drug seeking in an animal model that may closely mimic the human addiction condition, which supports the use of *in vivo* prefrontal cortex stimulation to treat compulsive drug use.

METHODS SUMMARY

Cocaine self-administration. Outbred male Wistar (Harlan) rats weighing 275–300 g at the beginning of the experiments were used for all experiments. Cocaine self-administration began 7 days after surgery. Rats were trained 6 or 7 days per week during the animal's dark cycle.

***Ex vivo* patch-clamp recording.** Rats were deeply anaesthetized, perfused with ice-cold sucrose-substituted buffer, decapitated, and 250-μm sections of the medial PFC were prepared. Whole-cell current-clamp recordings and nucleated patch-clamp recordings were performed using a potassium-based internal solution[26,27].

***In vivo* optogenetic prelimbic cortex stimulation or inhibition during behaviour.** Adeno-associated viral vectors (AAV5 CaMKIIα::ChR2–eYFP or AAV5 CaMKIIα::eNpHR3.0–eYFP) were packaged by the University of North Carolina Vector Core Facility. Following surgery to implant a jugular catheter for intravenous cocaine self-administration (see Methods), virus (0.5 μl) was injected bilaterally into the prelimbic cortex at 0.1 μl min^{-1}. Chronic optical fibres were implanted bilaterally directly above the prelimbic cortex. During optogenetic experiments, chronic optical implants were connected to optical patch cables coupled to 473-nm (for ChR2) or 532-nm (for eNpHR3.0) lasers modulated by behavioural hardware (Med Associates). Chronic optical implants were constructed on site as previously described[22].

Full Methods and any associated references are available in the online version of the paper.

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Supplementary Information is available in the online version of the paper.

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Author Contributions B.T.C., H.-J.Y., F.W.H. and A.B. designed, discussed and planned all experiments. B.T.C., H.-J.Y., I.K.-Y., C.H. and S.L.C. performed experiments. B.T.C. and C.H. analysed data. B.T.C., F.W.H. and A.B. wrote the manuscript.

Author Information Reprints and permissions information is available at www.nature.com/reprints. The authors declare no competing financial interests. Readers are welcome to comment on the online version of the paper. Correspondence and requests for materials should be addressed to B.T.C. (billy.chen@nih.gov) or A.B. (antonello.bonci@nih.gov).

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METHODS

Subjects. Adult male Wistar rats were singly housed in a climate-controlled facility. Experiments followed the guidelines of the National Institutes of Health Guide For the Care and Use of Laboratory Animals, and were performed during the animals' dark cycle.

Catheter surgery. Rats were implanted with a chronically indwelling intravenous catheter, as previously described[28]. Briefly, rats were anaesthetized with isoflurane (2.5%) and a unilateral, intrajugular catheter was inserted 2.7–3.0 cm into the jugular vein. Catheters were sutured to the underlying muscle tissue and the other end externalized between the scapulas. Catheters consisted of a guide cannula (Plastics-One) attached to MicroRenathane tubing (0.025-inch inside diameter, 0.047-inch outside diameter) and dental cement. The catheter was flushed daily with a saline solution containing gentamicin (5 mg ml^{-1}, Butler Schein) to help maintain catheter patency and reduce infection. Following surgery, animals were allowed to recover for one week. During the first 5 days of the recovery period, animals were housed in home cages and had ad libitum access to food and water. Beginning on day 6, rats were food restricted to 20 g per day of standard rat chow. Rats were monitored daily to ensure that weight did not fall below 90% of ad libitum weight. Food restriction remained until the end of the experiment. Cocaine self-administration training began 7 days after surgery. Rats were food restricted to promote self-administration behaviour[29].

Stereotaxic surgery. Rats designated for optogenetic experiments received virus injection and chronic optic fibre surgery on the same day as catheter surgery. Rats were placed into a stereotaxic frame (David Kopf Instruments) and remained under isoflurane anaesthesia. Microinjection needles were then inserted bilaterally targeting the prelimbic area (prelimbic cortex; coordinates from Bregma: +3.2 AP, ± 1.2 ML, −3.7 DV, 10° angle). Microinjections were performed using custom-made injection needles (26-gauge) connected to a 2-µl Hamilton syringe. Each prelimbic cortex hemisphere was injected with 0.5 µl of purified and concentrated AAV ($\sim 10^{12}$ infectious units ml^{-1}) encoding ChR2–eYFP or eNpHR3.0–eYFP under the control of the *Camk2a* promoter (AAV5 CaMKIIα::ChR2–eYFP or AAV5 CaMKIIα::eNpHR3.0–eYFP) to infect prelimbic cortex glutamatergic neurons. Injections occurred over 10 min followed by an additional 10 min to allow diffusion of viral particles away from the injection site. Chronic optic fibres were implanted bilaterally over the injection site (+3.2 anteroposterior, ± 1.2 mediolateral, −3.4 dorsoventral, 10° angle). Fibres were secured to the skull with dental cement.

