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Reversing compulsive cocaine seeking with low frequency deep brain stimulation of the subthalamic nucleus

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TEXT

Addictive individuals continue to seek and take drugs of abuse despite obvious negative consequences for their personal, professional and social life.[1] Although significant advances have been made in our understanding of brain mechanisms leading to compulsive drug seeking/taking, there is still no FDA-approved pharmacological treatment for cocaine addiction. Resurgence of surgical techniques such as deep brain stimulation (DBS) for the treatment of neurodegenerative and psychiatric diseases, including Parkinson's disease (PD), obsessive compulsive disorders (OCD) and depression,[2–4] has recently opened new therapeutic perspectives for the treatment of addictive disorders. Although the ideal brain target is still a matter of debate,[5–7] the subthalamic nucleus (STN) stands as a solid candidate since its 130 Hz DBS reduces drug-induced addiction-like behaviors.[8–10] Here we used a rat model of compulsive cocaine seeking[11,12] in which, after extended cocaine taking history, a subset of rats compulsively seek cocaine despite intermittent punishment by mild electric foot shock. We show that this pathological behavior is predicted by an abnormal increase in STN low frequency oscillations power, especially in the theta band (6-12 Hz), during prolonged cocaine consumption. Conversely, very low frequency (8 Hz) stimulation of the STN of 'shock-sensitive' rats during extended cocaine access triggers compulsive drug seeking upon subsequent re-exposure to the punishment, demonstrating that abnormal very low frequency oscillatory activity within the STN is causally involved in the emergence of compulsive cocaine seeking and represents a predictive marker of pathological drug seeking. Finally, we also demonstrate that 30 Hz, but not 130 Hz, STN DBS has a beneficial effect at reducing compulsive cocaine seeking in 'shock-resistant' animals. By evidencing a frequency-dependent bidirectional control of STN DBS on compulsive cocaine seeking, our results outline the critical contribution of the STN to the onset and maintenance of pathological cocaine seeking behaviors and further emphasize the therapeutic potential of STN DBS for the treatment of addiction.

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Whereas the majority of cocaine users quit as they experience the negative consequences of drug use, some lose control over their drug taking and compulsively seek drugs. Identifying possible predictive biomarkers of compulsive drug use thus remains a key challenge for both the prevention and the treatment of addiction. Here we used a rat model of addiction in which a subset of animals with an extended cocaine self-administration history keep seeking drug despite intermittent punishment by foot shock.[11,12] Following training in the cocaine seeking-taking chained schedule of reinforcement, consisting in 10-daily seeking cycles (2 h-sessions), which completion (see online methods for details) triggered access to the cocaine taking lever, animals were subjected to 6 h-daily access to cocaine for 15 days, allowing them to lose control over drug intake.[13] Then, after five sessions of baseline seeking, animals experienced eight daily sessions of punishment in which 50% of the cycles ended with delivery of a mild foot shock (0.5 mA, 0.5 sec) with no access to the taking lever (Fig. 1a). Punishment exposure revealed a small population of rats that kept seeking the drug despite the foot shock (Fig. 1b,c), as previously reported.[11,12,14,15] Indeed, the compulsivity score (*i.e.* averaged number of completed seeking cycles during the last four punishment sessions) identified ~1/3 of the animals as 'shock-resistant' or 'compulsive': they completed ≥30% of the 10-daily seeking cycles, in contrast to 'shock-sensitive' and control (no cocaine escalation, Extended data Fig. 1) animals which displayed a lower compulsivity score (Extended data Fig. 2a). Remarkably, 'shock-resistant' and 'shock-sensitive' rats could not be differentiated nor predicted in terms of cocaine intake during escalation, basal cocaine seeking (number of cycles completed or lever presses on the last baseline session) and there was no correlation between number of lever presses before exposure to punishment and compulsivity score (Fig. 1b,c; Extended data Fig. 2b-d). During seeking sessions, animals were independently given the opportunity to nosepoke for an unpunished sucrose reward. Importantly, sucrose seeking was not altered during punished cocaine seeking sessions in both 'sensitive' and 'resistant' populations (Extended data Fig. 1e), indicating that the effect of punishment was specific to the punished seeking response and did not reflect a general suppression of responding.

The STN has recently received much attention in compulsive-like behaviors, including addiction, since its high frequency (~130 Hz) DBS reduces the motivation to take cocaine.[9] It also prevents both escalation of cocaine intake and re-escalation of cocaine[8] or heroin[10] intake, normally observed after protracted abstinence. Furthermore, development of cocaine escalation induces a

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pathological increase in STN low frequency oscillations, measured with local field potentials (LFPs), suggesting a key contribution of the STN oscillatory activity to the development of loss of control over drug intake.[8] Thus, as cocaine escalation is a prerequisite to the emergence of compulsive drug seeking (Fig. 1a, Extended data Fig. 1), we hypothesized that STN oscillatory activity might also be involved in the onset of this behavior. To address this issue, we recorded LFPs, through DBS electrodes implanted in the STN, during the cocaine escalation protocol (15-min before and after cocaine access on days 1, 4, 8, 12 and 15, Fig. 2a). Following the punishment sessions and the characterization of rats' compulsivity status (Fig. 2b), analysis of STN LFPs power spectrum revealed two distinct patterns of activity during the development of cocaine escalation: 'shock-resistant' animals exhibited a progressive increase in STN low (6-40 Hz), but not high (65-90Hz), frequency oscillation powers during LFPs baseline recordings (*i.e*. before cocaine access) that was not observed in 'shock-sensitive' rats (Fig. 2c, Extended data Fig. 3a). Band-specific analysis revealed significant power increases of STN oscillations in alpha/theta (6-13Hz) and low/high beta (14-40Hz), but not in the gamma (65-90Hz), bands during escalation of cocaine intake in 'shock-resistant' rats (Fig. 2d-f). Interestingly, these pathological increases were no longer present after cocaine self-administration (Fig. 2d-f, Extended data Fig. 3b), in line with the ability of dopaminergic agonist treatment to reduce STN pathological oscillations in both PD monkeys and patients.[16–18] Rather than reflecting an enhanced craving following the daily 18 h-abstinence period experienced by all animals during the escalation protocol, these pathological increases of low frequency oscillation power could represent a predictive biomarker of compulsivity. In agreement with this, the STN alpha/theta (6-13 Hz) oscillatory activity has also been shown to correlate with symptom severity in OCD patients.[19,20]

