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Abstract

One of the key features of addiction is the escalated drug intake. The neural mechanisms involved in the transition to addiction remain to be elucidated. Since abnormal neuronal activity within the subthalamic nucleus (STN) stands as potential general neuromarker common to impulse control spectrum deficits, as observed in obsessive–compulsive disorders, the present study recorded and manipulated STN neuronal activity during the initial transition to addiction (i.e., escalation) and post-abstinence relapse (i.e., re-escalation) in rats with extended drug access. We found that low-frequency (theta and beta bands) neuronal oscillations in the STN increase with escalation of cocaine intake and that either lesion or high-frequency stimulation prevents the escalation of cocaine intake. STN–HFS also reduces re-escalation after prolonged, but not short, protracted abstinence, suggesting that STN–HFS is an effective prevention for relapse when baseline rates of self-administration have been re-established. Thus, STN dysfunctions may represent an underlying mechanism for cocaine addiction and therefore a promising target for the treatment of addiction.

Introduction

The subthalamic nucleus (STN) is a small nucleus within the basal ganglia that has been historically associated with basic motor functions. Abnormal neuronal activity within the STN, mostly increased beta oscillations (12–30 Hz), marks and drives motor symptoms of Parkinson's disease (PD) [1]. Although their exact origins remain largely unknown, these abnormal beta oscillations may be a secondary consequence of the PD-associated degeneration of midbrain dopamine (DA) neurons. Importantly, neutralizing beta activity by blocking STN activity with high-frequency stimulation (HFS) is a current effective treatment for PD [2, 3].

Over the past 15–20 years, the STN has progressively been shown also to be involved in non-motor functions, such as impulse control, encoding of reward, and motivational regulation [4–11]. As a result, abnormal STN activity has been reinterpreted as a general neuromarker common to impulse control spectrum deficits, as observed in obsessive–compulsive disorders (OCD) [6]. Since addiction is a form of impulse control spectrum disorder, the present study aimed first at identifying these possible abnormal activities within the STN during the transition to addiction. Previous research has shown that extended, as opposed to limited, access to drugs of abuse causes rats to develop several core addiction-like behavioral symptoms, including escalated drug intake and increased drug motivation despite the negative consequences [12, 13].

Since STN inactivation reduces the initial motivation to self-administer cocaine in individuals that have limited access to the drug, while simultaneously increasing the motivation for sweet food [14, 15], targeting STN has been proposed as potential therapeutic strategy for cocaine addiction [16]. Whether and to what extent STN inactivation can affect the transition to more compulsive cocaine self-administration remains to be demonstrated. Here, we thus tested the effects of STN lesions or HFS during both the initial transition to addiction (i.e., escalation of cocaine use) and re-escalation after protracted abstinence.

Materials and methods

Animals

Adult Lister Hooded males (~380 g, Charles River) were housed in groups of two in Plexiglas cages and maintained on an inverted 12 h light/dark cycle (light onset at 7 pm) with food and water available ad libidum, in a temperature-and humidity-controlled environment. 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 under #03129.01.

Surgery

As previously described [14, 15], animals were anesthetized with ketamine (Imalgen, Merial, 100 mg/kg, s.c.) and medetominine (Domitor, Janssen, 30 mg/kg, s.c.) to first implant homemade silicone catheter in the right jugular vein and were then placed in a Kopf stereotaxic apparatus for either lesion or electrode implantation surgery. Animals received a bilateral microinjection of ibotenic acid (9.4 μg/μl; 0.5 μl injected; $n = 33$) or vehicle

solution (PB; 0.1 M, $n = 27$) in the STN (AP: −3.7 mm; L: ±2.4 mm from bregma; DV: −8.35 mm from skull) [17]. Ninety-seven other animals were implanted with electrodes at these coordinates. Electrodes and four anchoring screws (one on the right frontal lobe designated as the electric reference allowing LFP recordings in some animals) were secured with dental cement.

After surgery, all rats were awakened with an injection of atipamezol (Antisedan, Janssen, 0.15 mg/kg, i.m.). They were allowed to recover for 10 days. Catheters were daily checked for blood reflux and flushed with heparin (Sanofi, 3 g/l) and enroflorilexine (Baytril, Bayer, 8 g/l) in 0.9% saline to prevent blood clots and infection during the recovery period and until the end of experiments. Catheters were also regularly tested with propofol (Propovet, Abbott, 10 mg/ml) to confirm their patency.

Self-administration paradigms

The classical self-administration chambers used are described in Supplementary materials. Each session started with the illumination of the house light and the delivery of a free infusion (250 μg/90 μl) of hydrochloride cocaine (Coopérative pharmaceutique française, dissolved in 0.9% NaCl). A continuous fixed-ratio 1 schedule of reinforcement (FR1) was used: a single nose poke in the active hole (randomized assignment) resulted in a 5-s illumination of the associated cue-light and cocaine delivery, that initiated a time-out period of 20 s during which nose pokes in the active hole were counted as perseverative responses. Alternatively, nose poke in the inactive hole had no programmed consequence. After acquisition of a stable baseline of cocaine consumption over the 2 h-short access (ShA) sessions, they were given daily 6 h-long access (LgA) to cocaine for 15 or 20 days.

One group of animals ($n = 13$ STN lesion and 11 sham) was tested during this procedure for preventive effect of the lesion on escalation. In parallel, another group ($n = 12$ ON and 12 OFF) was tested with STN–HFS.

An additional group of animals was only tested with STN–HFS ($n = 10$ ON and 8 OFF) applied during 15 ShA sessions to test the specificity of STN–HFS effects on escalation process.

In another experiment ($n = 16$), STN lesion ($n = 8$) was performed after rats had escalated their cocaine intake. They were then tested for ten LgA sessions after a post-surgical recovery period of 10 days.

In parallel, in a group of animals that had escalated their drug intake during 15 sessions of LgA ($n = 16$), STN–HFS was applied immediately from the 16th session for 15 consecutive sessions ($n = 10$) or remained OFF ($n = 6$).

