Title: PEER'S PRESENCE REVERSES ESCALATED COCAINE INTAKE IN RATS

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One Sentence Summary: The presence of a stranger peer drastically decreases cocaine intake after drug escalation, as does a subthalamic optogenetic modulation.

Abstract: The immediate social context at the time of drug consumption is critical at modulating it. The neurobiological substrate of such an influence is however poorly documented. The presence of a stranger peer, naïve to the drug, has been shown to reduce recreational cocaine intake in rats with similar results in human cocaine users. Here we assessed its influence in rats having previously lost their control over drug consumption. The subthalamic nucleus (STN) has been shown to play a critical role in cocaine motivation, escalation and re-escalation, as well as compulsive drug seeking. We show here that after escalation of cocaine intake, the presence of a stranger peer drastically reduced cocaine intake. The same effect was observed after both optogenetic inhibition and high-frequency stimulation of the STN in absence of a peer. We further show that the beneficial influence of social presence is mediated via the STN.

Main Text:

INTRODUCTION

There are currently no validated pharmacological agents for treating cocaine abuse. An alternative is to use behavioral and social interventions. These therapies have been shown to be effective in helping cocaine users regain the control on their drug consumption *(1, 2)*, highlighting the key influence of environmental factors on drug use and cessation. Although only few preclinical studies took into account this environmental, or contextual, influence *(3–5)*, social factors have been shown to play a critical role in initiation, maintenance, attempt to quit and relapse of drug use in both rodents and humans *(6, 7)*. Positive social context in everyday life (*e.g.* parenting in humans, maternal cares in rats) has a preventive effect on drug consumption *(8, 9)*, while aversive social interaction (*e.g.* children abuse in humans, repeated social-defeat in rats) or a lack of positive social contact (*e.g.* loneliness in humans, isolation rearing or maternal separation in rats) leads to an increasing risk to develop substance use disorders (SUD) *(10–13)*. Proximal social factors, such as the social presence during drug consumption, also affect the drug intake *(14)*, depending on the nature of the substance used *(15)*, the relationship between the subject and the stimulus peer *(16)* and the latter's behavior (*i.e.* self-administering or not; *15)*. In both rats and humans, the presence of a drug-naïve stranger peer seems to represent the situation that decreases the most the

recreational cocaine intake*(16)*. However, the influence of the peer's presence on addiction-like behaviors, such as after a loss of control over drug consumption, remains to be investigated in rats, as well as its neurobiological substrate.

Recent studies have suggested that the beneficial influence of proximal social factors on recreational drug use could involve the subthalamic nucleus (STN) *(18–20)*. Given its involvement in inhibitory control *(21–24)* and motivation processes *(25–27)*, the STN appears as a promising target for the treatment of cocaine addiction *(28)*. Rodent studies revealed that STN lesion or high-frequency (HF) deep brain stimulation (DBS) both decreases motivation for cocaine and increases motivation for sweet food in rats *(27, 29)*. STN HF-DBS was also shown to prevent the development of escalation and to further reduce re-escalation of cocaine or heroin intake *(30, 31)*. STN oscillatory activity would serve as a predictive biomarker of vulnerability to addiction. In fact, manipulating STN activity with DBS bi-directionally influences compulsive-like cocaine seeking *(32)*. Although the neural effects of STN DBS remain to be clarified, it seems legitimate to consider STN as a potential target to treat addiction.

Here, we assessed whether the presence of a stranger peer, naïve to the drug can reduce cocaine intake in rats having lost their control over cocaine intake. Following a cocaine escalation procedure, we investigated how the presence of a peer affects cocaine intake. We further examined the potential contribution of the STN in this social influence, using optogenetic to selectively modulate its neuronal activity in presence or absence of the peer.

RESULTS

27 rats received bilateral injections of AAV5-CaMKII-EYFP (n=9, EYFP-control group), AAV5-CaMKII-ArchT3.0-p2A-EYFP-WPRE (n=9, ARCHT3.0 group) or AAV5-CaMKII-hChR2(E123T/T159C)-p2A-EYFP-WPRE (n=9, hChR2 group) into the STN, with optic fibers implanted 0.4 mm above each injection site. They were trained to self-administer cocaine (250µg/90µL, *i.v.* injections) under a fixed ratio 1 (FR1) schedule of reinforcement, with a f2h-daily access to the drug. After stabilization of their basal cocaine intake (<25% variation for 5 consecutive days), animals were then subjected to an escalation procedure, consisting in a daily 6h-session of extended access to the drug during 20 days. Finally, they were tested for 2h self-administration sessions in presence of a drug-naïve stranger peer for 5 consecutive days ('*With peer'* condition). They were also allowed to self-administer cocaine for 5 consecutive days without the presence of a peer ('*Without peer'* condition). The order of '*With peer'* and '*Without peer'* conditions were counterbalanced between animals (**Fig. 1A**). The photo-modulation of STN activity was only delivered during these 2h-sessions.