General behavioural methods. Behavioural experiments were performed in standard operant chambers (Med Associates or Coulbourn Instruments) with two retractable levers set 5 cm above the floor. A cue light was positioned 6 cm above each lever. The two levers flanked a food hopper. One lever was paired with cocaine infusion (the take lever) while the other lever was designated as the seek lever. Each chamber was also equipped with a house light and tone-generator. All sessions continued for 6 h or until 30 or 80 cocaine infusions were earned (see below for detail methods on seek-take chain schedule), and were performed 6 or 7 days a week for approximately 2 months.

Acquisition of cocaine self-administration under the seek-take schedule. Seven days after surgery, rats began cocaine self-administration training using the seek-take chain schedule. The behavioural model used was modified from the procedures described previously[5]. Self-administration training was divided into four separate phases: acquisition of the taking response; training of the seek-take chain; extended training; and punishment.

Acquisition of the taking response. In this initial phase of training, each trial began with the insertion of the take lever. Depression of the take lever on a fixed ratio (FR-1) resulted in the delivery of a single cocaine infusion (0.50 mg kg^{-1} over 3 s). Cocaine infusions were paired with illumination of the cue light (20 s) above the take lever, retraction of the take lever and extinction of the house light. Following a 30 s time-out period, another trial was initiated with the insertion of the take lever and illumination of the house light. Training sessions terminated after either 30 earned cocaine infusions or 6 h, depending on which criterion was reached first. Training of the taking response continued until rats were able to earn 30 cocaine infusions across three consecutive sessions. Typically, training continued for five to seven sessions, after which rats advanced to training for the seek-take chain schedule.

Training of the seek-take chain. Each trial in the seek-take chained schedule began with insertion of the seek lever (opposite to the take lever), with the take lever retracted. A single press on the seek lever resulted in the retraction of the seek lever and insertion of the take lever. A single depression of the take lever then triggered delivery of a single cocaine infusion, illumination of the cue light above the take lever, retraction of the take lever and extinction of the house light. Following a 30 s time-out, another trial was initiated with the insertion of the seek lever and house light illumination.

Once the rats were able to earn 30 cocaine infusions, or had less than 20% variation in number of cocaine earned across three consecutive days, a random interval (RI) schedule was introduced into the seek link of the chain schedule. Under the RI schedule, a trial began with the insertion of the seek lever and illumination of the house light. The first seek-lever press initiated the RI schedule. Three RI schedules were used across the training period: RI5, RI30 and RI60. In the RI5 schedule, immediately after the first seek-lever press, the software randomly selected 0.1 s, 5 s, or 10 s as the 'random interval'. Seek-lever presses during the duration of the RI had no programmed consequences. The first seek-lever press following the end of the RI (in the case of RI5: 0.1 s, 5 s, or 10 s) terminated the seek link of the chain and resulted in the retraction of the seek lever and the insertion of the take lever. In RI30 schedule, the software could randomly select 15, 30, or 45 s, whereas for RI60 intervals of 45 s, 60 s or 75 s were used. One press on the take lever triggered cocaine infusion, paired with the identical cues as during the training of the take response. A time-out period followed each cocaine infusion. The time-out period started at 30 s but progressively increased to 7 min by the end of training. The RI schedule was not altered within any individual training session. Training on each RI schedule persisted until the rats were able to earn 30 cocaine infusions, or have less than 20% variation across three consecutive days. Once an animal achieved stable cocaine seeking behaviour at a RI schedule, usually 4–6 days, it was advanced to the next RI schedule. Typically, a rat would spend 5 days in RI5, receive another 5 days in RI30, and finally finish with another 5 days at RI60. Rats were given 10 min to complete each seek-take chain. Failure to complete the cycle within the 10 min resulted in a lost opportunity to self-administer cocaine. A new trial started after the time-out period. More than 90% of rats in the present study earned 30 infusions under all three RI schedules. Daily training sessions terminated after either 30 earned cocaine infusions or 6 h, depending upon which criterion was reached first.

Extended training. After reaching the criterion for RI60, rats were given extended access to cocaine. In this phase of training, rats were given eight sessions with only the take lever available. Rats could earn up to 80 cocaine infusions under an FR-1 schedule with a post-reinforcement time-out of 30 s. Interspersed in between the extended cocaine sessions were additional sessions of training on the seek-take chain schedule under RI60 schedule. At the conclusion of the extended training sessions, all rats received additional training on the seek-take chain schedule under RI60 schedule. Stable performance (earning all 30 cocaine infusions or >20% variation) across four consecutive days served as baseline for comparison for the effects of punishment and *in vivo* optogenetic stimulation.