Finally, in a last experiment, STN–HFS was tested on re-escalation in animals that had escalated their drug intake. After the 15 LgA session establishing escalation, a 35 days period of abstinence was observed before the animals were re-exposed to the drug for 10 LgA sessions under STN–HFS (ON, $n = 8$) or not (OFF, $n = 8$).

Recording and analysis of LFP activity

Bilateral bipolar platinum electrodes were used for LFP recordings and HFS (see Supplementary methods for details).

Animals ($n = 8$) were connected to the recording interface and placed in the operant chamber. LFPs were recorded for ten sessions of the ShA and for ten sessions (days 2, 3, 4, 6, 7, 8, 9, 11, 12, and 13) over the LgA. STN electric activity was recorded for 15 min before any access to cocaine and then during the first 30 min of the self-administration session. After the recording, the animals were immediately transferred to another operant chamber to complete their self-administration session.

In a separate experiment ($n = 9$), LFPs were recorded for 15 min before and after a single 6 h STN–HFS session (equivalent to an LgA session) with no access to cocaine. Additional recordings (15 min) were also performed 2 and 4 days after this HFS session.

Signals were amplified and filtered using a Neuralinx8 amplifier. Data were acquired using Sciworks software (Datawave Tech, USA) with a sampling rate of 1 kHz in the range of 1–475 Hz. Signals were filtered off-line with a Chebyshev low-pass filter (corner 98 Hz, order 10, ripple 0.5) and a notch filter was applied, to remove 50 Hz noise created by surrounding electrical devices, using Spike2 software (CED). Data were then carefully examined to ensure removal of electrical noise and ultimately treated using Matlab (Mathworks) software. As such, the analysis was limited to the following frequency bands: 5–45 Hz and 65–95 Hz. The specific activity of the STN, i.e., the difference of potential between the two wires within the same STN, was then calculated.

Correlational analysis

Relationships between behavioral performances during ShA or LgA (i.e., number of cocaine injections) and STN oscillatory power in the theta, beta, and gamma bands were assessed by plotting for each individual STN LFP powers and the number of cocaine injections taken either the day before each recording session ($n = 9$ for ShA and LgA) for the baseline recordings (i.e., 15 min before cocaine intake) or during the first 30 min of the self-administration sessions ($n = 10$ for ShA and LgA) for recordings during cocaine consumption. Power data used in these analyses were derived from all ten recording sessions during ShA and for ten sessions of LgA on days 2, 3, 4, 6, 7, 8, 9, 11, 12, and 13.

High-frequency deep brain stimulation

HFS 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 (see supplementary methods). Stimulation parameters were adapted from previous studies [15, 18]. Briefly, stimulation parameters were set with frequency at 130 Hz and 80 μs pulse width. Stimulation intensity (50–150 μA) was individually adjusted below the threshold of induction of hyperkinetic movements. STN–HFS was turned ON just prior the start of each behavioral session and applied during the entire session.

Histology

At the end of the experiment, the rats were killed with pentobarbital (Dolethal; i.p.). Brains were removed and frozen into liquid isopentane (−80 °C) to be further cut in 50-μm-thick frontal slices with a cryostat in a blinded manner. Histological controls, for extend of the lesions and position of the electrodes, were performed after staining with cresyl violet (Supplementary Figure S1). Out of 33 animals tested for STN lesions, ten were discarded from final analysis for missed lesion (no shrinkage and gliosis reaction within the STN). Eleven animals were excluded from the LFP or STN–HFS experiments for mislocation of the electrodes. The final *n* values after histological verification and exclusion of some animals due to catheter patency issue ($n = 18$) or head cap loss ($n = 3$) are indicated in the figure legends.

Statistical analyses

No statistical methods were used to predetermine sample size, but the samples are comparable to those reported in previous studies [14, 15, 19, 20]. However, power analysis (two groups, two tails, and equal variance model) was performed to confirm that our sample sizes were sufficient to detect reliable changes in escalation (power 80% at a level of confidence $P < 0.05$). Group assignment was mostly done in a random fashion, except for re-escalation procedure (Fig. 5), where rats were assigned to treatment group in a counterbalanced manner based on their first escalation level. Data are expressed as mean ± SEM with the sample size indicated for each group. Analyses were performed with Prism (GraphPad) and Matlab (Mathworks) softwares. Only *P* values <0.05 were considered significant. Most data were analyzed with two-tailed *t*-test or one- or two-way ANOVA with group (sham or STN lesion; HFS ON or OFF) as a between factor and sessions, and/or blocks of sessions, or time bins (LFP and locomotor activity experiments) as repeated measures, followed by Bonferroni post hoc test or Student's *t* test. Correlations between LFPs power and the number of cocaine injections were statistically validated with ANCOVA controlling for inter-individual differences, since rats were treated as a covariate.

Results

Oscillations within the STN during the process of escalation

To monitor STN oscillations during the development of cocaine escalation, rats implanted with bipolar HFS electrodes were trained to self-administer cocaine under an FR1 schedule of reinforcement with daily ShA to cocaine for 10 sessions and then for 15 LgA sessions (Fig. 1a). As expected, escalation in the drug intake was observed during LgA sessions (i.e., significant increased number of cocaine injections during the first 30 min spent in the electrophysiological recording chamber) (Fig. 1b).

Baseline activity (before access to cocaine): STN low-frequency oscillations as possible neuromarkers of reduced control over cocaine use?

LFPs recordings started 15 min before the self-administration sessions, allowing measure of the baseline activity in absence of cocaine (Fig. 1a). There were few variations in STN baseline oscillations recorded before the first ShA sessions, when the animals were naive to

cocaine. However, over the course of the experiment, the oscillatory activity significantly increased within the STN during the LgA phase to reach a plateau, in parallel with the increased cocaine self-administration (escalation). Indeed, in absence of cocaine, during the baseline recording, the oscillations in low frequencies increased progressively as a function of the sessions (Supplementary Figure S2), and this increase was even more striking in comparison to the first day of ShA, when the rats had no experience with cocaine (Fig. 1c). Of note, variations in STN oscillatory activities were only observed in low-frequency oscillations. Indeed, specific band analysis revealed a significant progressive power increase in both theta (6–11 Hz) and beta (specifically in its lowest part, 12–20 Hz) bands during the self-administration protocol (Fig. 1d, e), while the gamma band (60–95 Hz) remained stable (Fig. 1f).