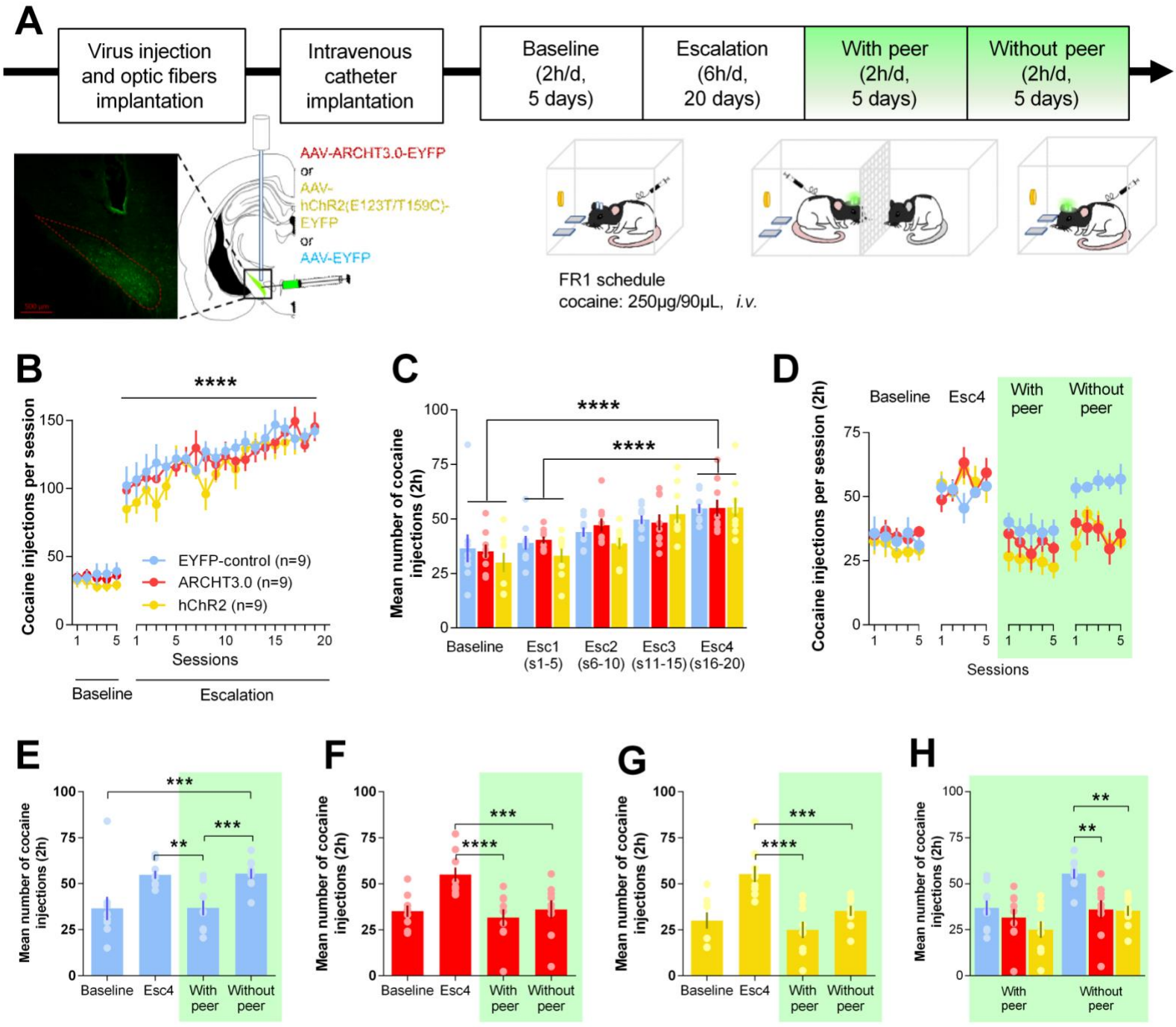

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Fig. 1 Peer's presence or STN optogenetic modulations reduce cocaine intake following drug escalation.

(**A**) Timeline of the behavioral experiment (upper part). Representative native fluorescence emitted by transfected STN neurons and implanted optic fiber trace, with schematic coronal section of the rat brain at the level of the STN, and schematic apparatus (bottom part). (**B**) Number of cocaine injections received by EYFP-control (n=9, blue), ARCHT3.0 (n=9, red) and hChR2 (n=9, yellow) expressing animals during the five 2-h sessions of the baseline and the twenty 6-h sessions of escalation (****: sessions effect during escalation) with the laser OFF. (**C**) Mean number of cocaine injections received during the two first hours of the sessions in blocks of 5-sessions (baseline, escalation: 'Esc1', 'Esc2', 'Esc3' and 'Esc4', corresponding respectively to sessions 1-5, 6-10, 11-15 and 16-20). (**D**) Cocaine injections received during the two hours of the baseline, the first two hours of 'Esc4', and the two hours sessions with the laser activated: 'With peer' and 'Without peer'. (**E,F,G**) Mean number of cocaine injections received during the two first hours of the sessions in 5-sessions blocks (baseline, 'Esc4', 'With peer', 'Without peer') respectively in EYFP-control (**E**), ARCHT3.0 (**F**) and hChR2 (**G**) animals. (**H**) Mean number of cocaine injections received during the two first hours of the sessions with the laser activated 'With peer' and 'Without peer' in EYFP-control, ARCHT3.0 and hChR2 rats. In **A**, **D**-**H**, the green areas correspond to the sessions with activation of the laser. In **C**,**E**,**F**,**G**: Each dot represents an individual mean per block of 5 sessions. ***: p<0.01; ***: p<0.001; ****: p<0.0001*

All rats escalated cocaine consumption

In absence of laser activation, all animals showed a stable baseline of cocaine intake (mixed 2-way ANOVA, sessions effect: $F_{(4,96)}=2.058$, $p=0.5001$) and drastically escalated their consumption during the 6h sessions of extended access to the drug ($F_{(19,456)}=12.59$, $p<0.0001$) (**Fig. 1B**). The three groups took similar amount of cocaine during both the baseline (group effect: $F_{(2,24)}=0.4996$, $p=0.6129$) and the 6h sessions of extended access to the drug ($F_{(2,24)}=0.6492$, $p=0.5314$). These results were further confirmed by analyzing the mean number of cocaine injections took during the two first hours of each session per block of 5 sessions (**Fig. 1C**; baseline, escalation: 'Esc1', 'Esc2', 'Esc3', 'Esc4', groups effect: $F_{(2,24)}=0.6225$, $p=0.545$, 5-sessions blocks effect: $F_{(4,96)}=25.15$, $p<0.0001$). Tukey's post-hoc comparisons confirmed that rats consumed more cocaine during 'Esc4' than during the baseline ($p<0.0001$), 'Esc1' ($p<0.0001$) and 'Esc2' ($p<0.0001$). We also controlled the fact that escalation of drug intake was due to the extended access procedure by maintaining other animals (EYFP-control, n=7 and ARCHT3.0, n=6) in a short access (2h) to cocaine during 20 days (same timeline used in the 'escalation' protocol, see Supplemental). Only rats that had a long access to the drug escalated their cocaine intake during the two first hours of the sessions (mixed 2-way ANOVA, long/short access x session interaction: $F_{(4.91,132.65)}=3.37$, $p<0.007$, **Fig. S1A, Table S1-2**).