We next tested the causal role of increased STN low frequency oscillation on the emergence of compulsive cocaine seeking by applying an artificial alpha/theta or gamma stimulation in the STN of 'shock-sensitive' animals during cocaine escalation. Following the determination of their seeking phenotype during the initial cocaine escalation-punishment protocol sequence (Fig. 3a), 'shock-sensitive' rats (Fig. 3b, Extended data Fig. 4a) were stimulated within the STN (0 Hz, 8 Hz or 70 Hz), during a second escalation of cocaine intake (escalation-2) before being re-exposed to the punishment protocol (punishment-2, Fig. 3a). STN DBS at 8 Hz or 70 Hz had no effect on cocaine intake during escalation-2 (Fig. 3b), indicating that only STN 130 Hz DBS can reduce re-escalation of drug intake,[8,10] and did not change the rats' pattern of cocaine consumption observed during

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escalation-1 (Extended data Fig. 4b). Likewise, there was no change in their level of cocaine seeking during baseline-2, compared to baseline-1 in the punishment protocol (Extended data Fig. 4c,d). However, when punishment was re-introduced, 8 Hz-stimulated rats became resistant to punishment (Fig. 3d), as evidenced by the switch in their compulsivity score (Fig. 3e), in stark contrast to control (0 Hz) and 70 Hz-stimulated animals that quickly stopped seeking cocaine. Of note, sucrose seeking levels were not altered in any groups between both punishment protocols (Extended data Fig. 4e). Although recent evidences revealed the role of STN in neuronal pain processing,[21] the resistance to the foot-shock induced by the STN 8 Hz stimulation did not reflect changes in pain perception, since such treatment (6 h/day, 14 days) of 'shock-sensitive' animals did not affect nociceptive responses on a hot-plate (Extended data Fig. 5). Thus, by specifically manipulating the STN alpha/theta band activity during cocaine escalation, we switched the compulsivity status of 'shock-sensitive' rats towards 'shock-resistant' phenotypes, thereby confirming that pathological increase in STN low frequency oscillation powers during escalation represents a causal predictive biomarker of compulsive cocaine seeking trait. Such marker may be of critical importance for the development of new preventive strategies and diagnostic tools in humans. Indeed, future investigations aiming at detecting cortical correlates of STN abnormal activities, as in PD,[22] may help to detect, in a non-invasive fashion (*e.g.* electroencephalography), vulnerable subjects that could develop pathological seeking despite its negative consequences.

In light of the frequency-dependent beneficial effects of STN DBS in PD and OCD patients,[2,3,20,23] we tested the ability of STN high (130 Hz) and low (30 Hz) DBS to affect punished cocaine seeking. Following the characterization of their compulsive status, half of the animals were then stimulated at 130 Hz-stimulation, while the other half at 30 Hz-stimulation (*i.e*. ON) in the STN during five punishment sessions. All animals then underwent five punishment sessions with no stimulation (*i.e*. OFF) before being subjected to another series of five-ON sessions at the other frequency. These sessions were followed by five-OFF sessions (Fig. 4a). Although 130 Hz STN stimulation acutely worsened cocaine compulsive seeking of 'shock-resistant' rats on the first two days of stimulation (Fig. 4b), it had no long-lasting effect on cocaine seeking in both groups (Fig. 4c,d). In contrast, 30 Hz STN stimulation progressively decreased pathological cocaine seeking of 'shock-resistant' animals, without affecting the behavior of 'shock-sensitive' rats (Fig. 4e-g). This effect was transiently reversed during the OFF-period, highlighting the reversibility of the DBS procedure.

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Interestingly, 30 Hz-STN had no noticeable side effects: it did not promote compensatory sucrose seeking (Extended data Fig. 6) and did not affect animals' locomotor activity (Extended data Fig. 7) nor nociceptive responses (Extended data Fig. 8).

Here, we uncover a STN DBS bi-directional control over compulsive cocaine seeking (Extended data Fig. 9), which is frequency (8 Hz *vs.* 30 Hz)- and addiction stage (escalation *vs*. punished seeking)-dependent. Our data also emphasize the necessity to correctly apply STN DBS to avoid deleterious effects, as previously observed in PD patients.[23] Indeed, 8 Hz STN DBS, which does not affect cocaine re-escalation, triggers compulsive cocaine seeking in non-compulsive animals. Likewise, 130 Hz STN DBS, which reduces cocaine escalation and relapse,[8] acutely increases pathological seeking of compulsive rats. Mechanisms sustaining these beneficial and deleterious effects remain to be elucidated. However, as in PD,[24,25] STN pathological oscillations may directly alter prefrontal neuron activity, which normally drives decision-making and reward seeking,[26] via antidromic propagation along the hyperdirect pathway.[27] Indeed, following cocaine escalation and punished cocaine seeking, the prefrontal cortex of 'shock-resistant' animals is hypoactive.[11] In these animals, 30 Hz STN DBS might thus antidromically 're-boost' prefrontal neuron activity to reduce compulsive cocaine seeking, as observed with local low frequency photostimulation.[11] To date, there is no FDA-approved pharmacological treatment for patients suffering from addictive disorders. We evidence here the STN critical contribution to the onset and maintenance of compulsive-like behaviors and further demonstrate the therapeutic potential of STN DBS for addiction. Given its efficiency and safety in other pathologies, our present and previous works[8,9] may shape the framework of a STN DBS-based therapeutic sequence, where specific frequencies would be applied at precise addiction stages to reduce or reverse addiction criteria in order to normalize pathological seeking and consummatory behaviors toward a more recreational/controlled pattern of use.

METHODS

Animals. Adult Lister Hooded males (~380 g, Charles River, N = 74) were paired housed, in Plexiglas cages and maintained on an inverted 12h light/dark cycle (light onset at 7 pm) with food and water available *ad libitum*, in a temperature- and humidity-controlled environment. All animal care and use conformed to the French regulation (Decree 2010-118) and were approved by local ethic committee and the University of Aix-Marseille (#3129.01).