A significant correlation was observed between baseline activity and the number of cocaine injections taken on the previous day during ShA sessions for theta and beta oscillations for most animals. This correlation disappeared during the LgA sessions, when the amount of cocaine injected was very high (Fig. 2a, b). No correlation was found for the gamma oscillations neither in ShA nor in LgA phases (Fig. 2c).

Oscillatory activity during cocaine access: loss of cocaine influence on STN theta and beta activity, but gamma activity as a possible neuromarker of cocaine intake

During the first 30 min of the ShA sessions, cocaine injections reduced the activity in the low-frequency bands (theta and beta) compared to baseline, with no effect on gamma band (Fig. 3a–d). However, during the first 30 min of the LgA sessions, following an increased baseline activity for theta and beta bands, the cocaine injections failed to reduce oscillatory activity (Fig. 3b, c). In contrast, while the baseline activity in the gamma band remained stable between ShA and LgA sessions, the access to cocaine induced a significant increase of the activity (Fig. 3a, d). Here, the increase in oscillatory activity in the gamma band was correlated with the number of cocaine injections taken during these 30 min of recording for most rats in ShA but not in LgA (Fig. 3e). In contrast, no correlation was found for theta and beta activities (Supplementary Figure S3). This result positions gamma oscillatory activity within STN as a discriminative signature of controlled cocaine consumption.

STN inactivation prevents cocaine intake escalation

STN lesions prevent cocaine intake escalation

Sham and STN lesion rats subjected to the escalation procedure took the same amount of cocaine during ShA sessions (Fig. 4a). In contrast, different patterns of intake were revealed when the animals were subjected to LgA sessions. As expected, sham control animals exhibited an escalation in their cocaine intake under extended access, showing a progressive increase in the total number of cocaine injections taken per 6 h session. In contrast, the STN lesion rats exhibited a lower intake than sham rats (Fig. 4a).

For a better comparison of the cocaine intake during ShA and LgA, the first 2 h of the LgA sessions were analyzed and pooled in blocks of five consecutive sessions and compared to the number of injections taken during the ShA sessions. Consistent with previous studies

[12], the sham control animals progressively increased their drug intake during the first 2 h of the LgA sessions and reached a level much higher than that observed during the ShA sessions (Fig. 4b). STN lesion had a preventive effect on escalation since STN lesion rats took less cocaine than the controls during these first 2 h of the LgA sessions with no escalation in their intake (Fig. 4b). It is important to note that this reduced cocaine intake was not observed in animals only subjected to repeated ShA sessions, but was seen in a progressive ratio procedure [14] and was not related to a possible reduced motor activity in animals with STN lesions, since the lesion has no effect on spontaneous or drug-induced locomotor activity (Supplementary Figure S4A–C).

STN–HFS prevents escalation in cocaine intake

To test the effects of STN–HFS on escalation, rats were divided into two groups: the control group remaining OFF stimulation and the group ON stimulation. The two groups were subjected to 15 LgA sessions. As expected, the OFF group exhibited an escalation of cocaine intake under extended access to the drug. Conversely, the ON group did not (Fig. 4c). STN–HFS significantly decreased the amount of cocaine consumed during the entire LgA sessions when compared with the controls (Fig. 4c), as well as during the first 2 h of the LgA sessions (Fig. 4d). This effect of STN–HFS was specific to escalation processes since it was not seen in animals subjected to 15 consecutive 2 h ShA sessions (Supplementary Figure S5).

STN–HFS not only prevented escalation, but also reduced the number of perseverative responses (nose pokes in the active hole during the time-out following an injection) in the ON group, compared to the increase in the OFF group (Supplementary Figure S6). These responses can be either interpreted as an index of compulsivity or motivation for cocaine. Similar to what was observed in the lesion experiment, STN–HFS did not affect spontaneous or cocaine-induced locomotor activity (Supplementary Figure S4D–F).

Effects of STN–HFS on STN oscillatory activity

Given that oscillations developed during escalation that is prevented by STN lesions and HFS, we tested whether or not STN–HFS could directly affect oscillatory activity. The results show that STN–HFS applied at 130 Hz immediately drastically diminishes the general oscillatory activity (Supplementary figure S7A) in an indiscriminate manner between bands (Supplementary figure S7B–D) and this effect recovers progressively over time.

Possible therapeutic effect of STN inactivation on cocaine intake after escalation?

To assess whether or not STN lesions could reduce cocaine intake even after a history of escalated intake, they were performed after rats had gone through the escalation procedure. As shown in Fig. 5a, while sham animals re-escalated their overall intake during the 6 h sessions, STN lesion rats did not re-escalate to the same extent. There was a trend toward significant group effect during re-escalation. Similar effects were observed during the first 2 h of the LgA sessions (Fig. 5b).

In the parallel experiment using STN–HFS, no significant effect of STN–HFS was observed when stimulation was activated immediately after escalation (Supplementary Figure S8).

Therapeutic effect of STN–HFS on re-escalation after protracted abstinence

Inactivation of STN immediately after escalation (Supplementary Figure S8) or after a 10-day period of abstinence (Fig. 5a, b) did not result in significant reduction of cocaine intake. We questioned whether or not a longer period of abstinence could allow possible therapeutic effect of STN manipulation. As shown in Fig. 5c, after an abstinence of 35 days, the OFF group exhibited re-escalation, unlike the ON group, indicating that STN–HFS prevented re-escalation of the number of cocaine injections taken overall. Similar effects were observed when analyzing the first 2 h (Fig. 5d).