To assess the effect of the peer's presence and the STN photo-modulation on cocaine intake, we compared the number of cocaine injections took by 3 groups during the sessions of the baseline, 'Esc4' (2 first hours), 'With peer' and 'Without peer' (**Fig. S1B;** mixed 3-way ANOVA). No effect of sessions was found (results reported in **Table S3-4**). We thus averaged the sessions in blocks of five sessions, corresponding to these various conditions. The results confirmed that the opsins expression modulates cocaine intake depending on the conditions (mixed 2-way ANOVA; group x condition interaction: $F_{(6,72)}=2.524$, $p=0.0284$). Similar results were found in animals maintained in short access (condition x optogenetic group $F_{(3,33)}=5.781$, $p=0.0027$) (**Fig. S1B**). In contrast, the number of errors or perseverations made by the animals remained unchanged during the entire course of the experiment for all groups (**Fig. S2**, **Table S5-6**), suggesting that these conditions and the STN photo-modulations specifically affected the responses towards the drug without side-effects.

Peer's presence decreases escalated cocaine intake…

In EYFP-control rats, the presence of a peer drastically reduced cocaine self-administration after drug escalation (**Fig. 1E**; Tukey's post-hoc analysis: $p=0.0014$, 'With peer' compared with 'Esc4'), similar to their basal level of consumption ($p>0.9999$ compared with baseline). In EYFP-control animals maintained in short access, peer's presence not only reduced cocaine intake compared with 'Esc4' ($p=0.0009$), but also compared with the baseline ($p=0.0046$) (**Fig. S1C**).

… Like the subthalamic nucleus optogenetic inhibition…

The laser activation did not affect cocaine intake in EYFP-control animals 'Without peer', which displayed an escalated-like level of cocaine intake ($p=0.9993$ 'Without peer' compared with 'Esc4'), consuming significantly more drug than during their baseline ($p=0.0009$).

In contrast, the STN photo-inhibition induced a drastic decrease in drug intake in ARCHT3.0 rats 'Without peer' (**Fig. 1F**; $p=0.0007$ *vs.* 'Esc4' and $p=0.9973$ *vs.* baseline). Cocaine intake was significantly lower in ARCHT3.0 rats than in EYFP-control ones in the 'Without peer' condition

(**Fig. 1H**; Sidak's post-hoc analysis: p=0.0027), confirming this beneficial effect of the STN photo-inhibition.

In contrast, the STN photo-inhibition did not reduce cocaine use in ARCHT3.0 animals maintained in short access sessions (**Fig. S1D**; p=0.9808, 'Without peer' compared to 'Esc4'). These rats consumed similar amount of drug than EYFP-control animals 'Without peer', despites the laser activation (**Fig. S1E**; p>0.9999). In line with previous reports *(27, 29, 30)*, these results show that the STN neuromodulation affects cocaine abuse, but not its recreational use.

… Or its optogenetic HF-stimulation

Interestingly, the STN optogenetic HF-stimulation also reduced cocaine intake in hChR2 transfected rats 'Without peer' (**Fig. 1G**; p=0.0003 *vs.* 'Esc4' and p=0.6759 *vs.* baseline). These animals also consumed less drug than EYFP-control rats 'Without peer' (p=0.0018). This results shows that optogenetic HF-stimulation induces a similar behavioral effect than optogenetic inhibition.

STN photo-modulations contribute to the beneficial effect of peer's presence

The presence of a peer also drastically reduced cocaine consumption in photo-activated ARCHT3.0 (p<0.0001 *vs.* 'Esc4' and p=0.8739 *vs.* baseline), as well as in hChR2-transfected rats (p<0.0001 *vs.* 'Esc4' and p=0.7119 *vs.* baseline). However, their level of consumption was not lower than EYFP-control rats in similar condition (p=0.5768 *vs.* ARCHT3.0 and p=0.0861 *vs.* hChR2). There was no significant difference between 'With peer' and 'Without peer' conditions for both ARCHT3.0 (p=0.7769) and hChR2-transfected (p=0.1361) rats, in contrast to EYFP-control ones (p=0.0009), confirming the lack of additivity in the effects of the STN photo-modulations and the peer's presence. This indicates that of the reduction in cocaine intake observed with STN photo-modulations or the peer's presence occluded each other.

Interestingly, the presence of a peer combined with STN photo-inhibition did not reduce cocaine intake in ARCHT3.0 rats maintained in short access (p>0.9999 *vs.* 'Esc4' and p=0.9996 *vs.* 'Without peer'). These animals consumed significantly more cocaine than the EYFP-control rats 'With peer' (p=0.0494), suggesting that STN photo-inhibition blocked the beneficial effects of social presence, for animals that had not escalated their cocaine intake.

These results suggest that the beneficial influence of the peer's presence is mediated via the STN. Nevertheless, in social context, the STN neuromodulation interferes in a different manner with recreational (*i.e.* suppression of social influence) and escalated drug use (*i.e.* occlusion of social influence).

Electrophysiological assessment of STN photo-inhibition

To assess the efficacy of our optogenetic manipulations on STN neuronal activity, we performed *in vivo* recordings in anesthetized animals and *ex vivo* patch-clamp recordings on brain slices. 30 naïve adult rats (not used for the behavioral experiment) were injected with AAV5-CaMKII-EYFP (n=3), AAV5-CaMKII-ArchT3.0-p2A-EYFP-WPRE (n=13) or AAV5-CaMKII-hChR2(E123T/T159C)-p2A-EYFP-WPRE (n=14). Recordings were performed 3-4 weeks following the opsins injection.