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Electrode design for STN DBS or LFP recordings. The electrodes were made out of Platinum-Iridium wires coated with Teflon (75 µm). Coating was removed over 0.5 mm at the tips and two wires were inserted into a 16 mm stainless steel tubing to form an electrode. Two electrodes, separated by a distance of 4.8 mm (*i.e.* twice the STN laterality), were soldered to an electric connector, allowing connection with both recording and stimulation devices. Electrodes (impedance = 20 kΩ ± 2.25) and connector were subsequently deeply bound, using a custom mold and dental cement. Finally, electrodes were tested with an isolated battery to avoid electrical short circuits.

Catheter and stereotaxic surgeries. Rats were implanted with a chronically indwelling intravenous catheter, as previously described.[8,9] Briefly, rats were anesthetized with ketamine (Imalgen, Merial, 100 mg/kg, *s.c.*) and medetominine (Domitor, Janssen, 30 mg/kg, s.c.) following a preventive long-acting antibiotic treatment (amoxicillin, Duphamox LA, Pfizer, 100 mg/kg, *s.c.*). A homemade silicone catheter (0.012-inch inside diameter, 0.025-inch outside diameter, Plastics-One) was inserted and secured into the right jugular vein. The other extremity of the catheter was placed subcutaneously in the mid-scapular region and connected to a guide cannula secured with dental cement. Animals were then placed in a stereotaxic frame (David Kopf apparatus) and maintained under ketamine/medetominine anesthesia. Electrodes were inserted bilaterally in the STN (in mm:[28] -3.7 AP, ±2.4 L from bregma, -8.35 DV from skull surface, with the incisor bar at -3.3 mm). Four anchoring screws (the one on the right frontal lobe designated as the electric reference allowing LFP recordings in some animals) were fixed into the skull. Electrodes, screws and skull were deeply bounded with dental cement. After surgery, rats were awakened with an injection of atipamezol (Antisedan, Janssen, 0.15 mg/kg *i.m.*) and allowed to recover for at least 7 days with *ad libitum* access to food and water. The catheters were daily flushed during the recovery period and just before and after each self-administration session with a saline solution containing heparin (Sanofi, 3 g/l) and enroflorilexine (Baytril, Bayer, 8g/L) to maintain their patency and to reduce infection. Catheters were also regularly tested with propofol (Propovet, Abbott, 10 mg/ml) to confirm their patency.

Behavioral apparatus.

Self-administration apparatus: behavioral experiments were performed during the dark phase and took place in standard rat operant chambers (MedAssociates), located in sound-attenuating cubicles, equipped with a house light, two retractable levers, which flanked a sucrose magazine, set 7 cm above the metallic grid floor through which an electric foot shock could be delivered via a generator

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(MedAssociates). A cue light was positioned 8 cm above each lever. For each rat, one lever was randomly paired with cocaine infusion (taking-lever) while the other one was designated as the seeking-lever. For intravenous drug administration, the stainless-steel guide cannula of the catheter was connected through steel-protected tygon tubing to a swivel (Plastics One) and then an infusion pump (MedAssociates). Data were acquired on a PC running MED-PC IV (MedAssociates). Sessions lasted for 2 h or 6 h (see below for detailed procedures).

Locomotor activity apparatus: Locomotor activity was measured as the distance traveled (in meters) in a circular home-made Perspex open field (60 cm diameter). A video tracking system was placed above the open field. Data were acquired by the software Bonsai (Open Ephys), recorded on a PC computer and analyzed offline with Matlab.

Hot plate apparatus: Animals were placed on a hot plate analgesia meter (Harvard apparatus) maintained at 52.0 ± 0.5 °C. Rats were constantly observed during the test to detect the first sign of pain (paw licking, rapid movements, escape…) to quickly remove them from the apparatus. Animal's behavior was also recorded by a video tracking system. Data were acquired by the software Bonsai (Open Ephys). Latency to react was quantified offline by an experimenter blind to the DBS treatment.

Acquisition of cocaine self-administration under the seek-take task schedule. At least one week after surgery, rats began cocaine self-administration training using the seek-take chain schedule, adapted from the previously described procedure.[12] Self-administration training was divided into four distinct phases: acquisition of the taking response; training on the seek-take chain; extended self-administration and punishment.

Acquisition of the taking response. In this initial phase, each trial started with the illumination of the house light and the insertion of the taking-lever. One press on the lever, under a fixed ratio schedule (FR-1), resulted in the delivery of a single infusion of cocaine (250 µg/90 µL over 5 s, Coopérative pharmaceutique française). Cocaine infusions were paired with illumination of the cue light (5 s) above the taking lever, retraction of the taking-lever and extinction of the house light. Following a 20 s time out-period, another trial was initiated with the insertion of the taking-lever. Training of the taking response continued, typically for six to eight sessions, until animals reached a stable level of cocaine intake (< 20% changes across 3 consecutive sessions), after which they advanced to the seeking-taking chain schedule.

Training on the seeking-taking chain schedule. Each cycle started with the illumination of the

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house light, insertion of the seeking-lever and retraction of the taking-lever. A single press on the seeking-lever resulted in the retraction of the seeking-lever and the insertion of the taking-lever, which activation then triggered cocaine delivery, illumination of the associated cue-light and retraction of the taking-lever and extinction of the house light. Following a 20 s time-out refractory period, another cycle was initiated with the insertion of the seeking-lever.

Once animals reached a stable level (< 20% variation in number of cycles completed across 3 consecutive sessions), a random interval (RI) schedule was introduced into the seeking-link of the chain schedule. Here, the first seeking-lever press initiated a RI schedule of 2 s, which progressively increased to 15, 30, 60 and 120 s during training. Seeking-lever presses within the RI had no programmed consequences. The first seeking-lever press following the end of the RI lead to the retraction of the seeking-lever and the insertion of the taking-lever, which press triggered the cocaine delivery, paired with the illumination of the associated cue-light as during the training of the taking response, thereby ending the seeking-taking cycle. Each cycle was followed by a 2 min time-out (TO) period, where both levers were retracted, which progressively increases to 4 and 10 min over consecutive training days, before initiation of the next seek-take cycle. Training on each RI-TO schedule persisted until animals displayed less than 20% variation in the numbers of cycle completed. Once an animal achieved stable cocaine seeking behavior at a RI-TO schedule, usually 5-7 days, it was advanced to the next RI-TO schedule.