Discussion

Escalation of drug intake is a hallmark of the transition from use to addiction. In animals, it is associated with decreased reward threshold, increased motivation to work for the drug and resistance to punishment, which are partly mediated by negative reinforcement [12, 21–24]. We show here that STN low-frequency (theta and beta) oscillations, measured before daily cocaine access, increase during the escalation process, while gamma oscillations increase during cocaine consumption. A 130 Hz STN–HFS reduces STN oscillatory activity on the one hand and prevents escalation of cocaine intake on the other hand, suggesting that increased STN oscillatory activity represents an important process in mediating escalation of drug intake. Finally, when applied after a period of protracted abstinence, STN–HFS can then reduce re-escalation of cocaine intake. These results suggest that STN inactivation prevents compulsive-like responding for cocaine possibly through reduction of oscillations in the STN and can restore a controlled consumption even after a history of drug intake escalation.

How can extended access to cocaine lead to oscillatory activity within the STN?

During the controlled consumption phase (ShA), basal low-frequency oscillations (theta and beta) correlate with the amount of cocaine taken the day before. Since the drug intake remained stable when rats were maintained in ShA for 15 days, it is highly probable that the LFPs would not change during this period for these animals according to the correlation observed between STN LFPs and cocaine intake. In contrast, these correlations disappear during the escalation phase (LgA) while low oscillation powers keep increasing, suggesting they are not a direct consequence of the increased drug intake. Consequently, this pathological increase may thus represent a biomarker of loss of control over drug intake.

Beta oscillations in particular have been associated with a DA depletion, as they are found in PD patients and animal models [1, 25, 26]. Interestingly, these oscillations are only present in STN after a chronic DA depletion, but not an acute DA blockade [26], suggesting their development required a long-term plasticity process. This could imply that repeated LgA exposures to cocaine may trigger a long-lasting process mimicking a DA depletion when recorded for baseline after an 18 h period of abstinence, a time-point during which reward efficacy is decreased [27]. In line with previous reports showing that DA replacement

therapy reduces PD-abnormal STN oscillations [1], we show here that cocaine consumption reduces STN beta and theta oscillations.

In contrast, access to cocaine increases STN gamma oscillations, in line with their proposed involvement in some aspects of addiction and motor behaviors related to DA activation [28–31]. As for basal low frequencies, gamma band power correlates with the amount of cocaine taken in the ShA phase, but no longer during the LgA phase. This loss of correlation can be either due to a ceiling effect resulting from high amount of cocaine intake or may be interpreted as another biomarker of loss of control over drug intake, independent on the amount of drug consumed.

What are the consequences in the brain and on behavior of STN oscillations?

Although they can be recorded in various sensorimotor regions of the basal ganglia structures, STN LFP oscillations correlate with cholinergic striatal interneurons as well as basal ganglia downstream structures (SNr, GPe, and GPi), but not with medium spiny striatal neurons [31, 32]. Beta activity induced by DA depletion is both higher in the STN than in its surroundings and correlates with increased single neuron or neuronal assembly activity in STN [26, 32, 33]. We thus hypothesize here that the progressive increased beta power observed during escalation process reflects an increased neuronal activity within the STN during baseline, which should result in a general reduction of motor activity. However, our results indicate that animals are not motorically impaired to a level that could prevent them from self-administering the drug since they are able to escalate their intake. In fact, beta oscillations and motor behavior do not always correlate, as acute DA antagonists seriously affect motor behavior without inducing any pathological beta activity in the STN, as in the case of catalepsy [34].

If one considers that STN activity increases in absence of cocaine in the escalated phase, this should lead to increased activity in the NAc, via the direct projections from STN to striatum. In contrast, NAc activity is progressively reduced during the development of escalation and also during re-escalation after protracted abstinence, especially in the shell [19]. This observation further suggests that the reduced NAc activity is probably due to another source of changes during escalation that might be more related to the DA system and confirms that STN oscillatory activity does not correlate with striatal activity [32]. However, STN oscillatory activity has been shown to correlate with motor cortical activity in PD rats [26]. It would thus be interesting to further investigate whether or not the STN biomarkers identified here have a correlate in the motor cortex or are indeed synchronized, as observed in PD, in which case desynchronization either at STN or cortical level might represent an interesting therapeutic strategy to prevent escalated drug intake.

The synchronization between STN and cortical activities has also been reported for gamma band oscillations [35]. Gamma oscillations have also been associated with arousal to emotional stimuli under DA influence in PD patients [36]. The increased gamma power observed here may therefore possibly affect the sensitivity to DA during cocaine intake and also prevent a proper treatment of emotional context. Indeed, lesion of STN reduces affective responses in rats [37].

STN lesions and HFS effects?

STN lesions and HFS have a preventive effect on escalation that is specific to the LgA phase since they have no effect on animals' consumption during ShA. One hypothesis to explain the efficacy of STN inactivation on blocking escalation of cocaine self-administration would be that abnormal synchronization within the STN network could be prevented by manipulating STN activity. Indeed, STN–HFS reduces oscillatory activity in all bands which is in line with former work showing that beta oscillations observed in PD patients are blocked by either L-DOPA [38] or STN–HFS [3]. L-DOPA and STN–HFS silence STN neurons in both PD macaques [25] and intact rats [20]. These physiological consequences have been classically associated with the beneficial effect of STN lesion or HFS on motor Parkinsonian symptoms [3, 39]. In a non-Parkinsonian brain, STN inactivation has here no effect on spontaneous or cocaine-induced locomotor activity, but has been shown to increase impulsivity of action that could favor drug self-administration [39, 40]. However, it also slows motor responses in tasks also requiring choice and reduces impulsivity of choice [5, 41, 42], which is often associated with compulsivity, consistent with the STN–HFS beneficial effect on compulsivity in OCD patients [43]. Here, STN–HFS reduces the number of perseverations during the cocaine escalation, an index of either compulsivity or motivation for the drug. Thus, our previous and present data confirm that STN inactivation reduces the animal's motivation for cocaine [14, 15].