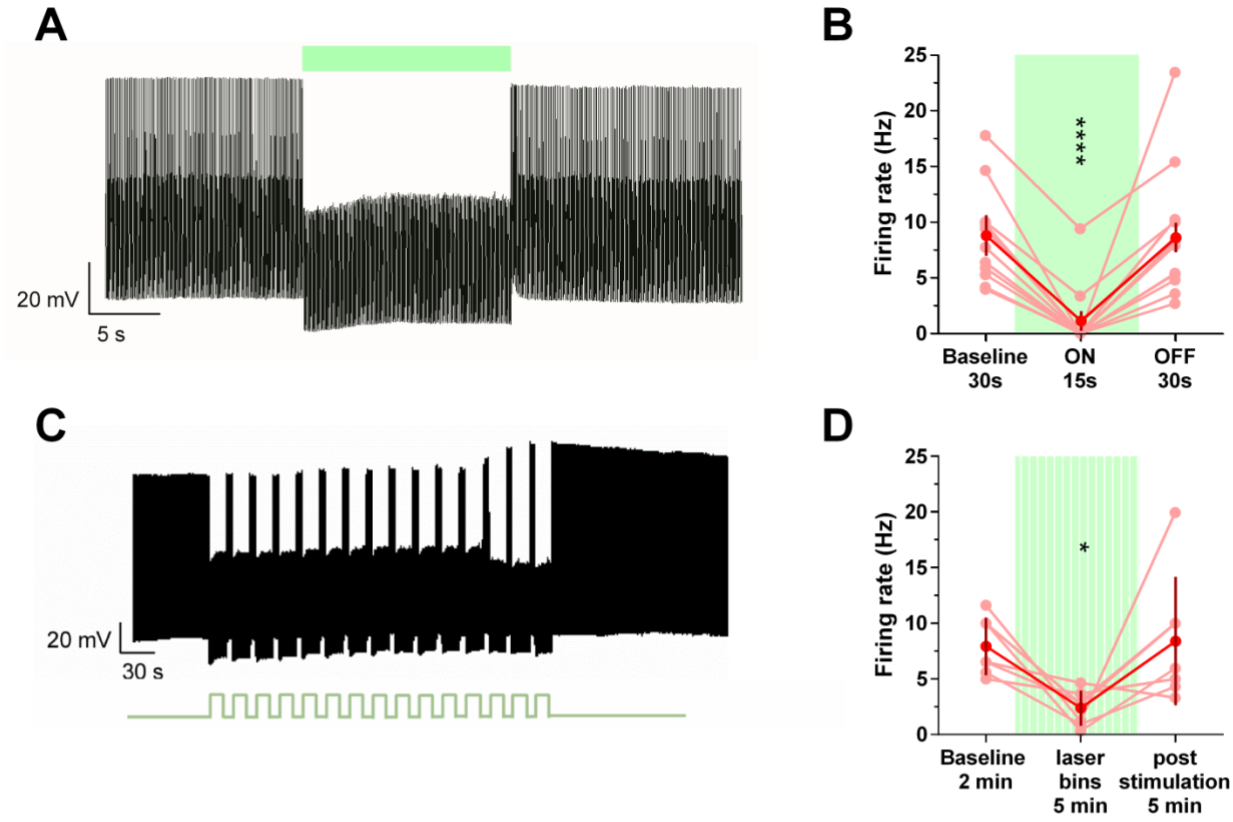
Opsins expression did not deeply change the electrophysiological properties of the STN neurons, as resting membrane potential (Kruskall-Wallis: p=0.8214), cell capacitance (p=0.0644),

membrane resistance (p=0.7397), access resistance (p=0.3158) and firing rate (mixed 2-way ANOVA, Frequency x Group interaction: $F_{(30,390)}$=0.2480, p>0.9999) were equivalent between EYFP-control (n=3 animals; n=8 cells), ARCHT3.0 (n=7 animals; n=11 cells) and hChR2 groups (n=5 animals; n=10 cells) (**Fig. S3**). To confirm the efficacy of the optogenetic modulation, we first applied discrete light pulses on STN neurons (**Fig. S4**). Longer pulse-width duration and higher light intensity elicited higher inhibitory currents in ARCHT3.0 positive cells (At 5mW, effect of pulse duration; $\chi^2(5)$=51.88, p<0.0001, Dunn's post-hoc test: p<0.0100 *vs.* 1s pulse and p<0.0010 *vs.* 10s, 15s or 20s pulse). Consistent with previous reports *(33)*, our these results suggest that higher laser activation (*i.e.* increased pulse duration and brighter illumination) leads to higher activation of opsins and increases the transmission through a larger volume of tissue, thereby recruiting a larger pool of neurons. To further assess the effect of optogenetic inhibition, electrical stimuli (10Hz, injected current: rheobase + 20-130pA) were delivered to STN neurons, to evoke actions potentials before applying photo-inhibition (**Fig. 2A**). A 15s light-pulse delivered to ARCHT3.0-expressing neurons successfully inhibited neuronal firing (**Fig. 2B**, p=0.0006). We then applied the same light pattern than during the behavioral experiments (*i.e.* alternation of a 15s period ON followed by a 5s period OFF, for 5min) on other ARCHT3.0-transfected cells (n=2 rats, n=7 cells) (**Fig. 2C**). Photo-inhibition of STN neurons leads to reduced firing rate of STN neurons (**Fig. 2D**; Dunn's post-hoc test: p=0.0323 *vs.* baseline), which was resumed when the laser was stopped (p=0.9999). This confirmed that our photo-inhibition protocols were efficiently at inhibiting STN neurons.