During these sessions, rats were also trained to nose poke into the sucrose magazine to obtain 0.04 ml of a 20% sucrose solution, which was delivered under a RI schedule, which parameter was progressively increased to 60 s. Sucrose seeking-taking behavior occurred concurrently and independently to the cocaine seeking-taking schedule, thereby allowing us to specifically investigating 'natural' reward seeking.

At the end of training under the seeking-taking schedule, animals were allowed to complete up to 10 cocaine cycles and 120 sucrose deliveries in each 2 h session of the RI120-TO10 schedule.

Extended cocaine self-administration. After reaching the RI120-TO10 schedule criteria of the seeking-taking schedule, all animals (except those Extended data Figs. 1 and 6, which underwent punishment protocol right after completed RI120-TO10 schedule), were submitted to escalation protocol, in which they were allowed to self-administer cocaine over 6 h for 15 consecutive days to loss control over their cocaine intake,[13] a cardinal criteria of human drug addiction.[1] Here, sessions

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started with the illumination of the house light and the insertion of the taking-lever. As in the training phase, each press on this lever triggered cocaine delivery and the illumination of the associated cue-light, followed by a 20 s TO. Sucrose seeking was unavailable during extended cocaine self-administration sessions. After 15 days, animals were re-subjected to the seek-take RI120-TO10 schedule, as described above, during five days to establish post-escalation cocaine seek-take baseline.

Punishment. After RI120-TO10 baseline, animals were subjected to 8 daily sessions under the resistance to punishment paradigm.[12] Here, half of the completed RI120-TO10 cycles resulted in randomly (50%) mild foot shock delivery (0.5 mA, 0.5 s) to the animals' paws: punishment was administered following the first press on the seeking-lever after the RI120 schedule has elapsed, with no access to the taking lever. The other half of the RI120 completed triggered the insertion of the taking lever, which press initiated cocaine delivery and illumination of the associated cue-light. Thus, animals randomly received up to five foot-shocks and five cocaine infusions. Compulsivity score was defined as the average number of completed cycles (foot-shocks + cocaine deliveries) during the last four sessions of the punishment paradigm. Animals with a compulsivity score > 2.5 (*i.e.*, completed more than 25% of the 10-daily seeking cycles during the last four sessions of the punishment paradigm) were classified as 'compulsive' or 'shock-resistant'.

Spontaneous- and cocaine-induced locomotor activity measurement. Treatment order (saline-OFF, cocaine-OFF, saline-ON, cocaine-ON) was counterbalanced between animals with a seven-day period between each treatment. Animals were first placed in the open field and connected to the stimulation for a 60 min period of habituation. They were then injected with either saline (0.9% NaCl, 1 ml/kg) or a low dose of cocaine (5 mg/kg, *s.c.*) and their locomotor activity was recorded for 60 h with DBS ON or OFF.

Hot plate analgesia measurement. Basal nociceptive response was determined before the first STN DBS session. Then, animals were subjected to 6 h-STN DBS (at 8 Hz) for 14 consecutive days accordingly with the procedure used to test the effects of this frequency applied during the escalation procedure. Their nociceptive responses were tested after the first and the last sessions of STN 8 Hz DBS (Extended data Fig. 5). In another set of animals, the effects of 2 h-STN DBS (at 0, 30 or 130 Hz) for 5 consecutive days accordingly with the procedure used to test the effects of these frequencies on the compulsivity measures were tested on nociceptive responses after the first and the last sessions of

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STN DBS (Extended data Fig. 8).

Recording and analysis of STN LFP activity. LFP recordings were performed in similar operant chambers as above with no access to cocaine. They were further equipped with wires connected to the acquisition setup. Animals were connected to the interface and placed in the chamber where they could freely move. STN electric activity was recorded 15-min before and after extended cocaine access on days 1, 4, 8, 12 and 15 of the escalation protocol.

Signals were amplified and filtered using a Neuralynx8 amplifier. Data were acquired using Sciworks software (Datawave Tech, USA) with a sampling rate of 1kHz in the range of 1- 475Hz. Signals were filtered off-line with a Chebyshev low pass filter (corner 98Hz, order 10, ripple 0.5) and a notch filter were applied, to remove 50Hz noise created by surrounding electrical devices, using Spike2 software (CED). Data were then carefully examined to ensure removal of electrical noise the specific activity of the STN (*i.e.* difference of potential between the two wires within the same STN) was then calculated and ultimately treated using Matlab (Mathworks) software. As such, the analysis was limited to the following frequency bands: 4-40 Hz and 65-90 Hz.

Deep brain stimulation. DBS was delivered to the STN by a digital stimulator (DS8000, WPI) via a stimulus isolator (DLS100, WPI) and a rotating commutator (Plastics-One) wired to the implanted electrodes. Stimulation parameters were adapted from previous studies.[8,9] Briefly, individual stimulation intensity parameters was determine using 130 Hz frequency and 80 µs pulse width stimulation. Intensity was progressively increased until the appearance of hyperkinetic movements. Stimulation intensity (50-150 µA) was setup just below the hyperkinetic movement threshold.

Before each behavioral session, animals were connected to the stimulation device, STN DBS was turned ON and stimulation intensity was progressively increased to reach the pre-determined stimulation parameters prior the start of the session. Depending on the condition tested, 8, 30 or 130 Hz were applied during 1, 2 or 6 h.

Histology. At the end of the experiment, the rats were euthanized with an *i.p.* injection of pentobarbital (Dolethal). Brains were removed and frozen into liquid isopentane (-80°C) to be further cut in 40 µm thick frontal slices with a cryostat. Histological controls for location of the electrodes were performed after staining with cresyl violet by an observer blind to treatment group (Extended data Figs. 3c, 4f, 5b, 6c, 7c, 8b). Animals with incorrect electrode placement (n = 10), as well as those with catheter clogging (n = 4) were excluded from statistical analysis. The final n values are indicated in the figure

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legends.