In addition to its local effects, HFS affects STN network activity because stimulation may propagate both orthodromically and antidromically along the axons to disrupt abnormal synchronization of the basal ganglia functional circuits [25, 31] and also to reduce excessive cortical coupling between beta oscillations and broadband activity observed in PD [44]. STN–HFS also reduces c-fos activity in the NAc shell in absence of drug [20] and abolishes the cocaine-induced increased c-fos expression in both NAc core and shell [45]. As such, during escalation, STN–HFS might therefore counteract the changes induced by cocaine in the NAc.

Immediately after cocaine escalation has developed, STN–HFS had no therapeutic-like effect in a procedure during which rats experienced a daily 18 h period of abstinence. Despite a trend, STN lesions also failed to significantly reduce re-escalation of cocaine intake after a 10-day period of abstinence. However, STN–HFS reduces re-escalation after a 35-day period of abstinence. This difference between STN lesions and HFS may be due to the procedure used, since excitotoxic lesions only affect STN neurons, while the HFS mostly inhibits the cells, but also activates the passing fibers. This highlights the issue that to obtain beneficial effects, it is important to reduce STN activity, while acting simultaneously on its network. However, it is more likely that the duration of abstinence is a more critical factor than the procedure. The 10-day period of abstinence imposed by the post-surgery recovery period presumably was too short. This suggests that a prolonged period of abstinence such as 35 days, possibly allowing a return to homeostasis resembling the naive state of the first escalation, seems required to allow STN–HFS effectiveness on cocaine relapse. Future experiments will be required to investigate this issue.

Deep brain stimulation is currently an FDA-approved surgical treatment for some neurodegenerative and psychiatric disorders [46]. Regarding addiction, the best target

remains a matter of debate [15, 47, 48, 49]. Since it is important to treat the addiction to a drug without diminishing the desire for alternative, non-drug rewards such as food [14, 15], the STN appears as a solid candidate [16]. Favoring our proposed target, STN–HFS can reduce L-DOPA addiction in PD patients [50]. Accordingly, we identified here an addiction-associated pattern of STN abnormal activity whose reduction may contribute to HFS preventive and therapeutic effects. Furthermore, since addiction evolves through multiple cycles of abstinence/relapse, our data evidence a potential clinical window for STN–HFS application, which may normalize drug intake after abstinence in cocaine abusers.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgements

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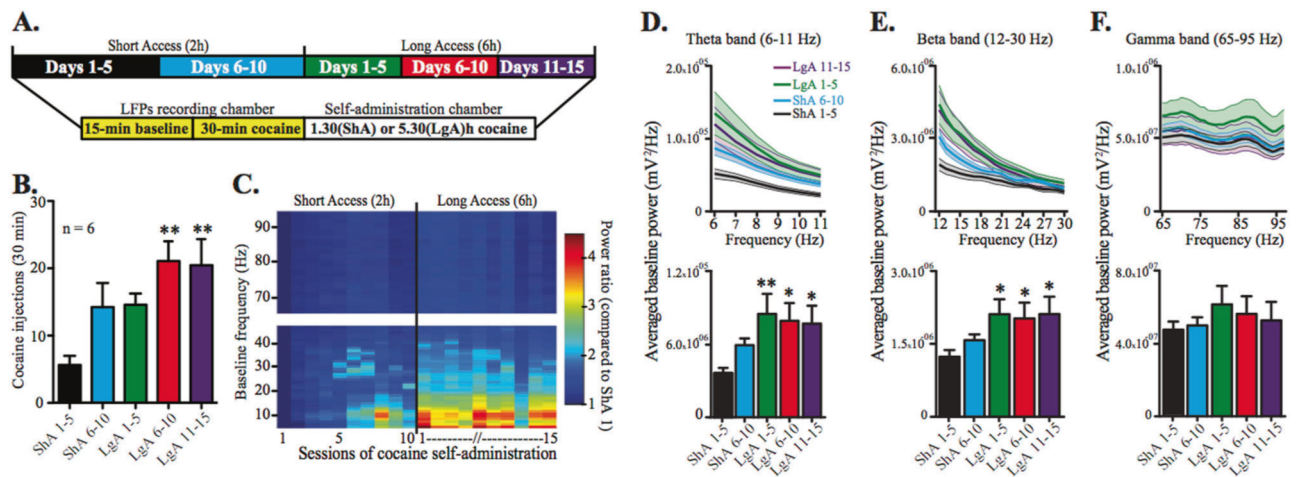
Fig. 1.
Progressive development of low-frequency oscillations within the STN during baseline recording, i.e., in absence of cocaine (15 min before access to cocaine) parallels escalation of cocaine intake. **a** Schematic of the experiment. **b** Increase in cocaine intake during the first 30 min of self-administration averaged by blocks of five daily sessions over ShA (black bar: days 1–5, blue bar: days 6–10) and LgA conditions (green bar: days 1–5, red bar: days 6–10, purple bar: days 11–15; block effect: $F_{4,20} = 6.952$, $P = 0.011$, $n = 6$, repeated measure one-way ANOVA). **c** Daily time-frequency analysis of the power spectrum, equivalent to a measurement of the signal amplitude. Values indicate the ratio of power spectrum normalized to the first day of ShA. **d–f** Highlights (upper panels, bold lines indicate average power values, light-colored areas indicate SEM; LgA 6–10 data have been omitted for clarity of presentation) and global quantifications of baseline power (bottom panels) for the theta (**d**), beta (**e**), and gamma (**f**) bands. Cocaine escalation induces an increase in oscillation power during LgA sessions only in the theta and beta bands (**d** $F_{4,20} = 4.308$, $P < 0.05$; **e** $F_{4,20} = 4.633$, $P < 0.01$; **f** $F_{4,20} = 1.917$, n.s., repeated measures one-way ANOVA). All bars are mean with SEM. $*P < 0.05$, $**P < 0.01$ vs. ShA 1–5 (Bonferroni post hoc test)
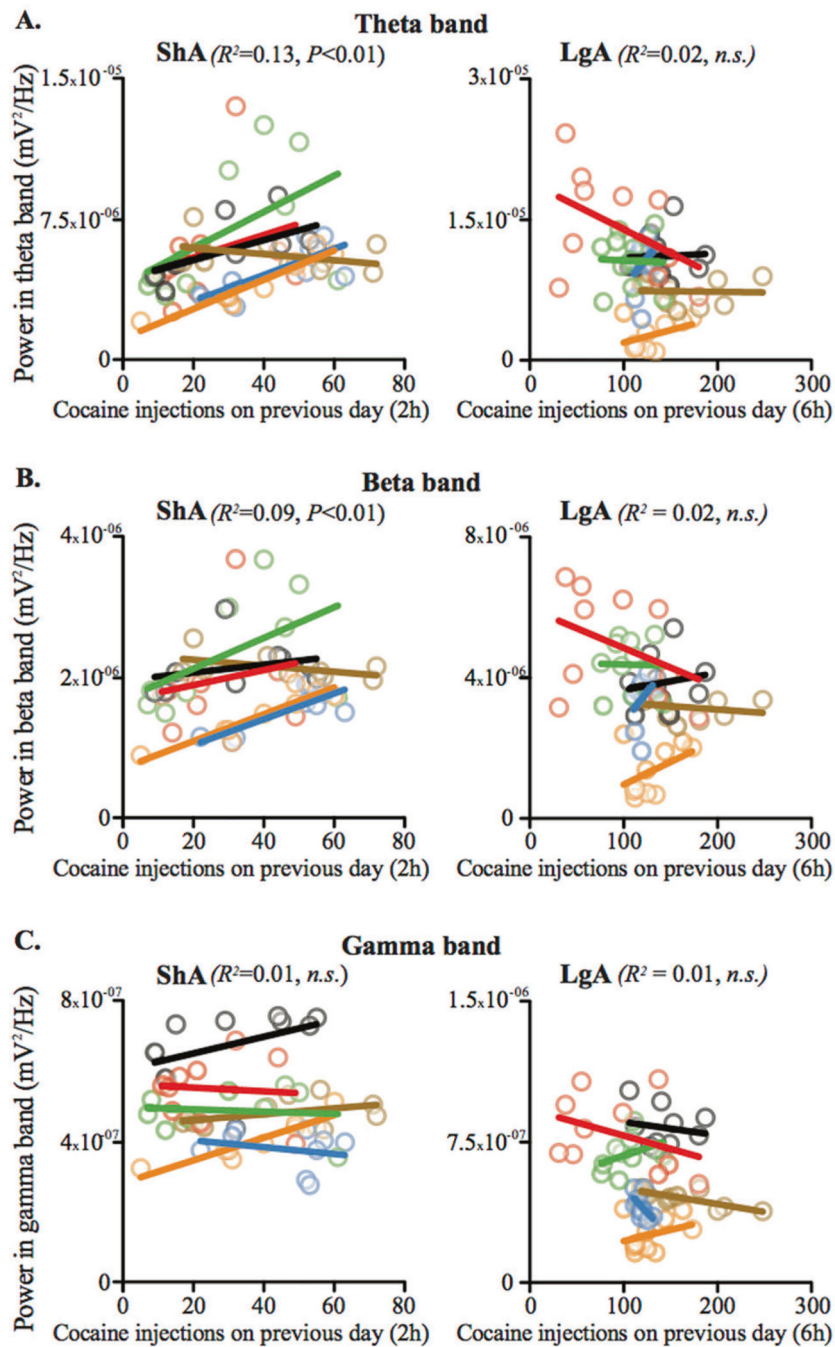
Fig. 2.
During baseline recording, theta and beta oscillations in the STN show a correlation with the number of cocaine injections taken the day before during ShA, but not LgA sessions. Diagrams showing the correlations (bold lines) between STN oscillation power of the theta (**a**), beta (**b**), and gamma bands (**c**) and the amount of cocaine taken on the previous day during the baseline recordings preceding ShA (left panels) and LgA sessions (right panels) for each animal (light shaded-color circles). A significant correlation was found for the theta and beta, but not for the gamma, bands for ShA (**a** $F_{1,42} = 9.2917$, $P < 0.01$; **b** $F_{1,42} = 7.55$, P

