Supplementary Figure 1. Distribution of the number of earned cocaine infusions in Shock-sensitive and Shock-resistant rats on the fourth day of shock session. All Shock-sensitive rats (SS, red bar) earned less than five cocaine infusions on the last day of the shock session. Shock-resistant rats (SR, green bars) earned more cocaine infusions. Our separation of SS and SR rats followed Belin and colleagues\(^3\) who found that responding for cocaine under punishment could be best modeled by two populations, one with very low responding and another more broadly distributed population of rats that continued to respond for cocaine despite pairing with footshock. In our existing data set, there was a clear group of rats with low responding (SS, with less than 5 responses per session), with another more diverse group that did respond for cocaine under punishment. However, the sample size was still too low to statistically separate SS and SR rats, which might be predicted from the lower n as discussed by Belin et al.\(^3\).

Supplementary Figure 2. Measures of prelimbic area (PL) neuronal activity correlate with cocaine rewards earned. a, Minimum current to evoke firing and, b, input resistance both significantly correlated with cocaine rewards earned on the fourth day of shock (minimum current: \(r^2 = 0.787, P = 0.003\); input resistance: \(r^2 = 0.532, P = 0.040\)). For this analysis, all cells from a given rat were averaged to give one value. These results indicate that compulsive cocaine seeking was related to hypoactivity in PL neurons independent from our designation of rats as SR or SS.
Supplementary Figure 3. The main composition of voltage-dependent K⁺-currents is not significantly different in Naïve, SS and SR rats. a, DIC image of a nucleated patch excised from a deep-layer pyramidal PL neuron. b, Illustration of custom-made glass barrels and a nucleated patch. A nucleated patch was successively inserted into each barrel of a custom-made multiperfusion system. In this way, voltage-clamp recordings could be made in the presence of channel blockers (TEA, 4-AP) to reveal different K⁺-current components. c, Example of digitally-subtracted traces from nucleated patch-clamp recording to reveal different K⁺-current components. d, Fraction of different K⁺-components from Naïve, SS and SR rats (P = 0.326 for 4-AP-sensitive currents; P = 0.117 for TEA-sensitive currents; P = 0.493 for TEA-resistant currents).
Supplementary Figure 4. Ba$^{2+}$-sensitive K$^+$ conductances in PL neurons are not significantly different in Naïve, SS and SR rats.  

a, (Top) representative sample current traces elicited using a slow voltage ramp (middle) in the absence and presence of 1mM Ba$^{2+}$ to reveal the voltage-independent, Ba$^{2+}$-sensitive K$^+$-current.  

b, No significant differences in Ba$^{2+}$-sensitive conductances were found between the three groups of rats ($P = 0.074$).
Supplementary Figure 5. PL neurons from SS rats and SS rats that receive extra cocaine show similar levels of excitability. Difference in neuronal excitability between SR and SS rats might reflect the additional cocaine infusions received by SR, but not by SS, rats during the four shock sessions. Thus, for a separate group of SS rats, the take lever was extended at the end of the four shock sessions to allow further cocaine SA (shock/cocaine-take session). Since footshocks were only paired with the seek lever, these SS rats readily pressed the take lever and could earn up to 30 cocaine infusions to match the daily intake of SR rats. PL excitability was evaluated twenty-four hours after the fourth shock/cocaine-take session.
**Supplementary Figure 5 (cont.).** a, Averaged amount of current required to reach action potential threshold was significantly higher only in SR rats (ANOVA F(3,45) = 18.53, P < 0.001; post-hoc comparison by Tukey’s multiple comparison yielded ***P < 0.001 for SR versus Naïve, SS and SS + coc) (Naïve, n = 10 cells; SS, n = 13 cells; SR, n = 16 cells; SS + Coc, n = 10 cells).

b, Input resistance was decreased only in SR rats (ANOVA F(3,60) = 3.986, P = 0.011; post-hoc comparison by Tukey’s multiple comparison yielded #P < 0.05 for Naïve versus SR and **P < 0.01 for SS versus SR) (Naïve, n = 12 cells; SS, n = 24 cells; SR, n = 16 cells, SS + coc, n = 12 cells). c, Averaged number of spikes elicited by a series of long-duration positive current injections (0 to 500 pA, Δ50 pA steps) (two-way ANOVA between all groups: treatment, F(3,59) = 17.45, P < 0.001; interaction, F(30,590) = 11.95, P < 0.001; two-way ANOVA between SR and Shock sensitive: treatment, F(1, 33) = 6.440, *P = 0.016; interaction group x input, F(10,330) = 6.201, P < 0.001; two-way ANOVA between SR and SS + coc: treatment, F(1, 27) = 5.09, #P = 0.032; interaction group x input, F(10,270) = 4.46, P < 0.001) (Naïve, n = 15 cells; SS, n = 19 cells; SR, n = 16 cells, SS + coc, n = 13 cells). Error bars show s.e.m.