Statistical analyses:

No statistical methods were used to predetermine sample size, but our samples are comparable to those reported in previous studies.[8,9,12] Group assignment was pseudo-randomized for most experiments, as rats were assigned to experimental group in a counterbalanced fashion based on their initial basal cocaine seeking levels. Treatment assignment was randomized between experimental groups. Data are expressed as mean ± s.e.m. with the exact sample size indicated for each group in figure legends. Using Prism 6.0 (GraphPad) and Matlab (Mathworks) softwares, data were analyzed two-tailed *t* test, one- or two-way repeated measures ANOVAs, followed by Bonferroni *post hoc* test when applicable. Only *P*-values < 0.05 were considered significant. Power analysis was further performed with the G*Power software to confirm that our sample sizes were sufficient to detect reliable changes for critical experiments (power ≥ 80% at a level of confidence p<0.05; values are reported in the statistical table).

REFERENCES

1. American Psychiatric Association. Diagnostic and Statistical Manual of Mental Disorders, 5th Edition. in *Diagnostic and Statistical Manual of Mental Disorders, 5th Edition* (American Psychiatric Publishing, Inc, 2013).

2. Limousin, P. *et al.* Effect of parkinsonian signs and symptoms of bilateral subthalamic nucleus stimulation. *Lancet Lond. Engl.* **345,** 91–95 (1995).

3. Mallet, L. *et al.* Subthalamic nucleus stimulation in severe obsessive-compulsive disorder. *N. Engl. J. Med.* **359,** 2121–2134 (2008).

4. Mayberg, H. S. *et al.* Deep brain stimulation for treatment-resistant depression. *Neuron* **45,** 651–660 (2005).

5. Luigjes, J. *et al.* Deep brain stimulation in addiction: a review of potential brain targets. *Mol. Psychiatry* **17,** 572–583 (2012).

6. Pelloux, Y. & Baunez, C. Deep brain stimulation for addiction: why the subthalamic nucleus should be favored. *Curr. Opin. Neurobiol.* **23,** 713–720 (2013).

7. Salling, M. C. & Martinez, D. Brain Stimulation in Addiction. *Neuropsychopharmacology* **41,**

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2798–2809 (2016).

8. Pelloux, Y. *et al.* Subthalamic nucleus high frequency stimulation prevents and reverses escalated cocaine use. *Mol. Psychiatry* (2018). doi:10.1038/s41380-018-0080-y

9. Rouaud, T. *et al.* Reducing the desire for cocaine with subthalamic nucleus deep brain stimulation. *Proc. Natl. Acad. Sci. U. S. A.* **107,** 1196–1200 (2010).

10. Wade, C. L. *et al.* High-Frequency Stimulation of the Subthalamic Nucleus Blocks Compulsive-Like Re-Escalation of Heroin Taking in Rats. *Neuropsychopharmacology* (2016). doi:10.1038/npp.2016.270

11. Chen, B. T. *et al.* Rescuing cocaine-induced prefrontal cortex hypoactivity prevents compulsive cocaine seeking. *Nature* **496,** 359–362 (2013).

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

13. Ahmed, S. H. & Koob, G. F. Transition from moderate to excessive drug intake: change in hedonic set point. *Science* **282,** 298–300 (1998).

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

15. Kasanetz, F. *et al.* Prefrontal synaptic markers of cocaine addiction-like behavior in rats. *Mol. Psychiatry* **18,** 729–737 (2013).

16. Priori, A. *et al.* Rhythm-specific pharmacological modulation of subthalamic activity in Parkinson's disease. *Exp. Neurol.* **189,** 369–379 (2004).

17. Brown, P. *et al.* Dopamine dependency of oscillations between subthalamic nucleus and pallidum in Parkinson's disease. *J. Neurosci. Off. J. Soc. Neurosci.* **21,** 1033–1038 (2001).

18. Tachibana, Y., Iwamuro, H., Kita, H., Takada, M. & Nambu, A. Subthalamo-pallidal interactions underlying parkinsonian neuronal oscillations in the primate basal ganglia. *Eur. J. Neurosci.* **34,** 1470–1484 (2011).

19. Rappel, P. *et al.* Subthalamic theta activity: a novel human subcortical biomarker for obsessive compulsive disorder. *Transl. Psychiatry* **8,** (2018).

20. Welter, M.-L. *et al.* Basal ganglia dysfunction in OCD: subthalamic neuronal activity correlates with symptoms severity and predicts high-frequency stimulation efficacy. *Transl. Psychiatry* **1,** e5 (2011).

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21. Pautrat, A. *et al.* Revealing a novel nociceptive network that links the subthalamic nucleus to pain processing. *eLife* **7,** (2018).

22. de Hemptinne, C. *et al.* Exaggerated phase-amplitude coupling in the primary motor cortex in Parkinson disease. *Proc. Natl. Acad. Sci. U. S. A.* **110,** 4780–4785 (2013).

23. Timmermann, L. *et al.* Ten-Hertz stimulation of subthalamic nucleus deteriorates motor symptoms in Parkinson's disease. *Mov. Disord. Off. J. Mov. Disord. Soc.* **19,** 1328–1333 (2004).

24. Li, Q. *et al.* Therapeutic deep brain stimulation in Parkinsonian rats directly influences motor cortex. *Neuron* **76,** 1030–1041 (2012).

25. Mallet, N. *et al.* Disrupted dopamine transmission and the emergence of exaggerated beta oscillations in subthalamic nucleus and cerebral cortex. *J. Neurosci. Off. J. Soc. Neurosci.* **28,** 4795–4806 (2008).

26. Otis, J. M. *et al.* Prefrontal cortex output circuits guide reward seeking through divergent cue encoding. *Nature* **543,** 103–107 (2017).

27. Kita, T. & Kita, H. The subthalamic nucleus is one of multiple innervation sites for long-range corticofugal axons: a single-axon tracing study in the rat. *J. Neurosci. Off. J. Soc. Neurosci.* **32,** 5990–5999 (2012).

28. Paxinos, G. & Watson, C. *The rat brain in stereotaxic coordinates*. (Elsevier, 2007).

END NOTES

Supplementary information is available on the online version of the paper.