< 0.01; **c** $F_{1,42} = 2.14$, n.s.; ANCOVA controlled for inter-individual differences). In contrast, no correlation was found during the baseline sessions preceding LgA sessions (**a** $F_{1,48} = 2.34$, n.s.; **b** $F_{1,48} = 1.09$, n.s.; **c** $F_{1,48} = 2.78$, n.s.)
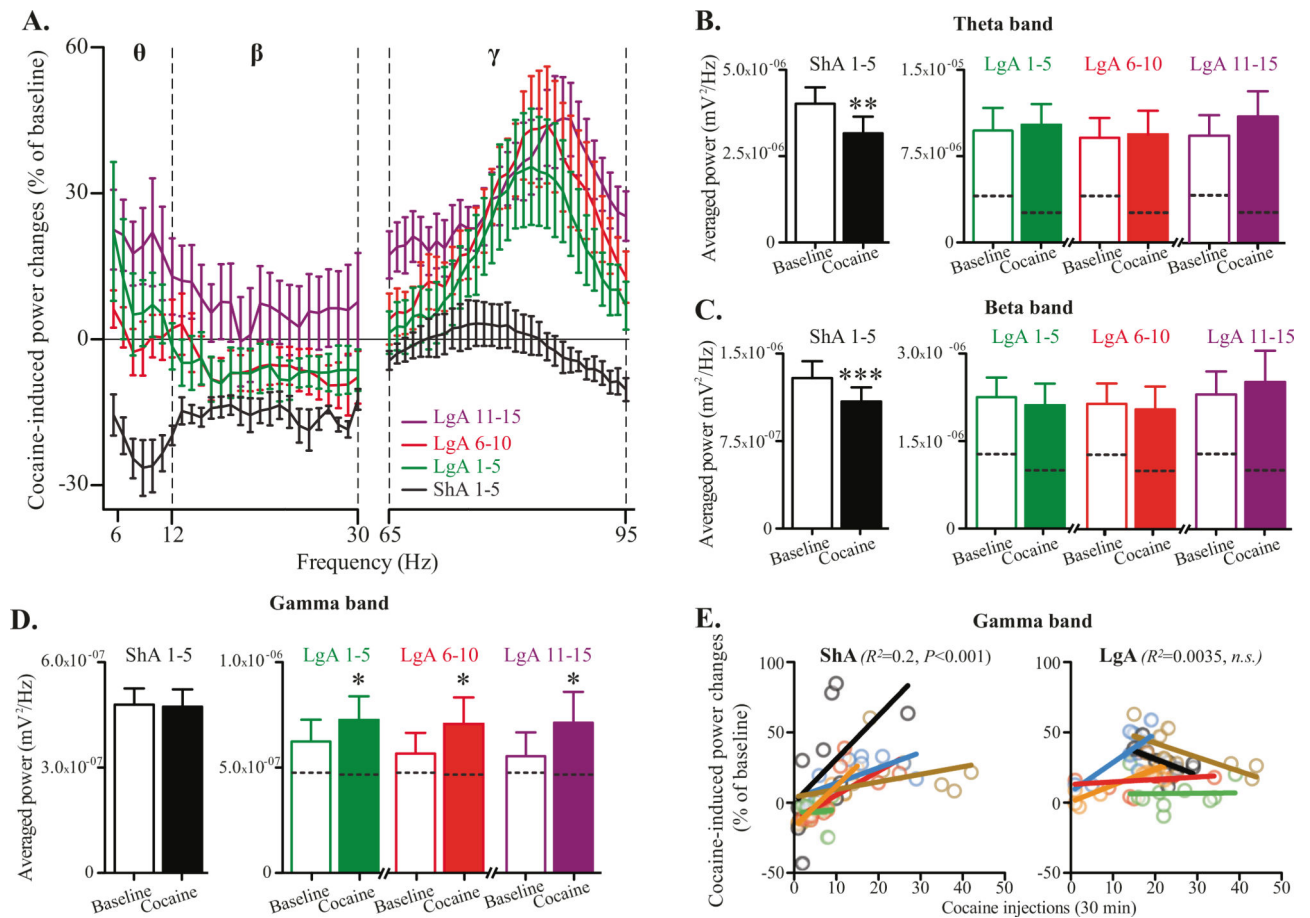
Fig. 3.
Cocaine induces STN theta and beta oscillations decrease when compared to baseline in ShA, but not LgA sessions, while STN gamma oscillations appear as a marker of cocaine intake. **a** Graph showing the variations in STN oscillation powers induced by cocaine consumption, compared to baseline recordings, during the first 30 min of ShA (days 1–5, black line) and LgA sessions (days 1–5, green line; days 6–10, red line; days 11–15 purple line). When expressed as percentage of the baseline, cocaine induced a decreases in LFPs power spectrum for the low (theta and beta bands) frequencies during ShA that disappeared during LgA sessions, where high-frequency oscillations increased in the gamma band. **b–d** Bar graphs showing the averaged STN oscillation powers during baseline (open bars) and cocaine recordings (filled bars) for ShA (days 1–5, black bars) and LgA sessions (days 1–5, green bars; days 6–10, red bars; days 11–15 purple bars) in theta (**b**), beta (**c**), and gamma (**d**) bands. (ShA 1–5: **b** $t_5 = 5.138$, $P < 0.01$; **c** $t_5 = 7.213$, $P < 0.001$; **d** $t_5 = 0.396$, n.s.; LgA 1–5: **b** $t_5 = 0.9415$, n.s.; **c** $t_5 = 1.416$, n.s.; **d** $t_5 = 2.873$, $P < 0.05$; LgA 6–10: **b** $t_5 = 0.5672$, n.s.; **c** $t_5 = 0.9004$, n.s.; **d** $t_5 = 3.607$, $P < 0.05$; LgA 11–15: **b** $t_5 = 2.256$, $P = 0.0738$, n.s.; **c** $t_5 = 0.9542$, $P = 0.3838$, n.s.; **d** $t_5 = 3.847$, $P = 0.012$, paired t-test). Dotted lines in the LgA bars indicate the level reached during ShA sessions. **e** Diagrams showing the correlations (bold lines) between STN oscillation power of the gamma band and the amount of cocaine taken during the first 30 min of ShA (left panel) and LgA sessions (right panel) for each animal (light shaded-color circles). A significant correlation was found during ShA ($F_{1,48} =$

18.39, $P < 0.001$; ANCOVA controlled for inter-individual differences) that disappeared during the LgA session ($F_{1,46} = 1.96$, n.s.). All data are means with SEM, $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ vs. baseline, paired t-test
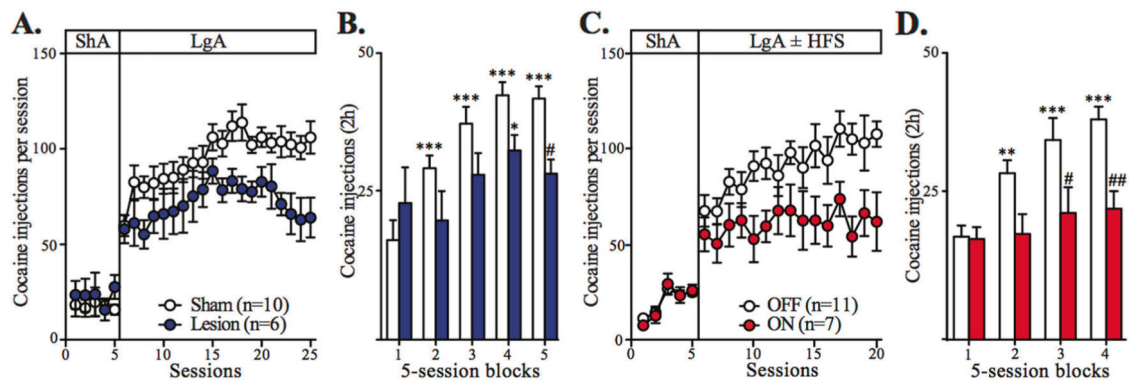
Fig. 4.