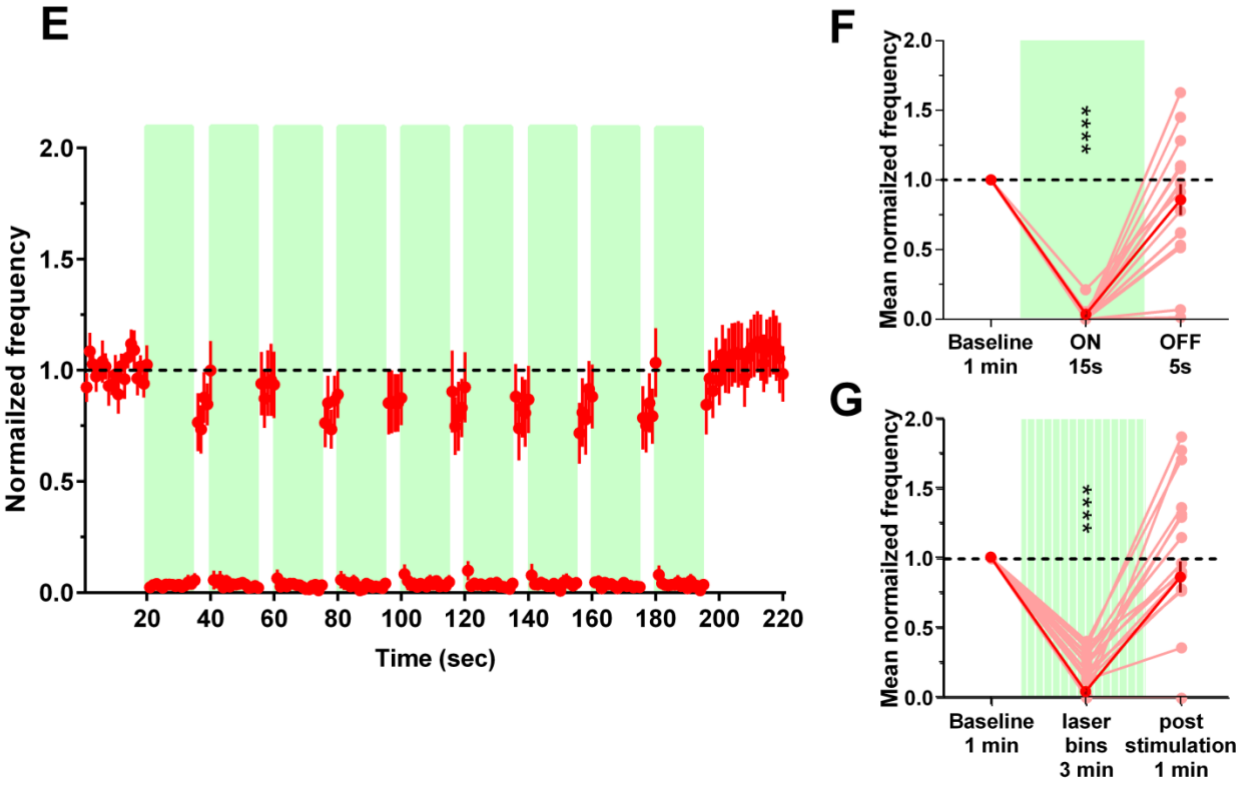
To control the effects of the STN photo-inhibition in *in vivo* conditions, we performed extracellular recordings in (n=4) anesthetized animals, which STN neurons had been transfected with ARCHT3.0-AAV. To estimate the population response, the neuronal firing of STN neurons was normalized to the 20s baseline prior photo-modulation. In ARCHT3.0 transfected rats, a 15s light-pulse (5 mW) successfully inhibited neuronal firing in STN neurons (**Fig. 2E,F**, Dunn's post-hoc test: p<0.0001 *vs.* baseline, n=16 cells) but did not impact neuronal activity during the intermittent 5s OFF periods (p=0.959), as observed in our ex vivo preparations. As a result, the normalized firing rate frequency in ARCHT3.0-transfected neurons significantly diminished during the 180s of laser bins (composed of alternation of 15s ON/5s OFF periods) compared with their baseline activity (**Fig. 2G**; p<0.0001 *vs.* baseline) . STN firing activity during the 20s following the laser bins was similar to the baseline (p>0.9999).

Fig. 2 Electrophysiological assessment of STN optogenetic inhibition.
(**A**) Typical response recorded in one STN neuron expressing ARCHT3.0 driven at a 10Hz frequency undergoing 15s of photo-inhibition (green line: laser ON). (**B**) Firing rate in ARCHT3.0-expressing STN neurons was reduced by a single 15s light pulse (n=11 cells). (**C**) Example of response for an ARCHT3.0-trasnfected STN neuron during 5 min of laser activation applied with the same pattern as during behavioral experiments (*i.e.* alternations of 15s ON, interleaved with 5s OFF, for 5min). (**D**) Firing rate in STN neurons expressing ARCHT3.0 was reduced by 5min of laser application at this pattern (n =7 cells). (**E**) Normalized frequency in ARCHT3.0-trasfected STN neurons during 20-s of baseline recording, followed by 180-s of laser activation with the same pattern and 20s post stimulation (n=16 cells). (**F**) 15s laser activation (ON-15s), but not 5s of obscurity (OFF-5s) reduced the mean normalized frequency in ARCHT3.0-transfected STN neurons, compared with their baseline activity (n=16 cells). (**G**) 180-s of laser bins diminished the mean normalized frequency in STN neurons, compared with their baseline activity. Following 180-s of laser bins, these cells returned to their baseline level of activity. Full green area corresponds to continuous laser activation. Hatched green area corresponds to laser bins. In **B**,**D**,**F**,**G**: Each light red dot and line represents an individual value. Dark red dot and lines correspond to the mean and SEM.
: p<0.05,**:p<0.0001*.

Ex vivo photo-inhibition of STN neurons trasnfected with ARCHT3.0



In vivo photo-inhibition of STN neurons trasnfected with ARCHT3.0



Electrophysiological assessment of STN optogenetic HF-stimulation

When discrete light pulses were applied *ex vivo* in (n=10) hChR2-expressing STN neurons, longer pulse-width duration and higher light intensity elicited higher depolarizing currents (intensity set at 10mW, effect of pulse duration: $\chi^2(5)=46.00$, $p<0.0001$, Dunn's post-hoc test: 0.5ms pulse $p<0.001$ *vs.* 4ms pulse and *vs.* 5ms pulse; at 1s pulse duration, effect of light intensity: $\chi^2(3)=28.92$, $p<0.0001$, 2.5mW $p<0.01$ *vs.* 10 mW and $p<0.001$ *vs.* 20mW, **Fig. S4D-G**). The effects of optogenetic stimulation were assessed in STN neurons with 10s of laser illumination at various frequencies (**Fig. 3A**). Interestingly, higher frequencies of laser stimulation did not change the number of action potentials compared to lower frequencies (**Fig. 3B**). However, higher frequencies significantly increased membrane potential ($p<0.001$ for 100-, 130- and 150-Hz *vs.* 10-Hz; **Fig. 3C**), suggesting that this pattern increased the excitability of STN neurons. To test this hypothesis, we applied a 10Hz electrical stimulation in other hChR2-transfected STN neurons (n=2 rats, n=8 cells) (injected current: Rheobase +0-50pA) to evoke a few action potentials. We then applied 130Hz laser stimulation for 5min (**Fig. 3D**), which significantly increased the firing rate of STN neurons ($p<0.0351$, **Fig. 3E**). Following cessation of the photo-stimulation, the firing rate was no longer different from the baseline period ($p>0.05$). This suggests that 130Hz optogenetic stimulation is unlikely to induce action potentials on its own but increases STN neurons excitability.