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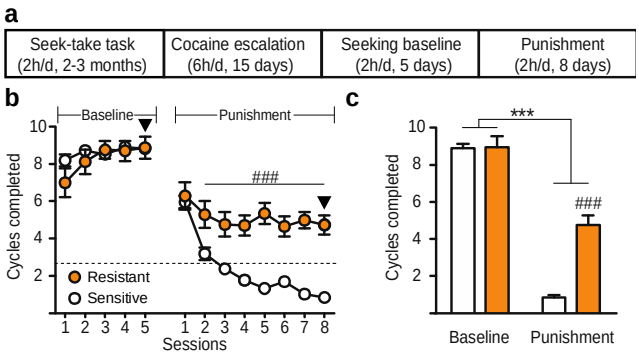

Figure 1. Extended cocaine self-administration promotes compulsive cocaine seeking in a subpopulation of rats. a, Experimental time course. Following extensive training on the seek-take task, animals were subjected to cocaine escalation protocol (Extended data Fig. 2a). After five days of baseline cocaine seeking, animals were then exposed to eight punishment sessions, during which foot shock (with no access to cocaine) was randomly delivered in 50% of the trials. Dashed line indicates compulsivity threshold below which animals are considered shock-sensitive (see methods). **b**, Punishment reduced the number of seeking cycles completed in all animals (punishment effect: $F_{(12,612)} = 117.2$, $P < 0.0001$). While both groups displayed equivalent level of cocaine seeking during baseline (group effect: $F_{(1,204)} = 0.559$, *n.s.*), shock-resistant rats (i.e. 'compulsive', $n = 17$) completed more seeking cycles than shock-sensitive rats ($n = 36$; punishment x group: $F_{(7,357)} = 7.927$, $P < 0.0001$; Bonferroni post hoc: $^{###}P < 0.001$ resistant *vs.* sensitive) during punishment. **c**, Number of seeking cycles completed during the last day of baseline and last day of punishment (arrowheads in **b**; session x group: $F_{(1,51)} = 35.63$, $P < 0.0001$; Bonferroni post hoc: $^{***}P < 0.001$ baseline *vs.* punishment, $^{###}P < 0.001$ resistant *vs.* sensitive). Line and bar graphs indicate mean ± s.e.m.

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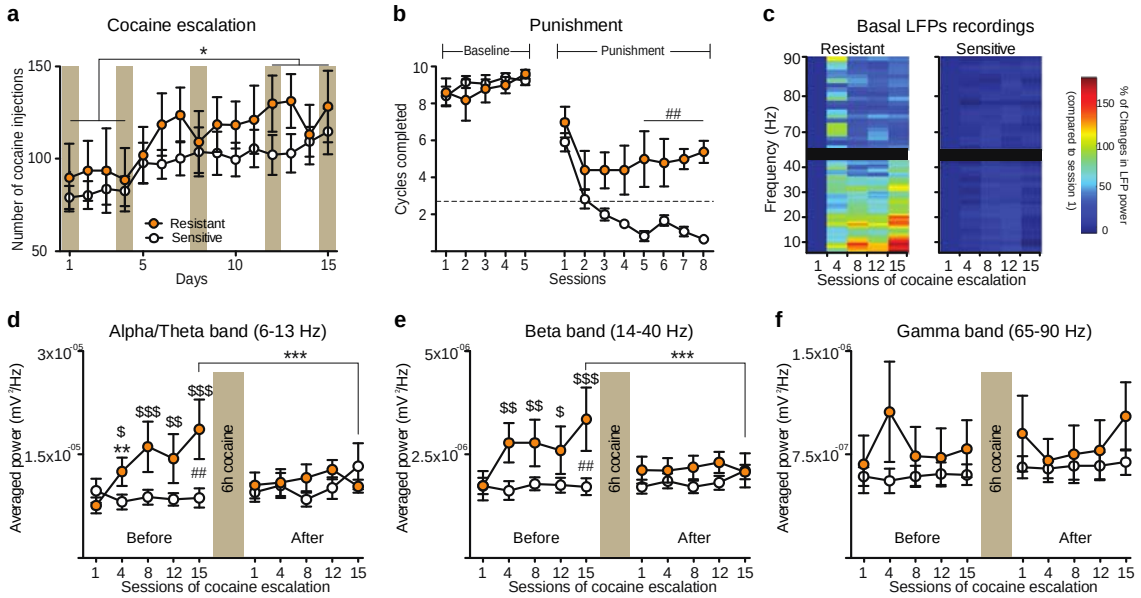

Figure 2. Compulsive rats exhibit pathological STN low frequency oscillations during cocaine escalation. a, Extended cocaine access (6 h/d, 15 d) induced similar cocaine escalation in 'future' shock-sensitive (n = 12) and -resistant (i.e. 'compulsive', n = 5) rats (session effect: $F_{(14,210)} = 6.675$, $P < 0.0001$; group effect: $F_{(1,210)} = 0.813$, *n.s.*; Bonferroni post hoc: $^{**}P < 0.01$). Brown rectangles indicate LFPs recording sessions. **b**, Punishment reduced the number of seeking cycles completed in all animals (session effect: $F_{(12,168)} = 76.12$, $P < 0.0001$). While both groups displayed equivalent level of cocaine seeking during baseline (group effect: $F_{(1,60)} = 0.204$, *n.s.*), shock-resistant rats completed more seeking cycles than shock-sensitive rats (punishment x group: $F_{(7,105)} = 3.947$, $P = 0.0007$; Bonferroni post hoc: $^{\#\#}P < 0.01$ resistant *vs.* sensitive) during punishment. Dashed line indicates compulsivity threshold below which animals are considered shock-sensitive. **c**, Session-frequency power spectrum showing basal (*i.e.* before cocaine) LFPs power changes normalized (in %) to session 1 in shock-resistant (left) and shock-sensitive (right) animals. **d-f**, Quantifications of LFPs power before and after cocaine consumption (6 h). During baseline recordings, shock-resistant rats showed a progressive power increase in alpha/theta (**d**, session effect: $F_{(4,60)} = 5.481$, $P = 0.0008$) and beta (**e**, session effect: $F_{(4,60)} = 4.119$, $P = 0.0051$; $^{\$}P < 0.05$, $^{\$\$}P < 0.01$, $^{\$\$\$}P < 0.001$ vs. session 1; $^{**}P < 0.01$ vs. session 15), but not in gamma band (**f**, session effect: $F_{(4,60)} = 0.909$, *n.s.*), which was not observed in shock-sensitive animals (group effect: **d**, $F_{(1,60)} = 5.454$, $P = 0.034$; **e**, $F_{(1,60)} = 6.035$, $P = 0.027$; Bonferroni post hoc: $^{\#\#}P < 0.01$ resistant *vs.* sensitive). Cocaine consumption reduces increased power on session 15 in both alpha/theta and beta bands (session x group effect: **d**, $F_{(9,135)} = 4.059$, $P =$

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0.0001; **e**, $F_{(9,135)}$ = 3.514, P = 0.0001; Bonferroni post hoc: ***P < 0.001) but has no effect in the gamma band (session x group effect: $F_{(9,135)}$ = 1.393, *n.s.*). Line graphs indicate mean ± s.e.m.