These experiments examined whether in vitro changes in excitability in neurons from SR versus SS rats might reflect differences in cocaine intake. We did not pursue experiments where SS rats received a similar number of foot shocks as the SR group. In particular, passively administered unpredictable footshock administration is known to be a significant stressor to the animal relative to more predictable footshock⁴⁻⁷ and thus this added stressor could prevent clear interpretation of any results.
Supplementary Figure 6. Expression and function of ChR2-eYFP in PL neurons.  

**a**, Illustration of the location of *ex vivo* electrophysiological experiments in a coronal brain slice showing expression of ChR2-eYFP (green) after virus injection into the PL.  

**b**, Sample trace (*top*) and average data (*bottom*) of action potentials in deep-layer pyramidal neurons in the PL evoked by a 5-ms light pulse (n = 7 cells) delivered at different frequencies.  

**c**, Illustration of *in vivo* optrode recording setup.  An optrode was stereotaxically lowered into the prefrontal cortex to detect light-evoked neuronal responses.  

*Right*: voltage trace from 10 pulses (5 ms), 1 Hz light stimulation. Light evoked extracellular spikes were only detected at depths below 3000 μm, the target depth for the PL, and not in regions above the site of injection.  Cyan bars in (c) represent light stimulations. Error bars show s.e.m.
Supplementary Figure 7. Nucleus accumbens (NAcc) field potentials evoked by PL photostimulation are unaffected by a 1 Hz period of light stimulation in Naïve, SS and SR rats.  

a. An optical fiber was stereotaxically inserted into the mPFC to activate ChR2-expressing PL neurons. A tungsten recording electrode was inserted into the NAcc to record PL-evoked excitatory field potentials. We examined the PL-NAcc projection because this input has been implicated as an important regulator of drug-related behaviors\(^9,10\). Also, we examined LTD because of the possibility that compulsive drug use is generated by synaptic remodeling, in particular through changes in LTD in the NAcc\(^11,12\). 

b. In naïve rats, baseline field potentials were evoked at 0.1 Hz (10 ms duration). Although other studies have observed LTD after lower-frequency stimulation protocols in the PL and NAcc\(^11,12\), a 1 Hz, 10 min PL photostimulation (onset is indicated by arrow) did not induce plasticity in glutamatergic responses in the NAcc core. Sample traces above the graph show recorded field potential in the NAcc following 10 min baseline and after 1 Hz, 10 min PL stimulation. This result could reflect that previous studies examined electrically-evoked glutamate currents\(^12\) that activated all glutamatergic inputs to the NAcc, while ours used ChR2 to specifically examine PL inputs, and different inputs to the NAcc are known to have different functional properties\(^8,13\). 