In a set of hChR2-transfected rats (n=7 animals; n=16 cells), the impact of STN HF-stimulation (130 Hz during 3 min) was also assessed *in vivo*. To estimate the population response, the neuronal firing of STN neurons was normalized to the 1 min baseline prior to photo-modulation. Although the overall population increased its firing rate during light application (**Fig. 3F,G**), about half of the cells (n=7) did not modify their firing rate (non-responding cells; $p>0.9999$ baseline *vs.* laser bins). In contrast, the impact of STN HF photo-stimulation was strongly marked in 9 neurons (responding cells; $p=0.0019$ compared with baseline). These cells returned to their basal level of activity when the photo-stimulation was turned OFF ($p>0.9999$ compared with baseline).

Fig. 3 Electrophysiological assessment of STN optogenetic HF-stimulation.
(**A**) Typical excitatory response recorded in current-clamp for a STN neuron expressing hChR2 and facing 10s of laser stimulation at various frequencies: 20 (light yellow), 50 (dark yellow) and 130Hz (black), eliciting a few action potentials but increasing membrane potential. (**B**) Number of action potentials elicited during 10s of photo-stimulation (n=10 cells). (**C**) Variation of membrane potential caused by the 10s optogenetic stimulation at various frequencies (n=10 cells). (**D**) Example of response in hChR2 positive STN neuron at onset (left panel) and offset (right panel) of 5min laser stimulation at 130Hz (green line) used during behavioral testing. (**E**): Average changes in firing rate induced by 5 min of 130Hz laser stimulation (n=8 cells). Light dot and lines represent individual values. Dark line and dot correspond to the mean and SEM. (**F**) Normalized frequency in responding (light yellow; n=9) and non-responding (dark yellow; n=7) hChR2-expressing STN neurons during 1min of baseline recording, followed by 180s of HF photo-stimulation (at 130Hz) and 1 min post stimulation. (**G**) Mean normalized frequency during baseline, HF photo-stimulation and post-stimulation in responding and non-responding cells.
Full green area corresponds to 130Hz laser stimulation.
**: p<0.05, **:p<0.01, ***:p<0.001.*

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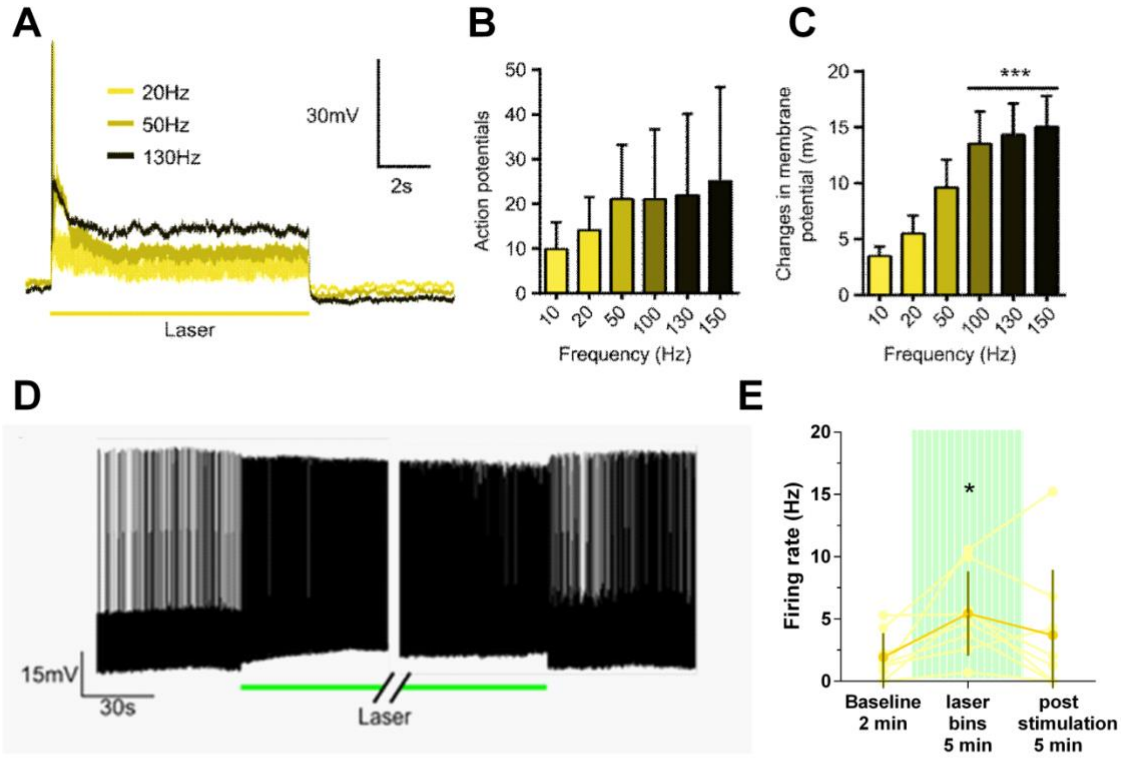
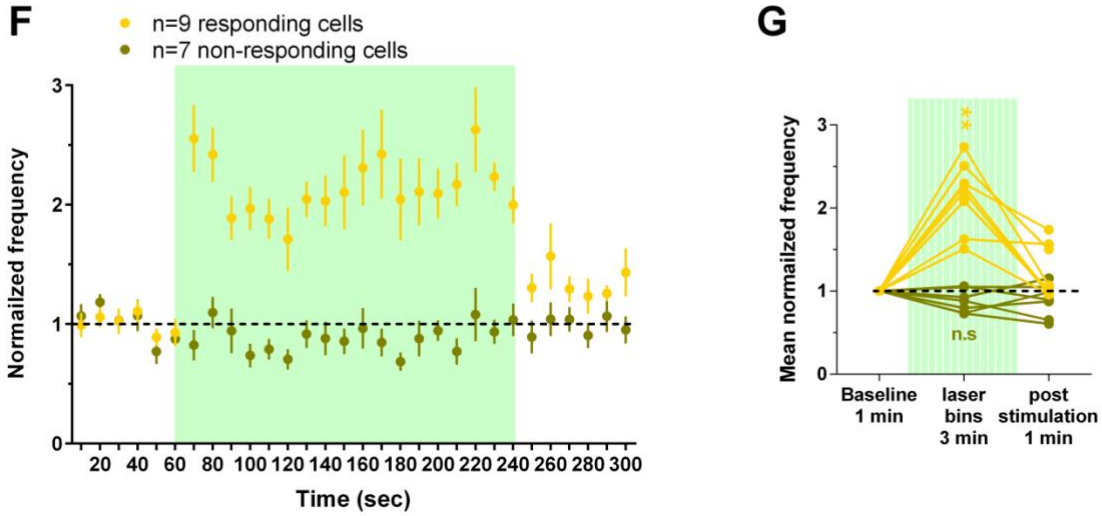
Ex vivo HF photo-stimulation of STN neurons transfected with hChR2