Punishment. During each punishment session, mild foot shocks (0.4 mA, 0.5 s) were administered in 30% of the trials. Shocks were delivered after the first lever press on the seek lever that occurred after the RI60 period was finished, and before extension of the take lever. Each punishment session consisted of 30 trials, and rats were given access to the take lever for cocaine infusions in all trials. Rats were given four punishment sessions. At the end of four days, shock-sensitive and shock-resistant rats were grouped based on the number of earned cocaine rewards.

***In vivo* optical excitation sessions with ChR2.** Shock-resistant rats received *in vivo* optogenetic excitation of the prelimbic cortex in which light stimulation (1 Hz, 10 ms, 10–15 mW, 473 nm)[21,22] was administered in two separate sessions. The first session was administered one day after last RI60 baseline sessions (Fig. 3a). A separate session was administered one day after the fourth shock sessions; thus, there was a fifth day of foot shock during which ChR2 was stimulated using a laser. In each of the two ChR2 stimulation sessions, light stimulation was administered only during the seek link of the seek-take chain schedule. Insertion of the seek lever initiated activation of the laser. A 1 Hz stimulation was present throughout the seek link. Photostimulation was terminated at the end of the seek link.

***In vivo* optical inhibition sessions with eNpHR3.0.** Shock-sensitive rats received *in vivo* optogenetic inhibition of the prelimbic cortex, in which light stimulation (continuous, 10–15 mW, 532 nm)[21,22] was administered in two separate sessions. The first session was administered one day after the last RI60 baseline sessions (Fig. 4a). The second session was administered one day after the four shock sessions; thus, there was a fifth day of foot shock during which eNpHR3.0 was stimulated using a laser. Insertion of the seek lever initiated activation of the laser. Laser stimulation was continuously present throughout each seek link of the seek-take chain schedule. Photostimulation was terminated at the end of the seek link.

Drugs. Cocaine HCl, picrotoxin and 6-cyano-7-nitroquinoxaline-2,3-dione disodium salt hydrate (CNQX) were purchased from Sigma-Aldrich.

Slice preparation. Rats were euthanized one day after the last shock session. Rats were anaesthetized with 40 mg kg^{-1} pentobarbital (intraperitoneally) and transcardially perfused with ~30 ml of nearly frozen (~0 °C) modified artificial cerebrospinal fluid (aCSF) at a rate of ~20 ml min^{-1}. The modified aCSF for perfusion contained (in mM): 225 sucrose, 119 NaCl, 2.5 KCl, 1.0 NaH_2PO_4, 4.9 $MgCl_2$, 0.1 $CaCl_2$, 26.2 $NaHCO_3$, 1.25 glucose. After perfusion, the brain was quickly removed and placed into ice-cold aCSF for 1–2 min. Coronal sections containing the medial PFC (250 µm) were prepared with VT-1200 vibratome (Leica). Slices

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were placed in a holding chamber (containing aCSF with 1 mM ascorbic acid added 15 min before brain dissection), and allowed to recover for at least 30 min before being placed in the recording chamber and superfused with a bicarbonate-buffered solution saturated with 95% O_2 and 5% CO_2 and containing (in mM): 119 NaCl, 2.5 KCl, 1.0 NaH_2PO_4, 1.3 $MgCl_2$, 2.4 $CaCl_2$, 26.2 $NaHCO_3$, and 11 glucose, at 32–34 °C.

Electrophysiology. Picrotoxin (100 μM) and CNQX (10 μM) were present throughout the experiment to block inhibitory and excitatory synaptic transmission, respectively. Cells were visualized using infrared differential interference contrast video microscopy. Whole-cell current-clamp recordings were made using a MultiClamp 700B amplifier (Molecular Devices). Electrodes (2.8–4.0 MΩ) contained (in mM): 120 potassium methanesulphonate ($KMeSO_4$), 20 HEPES, 0.4 EGTA, 2.8 NaCl, 5 TEA-Cl, 2.5 MgATP, and 0.25 NaGTP, biocytin (1%), pH 7.2–7.3 (270–285 mOsm). Series resistance (10–40 MΩ) was continually monitored on-line with a −20 pA, 300 ms current injection given after every current injection step; if the series resistance changed by more than 20%, data were not included in the analysis. Membrane potentials were not corrected for junction potentials (estimated to be 10 mV). After breaking into a neuron, the resting membrane potential was set to −80 mV by injecting DC current through patch amplifier. To measure the amount of current required to reach action potential threshold, a series of current steps (2 ms duration at 2.5 Hz, 0 to 2500 pA range with +10 pA step increments) were injected into the cell until an action potential was generated. To determine the input resistance, hyperpolarizing current injections (1 s duration, 0 to −100 pA in −25 pA step increments) were injected into the cell. Input resistance was taken at the linear part of the trace. Sustained firing was determined from a series of 11 current injections (1 s duration, 50 pA steps). All values were obtained after the cells had reached a stable response and were averages of three cycles for each cell.