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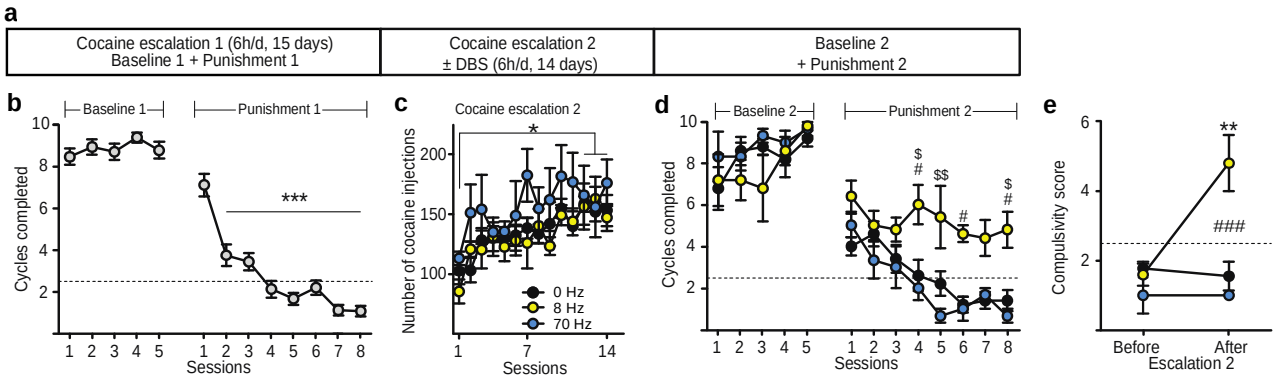

Figure 3. STN 8 Hz DBS triggers cocaine compulsive seeking in shock-sensitive rats. a, Experimental time course. Following cocaine escalation and punishment protocols and characterization of the compulsive status of the animals, shock-sensitive rats were subjected to a second escalation procedure during which some of the animals received STN DBS at 8 Hz (n = 5) or at 70 Hz (n = 3), while the others remained OFF stimulation (n = 5). They were then all tested a second time in the punishment paradigm. **b**, Shock delivery during punishment 1 abolished cocaine seeking in all shock-sensitive rats (n = 13, $F_{(12,144)}$ = 150.7 P < 0.0001; Bonferroni post hoc: ***P < 0.001, *vs.* baseline). **c**, STN DBS (black dots: 0 Hz, n = 5; yellow dots: 8 Hz, n = 5; blue dots: 70 Hz, n = 3) during cocaine escalation 2 did not alter escalation of cocaine intake (session effect: $F_{(13,130)}$ = 5.936, P < 0.0001; group effect: $F_{(2,130)}$ = 2.099, *n.s.*; Bonferroni post hoc: *P < 0.05). **d**, Punishment 2 reduced the number of seeking cycles completed in all groups (punishment effect: $F_{(12,120)}$ = 40.94, P < 0.0001). While all groups displayed equivalent level of cocaine seeking during baseline 2 (group effect: $F_{(2,40)}$ = 0.529, *n.s.*), 8 Hz-stimulated rats (yellow dots) completed more seeking cycles than 0 Hz- (black dots) and 70Hz-stimulated rats (blue dots) (punishment effect: $F_{(7,70)}$ = 9.108, P < 0.0001; group effect: $F_{(2,70)}$ = 10.48, P = 0.0035; Bonferroni post hoc: $^{\#}P$ < 0.05, 8 Hz *vs.* 0 Hz; $^{\$}P$ < 0.05, $^{\$\$}P$ < 0.01, 8 Hz *vs.* 70 Hz) during punishment 2. **e**, Compulsivity score before and after cocaine escalation 2 (before/after x group effect: $F_{(2,10)}$ = 7.705, P = 0.0094; Bonferroni post hoc: **P < 0.001, after *vs.* before, $^{\#\#\#}P$ < 0.001, 8 Hz vs. 0 / 70 Hz). Dashed lines indicate compulsivity threshold below which animals are considered shock-sensitive. Line graphs indicate mean ± s.e.m.