In vivo HF photo-stimulation of STN neurons transfected with hChR2



DISCUSSION

Here, we showed that the presence of a stranger peer, naïve to the drug, leads to a drastic reduction of cocaine consumption in rats after they had previously lost the control over their drug intake during cocaine escalation. In these animals, although the duration of the sessions was then reduced (from 6 to 2h sessions), cocaine intake remained higher than for animals not exposed to escalation procedure, in line with Ahmed et Koob *(34)*. Laser activation, with various parameters, had no effect in EYFP-control, indicating that laser light did not influence cocaine self-administration *per se*. In contrast, both STN optogenetic inhibition and HF-stimulation reduced cocaine consumption after escalation, in line with electric DBS former study *(30)*. This reduction was however not further modulated by the presence of a peer.

Expanding upon our former results showing that the presence of a drug-naïve stranger observer could reduce cocaine intake in rats only exposed to short access sessions *(16)*, we showed here a similar effect, even after they had lost the control over their drug consumption in the escalation procedure. In this present experiment, the stranger peer has been changed every day, preventing a possible habituation effect, as confirmed by the statistical analysis showing no changes across the five repeated sessions. This lack of familiarity with the peer present during drug consumption could be responsible for the beneficial influence of the presence of a peer. Social novelty has indeed been shown to be highly rewarding in laboratory rats *(16, 35)*. Of note, we only tested male individuals, mainly because they are known to be more sensitive to social context *(35)*. To fully address the influence of social context and gender differences on drug use, female subjects would have to be included in future investigations. Indeed, female rats acquire self- administration behavior more rapidly *(36)*, and escalate their drug intake *(37)* quicker than male rat, a feature also observed in human drug users *(38)*. Nevertheless, from our results, the presence of a stranger individual, not consuming the drug, would have a beneficial effect against SUD, in favor of the wider implementation of consumption rooms and socially based SUD therapeutics.

However, the presence of a peer does not always play a protective role on drug use. It was shown that when two rats learn to take drug together, the peer's presence facilitates the acquisition of drug self-administration *(14, 39)*. Similarly, when cocaine availability has been paired with the presence of an observing congener, the latter can later induce drug craving and relapse *(40)*. Altogether, these findings indicate that the presence of a peer differentially influences drug intake, depending on the contingency between social contact and drug use, but also depending on the observer's behavior (*e.g.* also self-administering drug or not) and its relationship with the focal rat (*e.g.* familiarity or dominancy). Like others *(4, 41)*, we thus argue here that considering the social context in animal models of SUD is necessary to address the translational crisis in addiction research, and would, ultimately, lead to the development of effective treatments for SUD.

STN photo-inhibition and HF photo-stimulation drastically reduced cocaine consumption after a loss of control over drug intake. This result is somewhat surprising, because we previously showed that electric STN HF-DBS reduced cocaine intake after escalation only after a protracted abstinence period (35 days), but not when applied immediately after drug escalation *(30)*. Yet, a key finding from Pelloux et al., *(30)* study was the wider development of low frequency neuronal oscillations in the STN during the process of cocaine escalation. These oscillations are known to drive abnormally synchronized oscillations in the basal ganglia network of parkinsonian patients, as well as parkinsonian-like rats *(42)* and are further suppressed by STN pharmacological lesion or reduced by its electrical HF-DBS *(43, 44)*. A recent computational study *(45)* showed that both STN optogenetic HF-stimulation and inhibition are more efficient than its electrical HF-DBS to

suppress this abnormal synchronized oscillatory activity in basal ganglia. Here, the fact that STN optogenetic photo-modulations drastically decreased cocaine consumption immediately after drug escalation could thus rely on this higher efficiency at suppressing STN-driving low frequency oscillatory activity in the basal ganglia network. Nonetheless, from a translational point of view, our results confirm the beneficial effect of STN neuromodulations as a potential treatment for SUD, as suggested previously *(27–32, 46, 47)*.

Surprisingly, photo-inhibition and photo-activation of STN neurons in ARCHT3.0 and hChR2 groups, respectively, both reduce the cocaine intake of animals that had previously escalated their cocaine intake. Of note, our behavioral experiment relies on the use of home-made self-administration chambers and control system. This latter did not allow us to discriminate rats' behaviors during the laser bins (*i.e.* 5min ON and OFF periods). Such kind of analysis would allow us to better understand the similar effect induced by these opposite STN neuromodulations. Yet, it has already been shown that chemically-induced inhibition of the STN or its electrical HF stimulation both reduce motivation to work for cocaine (see for instance *24, 26)*. This was explained by the fact that HF-DBS is consensually thought to inactivate the cell bodies, while stimulating the passing fibers. On the other hand, our electrophysiological recordings of STN neuronal activity confirmed the efficacy of the opsins used in the behavioral experiment and showed inhibition of STN neurons activity in ARCHT3.0 and neuronal activation in hChR2 groups, even when the stimulation was applied at 130 Hz. A general side-effect such as tissue heating due to long-lasting laser activation *(48)* in behavioral experiments seems unlikely since the different patterns of laser activation had no effect in the control group. Nevertheless, in a study using different parameters of laser stimulation, repetitive optogenetic stimulation of CHETA-transfected amygdala inputs to the prefrontal cortex was shown to induce a long-lasting synaptic depression *(49)*. Further electrophysiological recordings of the STN-related network efferent structures would be necessary to highlight their possible inhibition by STN optogenetic HF-stimulation. For example, it is plausible that photo-activation of STN neurons would stimulate GABAergic neurons of the external Globus Pallidus, which would in turn increase their inhibitory tone on STN neurons, thus mimicking the effects of photo-inhibition of STN neurons. Alternatively, both STN optogenetic HF-stimulation and inhibition may effectively suppress abnormal low frequency oscillations (40) associated with cocaine escalation (27), through different mechanisms of action, thereby reversing the loss of control over drug abuse.