STN manipulations have preventive effects on cocaine escalation. **a** Summary time graph presenting the effect of STN lesion on the total cocaine intake throughout the 5 last ShA and 20 LgA sessions. While STN lesion has no effect on ShA, it reduces the development of cocaine escalation (ShA: group effect: $F_{1,56} = 0.5683$, n.s., LgA: session effect: $F_{19,266} = 5.231$, $P < 0.0001$; group effect, $F_{1,266} = 8.939$, $P < 0.01$, mixed two-way ANOVA; sham group, $n = 10$, white dots; STN lesion group, $n = 6$, blue dots). **b** Summary bar graph showing that STN lesion also reduces the cocaine intake during the first 2 h of each session averaged per blocks of five ShA (block 1) and LgA (blocks 2–5) sessions (block × group interaction: $F_{4,56} = 4.329$, $P < 0.01$, mixed two-way ANOVA). **c** Summary time graph showing the effect of STN–HFS applied during 15 LgA sessions on the total cocaine intake (OFF group: $n = 11$, white dots; ON group: $n = 7$, red dots). While both groups exhibit comparable intake during ShA, STN–HFS blocks the development of cocaine escalation (ShA: group effect: $F_{1,64} = 0.01819$, n.s.; LgA: session effect: $F_{14,224} = 1.863$, $P < 0.05$; group effect, $F_{1,224} = 13.51$, $P < 0.01$, mixed two-way ANOVA). **d** Summary bar graph of averaged cocaine intake during the first 2 h of each session per blocks of five ShA (block 1) and LgA (blocks 2–4) also shows that STN–HFS also blocks cocaine escalation development (block × group interaction: $F_{3,48} = 3.081$, $P < 0.05$, mixed two-way ANOVA). All data are means with SEM. *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs. ShA (block 1); #$P < 0.05$, ##$P < 0.01$ vs. sham group or OFF group (Bonferroni post hoc test)
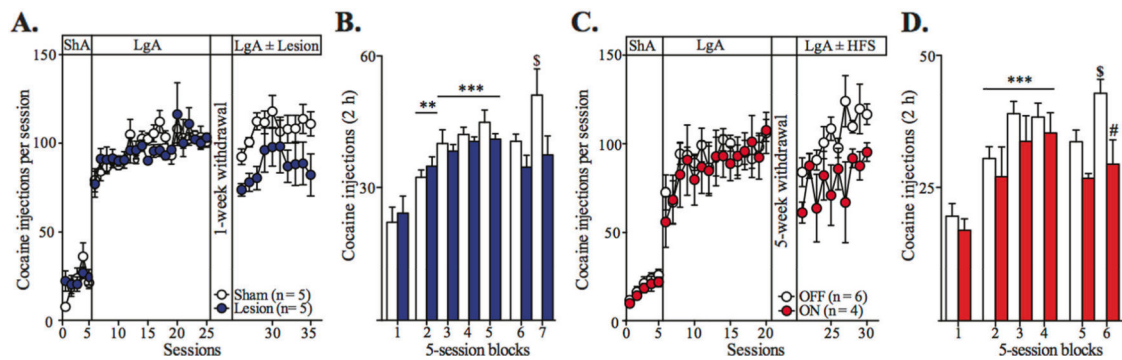
Fig. 5.

STN manipulations have therapeutic effects after cocaine escalation. **a** Summary time graph presenting the effect of STN lesion, performed after escalation, on the total cocaine intake during ten LgA sessions following the surgery recovery period (10-day abstinence). Both groups (sham group: $n = 5$, white dots; STN lesion group: $n = 5$, blue dots) display comparable cocaine intake during escalation (session effect $F_{19,152} = 4.084$, $P < 0.0001$, session × group interaction $F_{19,152} = 0.8126$, n.s., mixed two-way ANOVA). Although not significant, STN lesion tends to reduce cocaine re-escalation observed in sham control animals after abstinence (session effect: $F_{9,72} = 3.083$, $P < 0.01$; group effect: $F_{1,72} = 4.762$, $P = 0.06$, mixed two-way ANOVA). **b** Summary bar graph showing the cocaine intake during the first 2 h of each session averaged per blocks of five ShA (block 1), four blocks of five pre-surgical LgA (blocks 2–5) and two blocks of five post-surgical re-escalation LgA (blocks 6–7) sessions. Both groups escalate their cocaine intake before surgery (block effect $F_{4,32} = 24.27$, $P < 0.0001$; group effect $F_{1,32} = 0.07691$, n.s., mixed two-way ANOVA). Again, STN lesion tends to reduce cocaine re-escalation after abstinence (block effect: $F_{1,8} = 6.564$, $P < 0.05$, group effect: $F_{1,8} = 3.756$, $P = 0.08$, mixed two-way ANOVA). **c** Summary time graph showing the effect of STN–HFS on the total cocaine intake during ten LgA sessions following a 35-day period of protracted abstinence (OFF group: $n = 6$, white dots; ON group: $n = 4$, red dots). While both groups display comparable intake during escalation (session effect: $F_{14,112} = 2.861$, $P = 0.01$; group effect: $F_{1,112} = 0.1888$, n. s., mixed two-way ANOVA), STN–HFS reduces the re-escalation of cocaine escalation (session effect: $F_{9,72} = 2,546$, $P < 0.05$, group effect: $F_{1,72} = 10.01$, $P < 0.05$, mixed two-way ANOVA). **d** Summary bar graph, averaging the first 2 h of each session per blocks of five ShA (Block 1), three LgA of pre-HFS LgA (Blocks 2–4) and two blocks of five HFS LgA sessions, shows that STN DBS also reduces the cocaine re-escalation (block effect: $F_{1,8} = 7.086$, $P < 0.05$, group effect: $F_{1,8} = 9.186$, $P < 0.05$, mixed two-way ANOVA). All data are means with SEM. **$P < 0.01$, ***$P < 0.001$ vs. ShA (block 1); $^{\$}P < 0.05$ vs. first block of re-escalation, $^{\#}P < 0.05$ vs. OFF group (Bonferroni post hoc test)