Nucleated patch recordings were performed as described before to dissect K^+ current components[26]. Briefly, recording pipettes were filled with K^+ based internal solution (in mM): 140 $KMeSO_4$, 5 KCl, 0.05 EGTA, 2 $MgCl_2$, 2 Na_2ATP, 0.4 NaGTP and 10 HEPES (pH adjusted to 7.3). Recordings were made at room temperature (23–24 °C). Immediately after establishing whole-cell configuration from identified pyramidal cells, a negative pressure (−150 to −190 mbar) was applied through patch pipette to bring the nucleus close to the pipette tip. The patch pipette was then slowly withdrawn until the membrane patch surrounding the nucleus was completely pulled out of the cell. After excision, nucleated patches were held with a small constant negative pressure (−30 to −60 mbar) and were voltage clamped at a holding potential of −90 mV. P/−4 protocol in pClamp 10 was used for on-line subtraction of leakage and capacitive currents. A custom-made multi-barrel system consisting three glass capillaries was used to apply drugs to isolate different voltage-gated K^+ currents. Tetrodotoxin (TTX, 500 nM) and cadmium (Cd^{2+}, 100 μM) were included in HEPES-buffered ACSF (in mM: 138 NaCl, 10 HEPES, 2.5 KCl, 2 $CaCl_2$, 1 $MgCl_2$ and 25 glucose, pH 7.3) in each barrel to block voltage-gated Na^+ and Ca^{2+} currents, respectively. The K^+ current blockers 4-aminopyridine (4-AP, 0.5 mM) and tetraethylammonium (TEA, 20 mM) were included in different barrels, and nucleated patches were inserted into the barrels sequentially. Macroscopic voltage-gated K^+ current was elicited by a 150 ms test pulse stepping from −90 mV to +70 mV and up to 10 sweeps of recordings were collected and averaged to represent the current in each drug treatment. Drug-sensitive K^+ currents were then obtained by off-line subtraction and peak currents were measured to calculate the respective contribution in macroscopic K^+ current. To study background K^+ conductance, voltage-clamp recordings were made in the presence of 500 nM TTX, 0.1 mM Cd^{2+}, 10 mM TEA and 1 mM CsCl to block voltage-gated Na^+, Ca^{2+}, K^+ currents and I_h/inward rectifier currents, respectively. Cells were voltage-clamped at −70 mV. Under this condition, a slow voltage ramp from −130 mV to −70 mV was applied across 1 s before and after 1 mM barium (Ba^{2+}) application. Pharmacological subtraction revealed Ba^{2+}-sensitive current and background K^+ conductance was then calculated at −70 mV according to ref. 27.

All recorded neurons were filled with biocytin and processed for post-hoc immunohistochemical staining to confirm the presence of apical dendrites. Only neurons exhibiting a clear apical dendrite, indicative of pyramidal neurons, were included in the analysis. All data were acquired at 20 kHz and filtered at 10 kHz using Clampex 10.2 software (Molecular Devices).

Data analysis. Statistical significance was primarily assessed using ANOVA, followed by post-hoc test when applicable. Data were analysed with Prism (GraphPad Software).

Virus expression and histology. Following the completion of behavioural experiments, rats were deeply anaesthetized with euthasol and perfused transcardially with PBS followed by 4% paraformaldehyde dissolved in PBS. Brains were removed and fixed in 4% paraformaldehyde for an additional 24–48 h. Fifty-micrometre-sections of the PFC were made on a vibratome. Some slices were stained for 1 h with 2% Neurotrace fluorescent Nissl stain (Invitrogen; excitation 530 nm, emission 615 nm) diluted in PBS. Slices were then washed and mounted on gelatin-coated slides, treated with fluorescent-mounting media and mounted. Some slices were not stained with Neurotrace and were directly washed with PBS and mounted. In these slices, 4′,6-diamidino-2-phenylindole (DAPI) (0.15 μg ml^{-1}) was included in the fluorescent-mounting media. Expression of ChR2–eYFP and eNpHR3.0–eYFP was examined for all rats using a Nikon inverted fluorescent microscope with a ×4 objective or an Olympus Fluoview FV1000 with a ×4 objective. Rats showing no bilateral eYFP expression in the PFC owing to faulty microinjections, and showing bilateral fibre placement outside the PFC were excluded from analysis.

Reconstruction of optical stimulation sites in the prelimbic cortex. Optical stimulation sites of *in vivo* optogenetic experiments were determined as described previously[30]. Briefly, fixed and stained coronal brain sections containing the mPFC were examined with Nikon inverted fluorescent microscope or an Olympus Fluoview FV1000. Optic fibre tracks were located in the slice and optical stimulation sites were determined as 0.5 mm below the fibre tip (Supplementary Figs 8, 9 and 11).

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