c, d. In SS (c) and SR (d) rats, 1 Hz, 10 min PL photostimulation did not induce plasticity in the NAcc core responses. Scale bars correspond to 0.1 mV and 20 ms. Graph showing normalized PL-evoked field recording before and after the 1 Hz, 10 min PL optical stimulation. No changes in field potentials were observed. Each point represents an average of a 2-min time bin. Data are shown as mean ± s.e.m.
**Supplementary Figure 7 (cont.).** Cocaine-addicted rats show altered AMPAR-NMDAR ratios and LTD induction in the PL and NAcc\textsuperscript{11,12}. Here, we observed a dramatic decrease in intrinsic excitability in PL neurons from compulsive SR rats, and thus increased glutamatergic function might serve to help partially overcome the reduced intrinsic excitability. However, the observation that our low-frequency PL stimulation was able to reduce compulsive seeking suggests that greater glutamatergic function which would be activated by whatever level of activity occurs in the behaving rat is insufficient per se to overcome compulsive seeking. Thus, 1Hz PL stimulation did not itself cause LTD in SR, SS or Naïve rats, and thus was unlikely to be interacting with LTD mechanisms\textsuperscript{11,12} to reduce cocaine seeking. In addition, some studies from the NAcc have suggested that cortical hypoactivity can be responsible for "scaling up" of AMPAR transmission during cocaine withdrawal\textsuperscript{14,15}. Here, we did not directly examine glutamatergic function in the NAcc in cocaine-addicted rats, but our results suggest at least that the 1 Hz stimulation of PL ChR2 did not alter basal glutamatergic function, and thus that 1 Hz stimulation did not reduce compulsive cocaine seeking by interactions with LTD mechanisms.
Supplementary Figure 8. Optical stimulation sites in the PL for compulsive rats. Diagram of optical stimulation sites located 0.5 mm from the optic fiber tip for ChR2-eYFP used in Shock-resistant rat experiment.
Supplementary Figure 9. *In vivo* optical stimulation of PL is not reinforcing. A separate group of rats were injected with AAV-*CamKIIα*-ChR2-eYFP into the PL and implanted with chronic optic fibers into the PL, and then were tested for optical self-stimulation behaviour. A single lever press on the Active lever triggered optical stimulation (1 Hz, 5 ms, 30 pulses) to activate ChR2-expressing PL neurons. Inactive lever responses had no programmed consequence. **a**, The average number of lever presses (Active and Inactive) decreased across five consecutive optical self-stimulation sessions, in contrast to other brain regions where photostimulation supports motivated responding. **b**, Averaged active and inactive lever presses on day 5 of training. *n* = 5 rats. Dashed lines and error bars represent s.e.m. **c**, Optical stimulation sites in the PL for self-stimulation experiment. Diagram of optical stimulation sites located 0.5 mm from the optic fiber tip for ChR2-eYFP used in self-stimulation experiment.

These control experiments suggest that *in vivo* optical stimulation of PL did not have non-specific effects on motivation. In agreement, optogenetic stimulation or inhibition of the PL had no effect on control, baseline responding for cocaine (Fig. 3 and 4), while significantly altering seeking during footshock sessions; these within-animal comparisons thus also suggest a lack of non-specific effects of optogenetic activation on operant behavior.
Supplementary Figure 9 (cont.). Regarding the possibility of other non-specific effects, e.g. altered sensation of shock, our optogenetic stimulation or inhibition was applied during the seek period of the seek-take chain, and thus optogenetic manipulation did not occur when the shock was administered. Also, had optogenetic manipulations impacted sensation more generally, they would likely impact the rewarding value of cocaine and thus modify baseline operant responding. However, we found no changes in baseline operant responding for cocaine with optogenetic manipulation, again indicating a specific effect of optogenetic stimulation and inhibition of PL neurons on compulsive intake.

Supplementary Figure 10. Time course of decreased compulsive responding during PL ChR2 stimulation. a, There were 30 trials per session, and for this analysis we have averaged the latency to first response for each successive three trials to reduce variability. Fig. 3 shows that PL ChR2 overall significantly reduced compulsive cocaine intake, and an ANOVA across sessions found highly significant effects of time ($F_{(9,45)} = 7.672, P < 0.001$) and group ($F_{(1,45)} = 19.52, P = 0.007$) but no interaction ($F_{(9,45)} = 0.26, P = 0.982$), suggesting that stimulation of PL ChR2 increased latency to respond for cocaine throughout the intake session. b, In addition, the latency to respond was significantly increased during the first time bin of the session (paired t-test, $P = 0.035$). Thus, PL ChR2 reduction of compulsive intake may not have required learning or other processes which could take more time to develop across the cocaine intake session, although rapid learning cannot be ruled out.
Supplementary Figure 11. Optical stimulation sites in the PL for Shock-sensitive rats. Diagram of optical stimulation sites located 0.5 mm from the optic fiber tip locations for eNpHR3.0-eYFP used in Shock-sensitive rat experiments.

Supplementary Figure 12. Expression and function of eNpHR3.0-eYFP in PL neurons. a, 1 sec pulse of 532-nm light elicits robust outward current in vitro. b, Left: injected current steps (500 ms, +200 pA steps) reliably evoked action potentials in PL neurons. Right: in the same neuron, eNpHR3.0 activation inhibited evoked action potential. Green bar denotes time of light activation. c, A linear relationship was observed between eNpHR3.0-mediated currents and membrane hyperpolarization. Data are from 7 neurons from 3 rats.
Supplementary Table 1. No differences in action potential waveform in compulsive cocaine rats versus controls (all $P > 0.05$ across groups). In particular, lack of changes in action potential threshold and height suggest that there were no changes in Na$^+$ channel activation$^{1,2}$.

<table>
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Supplementary references