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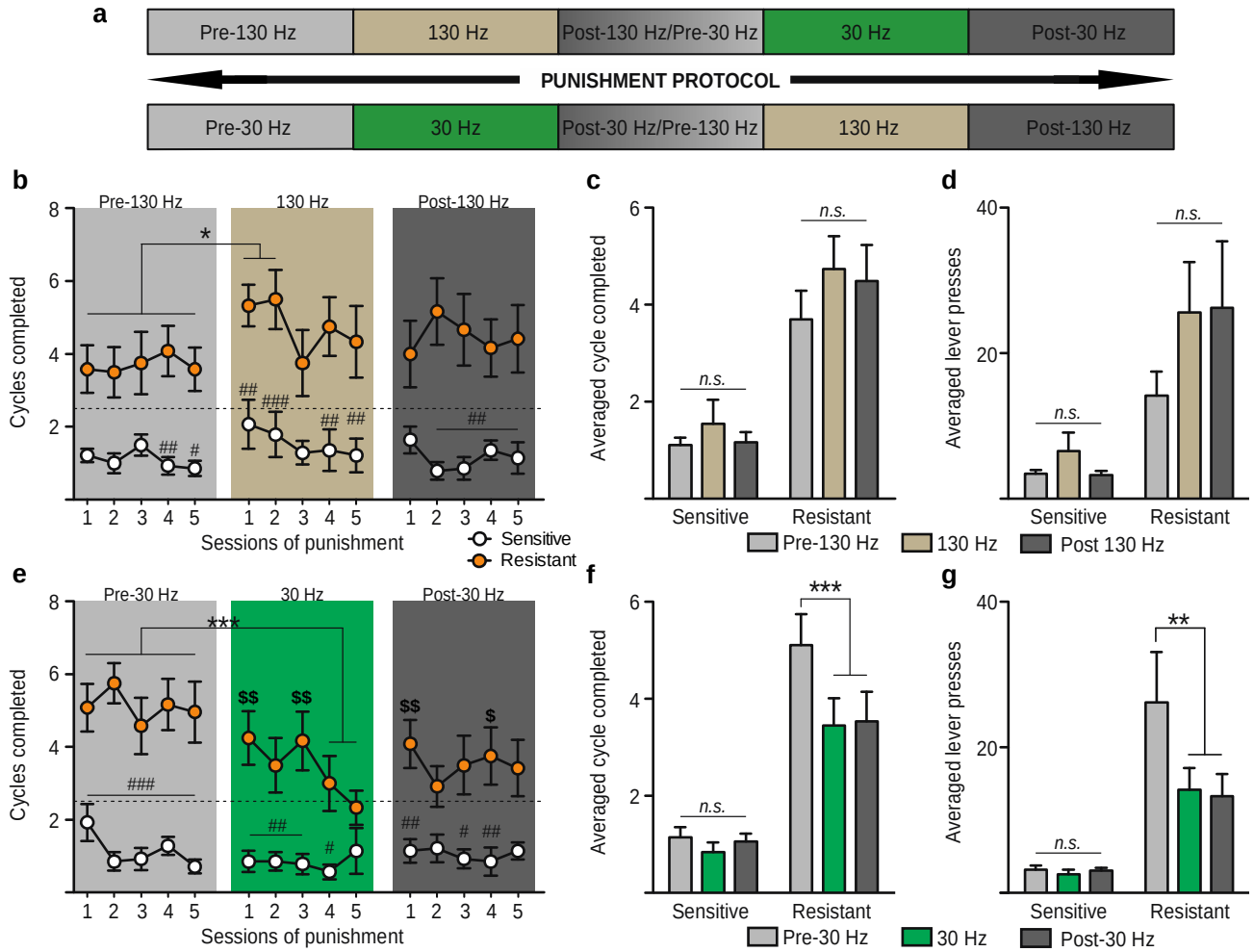

Figure 4. STN low frequency DBS reduces compulsive cocaine seeking during punishment. a, Experimental time course. Top: After five days of seeking under punishment and characterization of their compulsive status, animals were subjected to five days of 130 Hz STN DBS followed by 5 days with no DBS (Post-130 Hz/Pre-30 Hz). Animals were then stimulated for five sessions with 30 Hz DBS followed by 5 days with no DBS (Post-30 Hz). Bottom: Treatment order was counterbalanced for ~half of the rats. **b**, 130 Hz STN DBS acutely increased the number of seeking cycles completed by shock-resistant rats (orange dots; n = 12, DBS effect: $F_{(14,336)}$ = 1.764, P = 0.0427), but had no effect in shock-sensitive animals (white dots; n = 14, group effect, $F_{(1,336)}$ = 26.01, P < 0.0001; DBS x group effect: $F_{(14,336)}$ = 0.911, *n.s.*; Bonferroni post hoc: $^{*}P$ < 0.05; $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01, $^{\#\#\#}P$ < 0.001, resistant *vs*. sensitive). **c**, Averaged number of cycle completed by shock-sensitive (left) and -resistant (right) rats before (pale grey), during (brown) and after (dark grey) 130 Hz STN DBS (DBS effect: $F_{(2,48)}$ = 2.489, *n.s.*; group effect: $F_{(1,48)}$ = 26.01, P < 0.0001; DBS x group effect: $F_{(2,48)}$ = 0.677, *n.s.*). **d**,

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Averaged number of lever presses by shock-sensitive (left) and resistant (right) rats before (pale grey), during (brown) and after (dark grey) 130 Hz STN DBS (DBS effect: $F_{(2,48)}$ = 1.716, *n.s.*; group effect: $F_{(1,48)}$ = 15.48, P = 0.0006; DBS x group effect: $F_{(2,48)}$ = 1.118, *n.s.*). **e**, 30 Hz STN DBS reduced the number of seeking cycles completed by shock-resistant rats (DBS effect: $F_{(14,336)}$ = 3.494, P < 0.0001), but had no effect in shock-sensitive animals (group effect, $F_{(1,336)}$ = 33.67, P < 0.0001; DBS x group effect: $F_{(14,336)}$ = 2.848, P = 0.0005; Bonferroni post hoc: ***P < 0.001; $^{\$}P$ < 0.05, $^{\$\$}P$ < 0.01 *vs*. last session of 30 Hz DBS; $^{\#}P$ < 0.05, $^{\#\#}P$ < 0.01, $^{\#\#\#}P$ < 0.001, resistant *vs*. sensitive). **f**, Averaged number of cycle completed by shock-sensitive (left) and -resistant (right) rats before (pale grey), during (green) and after (dark grey) 30 Hz STN DBS (group effect: $F_{(1,48)}$ = 33.67, P < 0.0001; DBS effect: $F_{(2,48)}$ = 9.416, P = 0.0004; DBS x group effect: $F_{(2,48)}$ = 5.75, P = 0.0058; Bonferroni post hoc: **P < 0.01). **g**, Averaged number of lever presses by shock-sensitive (left) and -resistant (right) rats before (pale grey), during (green) and after (dark grey) 30 Hz STN DBS (group effect: $F_{(1,48)}$ = 19.1, P = 0.0002; DBS effect: $F_{(2,48)}$ = 4.996, P = 0.0107; DBS x group effect: $F_{(2,48)}$ = 4,415, P = 0.0174; Bonferroni post hoc: **P < 0.01). Dashed lines indicate compulsivity threshold below which animals are considered shock-sensitive. Line and bar graphs indicate mean ± s.e.m.

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