In both ARCHT3.0 and hChR2 groups, cocaine intake was reduced in such manner that it could not be further diminished by the presence of a peer. This could be due to a floor effect: the STN optogenetic modulation by itself could indeed have reduced cocaine consumption at its maximum. Indeed, in the control group, the presence of a peer diminished the drug intake to its baseline level, whereas the laser activation similarly reduced it in the 'Without peer' condition in both STN optogenetic HF-stimulation and inhibition groups. At such a low level of drug use, it is therefore possible that any additional protective factor would not reduce further drug consumption. Nevertheless, the fact that peer's presence decreased recreational cocaine consumption (in those animals remaining in short access sessions with no escalation; see Supplemental) only in EYFP-control, but not in ARCHT3.0 rats, makes it unlikely. Thus, the fact that STN optogenetic manipulations and peer's presence induce a similar effect without potentiating each other strongly suggests an interaction between these manipulations, implying that the influence of social contact on drug use relies on a neurobiological network involving the STN. It is noteworthy that in Giorla

et al. *(16)*, STN-lesioned rats only exposed to short access sessions of cocaine self-administration reduced their recreational cocaine intake in presence of a peer, like sham-control animals. This is different to what is reported here when STN neurons were inhibited in the short access group. This discrepancy can be explained by the fact that our experimental designs were drastically different (*e.g.* same stranger observer for 5 days *vs.* novel stranger every day). Notably, it is possible that the STN lesions induced compensatory mechanisms in the STN network, that are not observed during its acute optogenetic inhibition.

Finally, further studies are needed to unravel the role of the STN in the influence of social contact on drug use, as well as the cellular mechanisms involved. It has been shown that social reward induces an oxytocin release in the brain reward system in mice *(50, 51)*, highlighting an overlap between social and drug rewards neural circuitry *(52)*. Interestingly, oxytocin also acts within the STN to locally regulate dopaminergic neurotransmission in rats *(53)*. An acute intra-ventricular injection of oxytocin was shown to be sufficient to normalize cocaine-induced *c-fos* activity in the STN, and to prevent reinstatement of cocaine seeking *(54)*. Oxytocin transmission within the STN may thus play a critical role in the results obtained in the present study.

MATERIALS AND METHODS

Study design

This study aims at assessing whether the presence of a peer and the modulation of STN neuronal activity can modulate cocaine intake in rats having lost their control over cocaine intake. Animals were thus injected AVV in their STN and implanted with optic fiber to allow the pho-modulation of STN activity. For the all behavioral experiment, hChR2 and ARCHT3.0 groups were interleaved with EYFP-control ones. Group assignment for viral injections was also randomly assigned, such as animals were randomized by cage during the all experiment. In case of missing data for an animal in a given session (due, for instance, to catheter disconnection), the average of the two closest days of cocaine injections in the same condition was used. Animals were excluded from the study after histological assessments (see section Histology) by an experimenter blinded to the experimental conditions. Experimental group sizes were determined by taking into account historical accuracy within our group for stereotactic targeting of a given brain region.

Animals

72 male Lister Hooded rats (Charles River Laboratories, Saint-Germain-sur-l'Arbresle, France) weighing ~320 g at their arrival were used in the behavioral experiment. Only male rats were used in this study, because social interaction has been reported to be more rewarding for them than for females *(35)*. Animals were housed in pairs with unlimited access to water and food (Scientific Animal Food and Engineering, Augy, France). They were maintained in a temperature-controlled room, under a 12-hour inverted light/dark cycle, with all experiments conducted during the dark cycle (7am-7pm). Rats used as observer peer and electrophysiological recordings lived in the same conditions and had similar age and weight. Animals were handled daily. All animal care and use were conformed to the French regulation (Decree 2010-118) and approved by the local ethic committee and the French Ministry of Agriculture under the license #3129.01 and followed the 3R European rules.

Virus

To transfect STN neurons, we used AAV5 with the recombinant protein expression under CamKIIa-promoter control (UNC Vector Core, Chapel Hill, USA). The EYFP-control, ARCHT3.0 and hChR2 groups received respectively the following AAV5 constructs: AAV5-CaMKII-EYFP, AAV5-CaMKII-ArchT3.0-p2A-EYFP-WPRE and AAV5-CaMKII-hChR2(E123T/T159C)-p2A-EYFP-WPRE.

Surgeries

Once rats reached ~400g, they were anesthetized with ketamine (Imalgene, Merial, 100 mg/kg, *i.p.*) and medetomidine (Domitor, Janssen 30 mg/kg, *i.p.*), reversed at the end of the surgical procedure with an injection of atipamezole (Antisedan, Janssen, 0.15 mg/kg, *i.m.*). They also received an antibiotic treatment with amoxicillin (Duphamox, LA, Pfizer, 100 mg/kg, *s.c.*) and were administered meloxicam (Metacam, Boehringer Ingelheim, 1 mg/kg, *s.c.*) for analgesia. Animals were then placed in the stereotaxic frame (David Kopf apparatus) for 0.45µL bilateral injections of virus into the STN (with tooth bar set at -3.3 mm), at the following coordinates : anterior/posterior = -3.7 mm ; lateral = ±2.4 mm from bregma ; dorsoventral = -8.35 mm from skull; from Paxinos & Watson, *(55)*. For behavioral tests, optic fibers were implanted 0.4 mm above each injection site and maintained within a head-cap made of dental cement anchored on the skull.

After a week, rats assigned to behavioral testing were also subjected to intra-jugular implantation of a catheter. Using standard surgical procedures *(27)* and the same anesthetic procedure than for the stereotaxic surgery, silicon catheters were inserted into the right jugular vein and exited dorsally between the scapulae. The catheters were then flushed daily with a sterile saline solution containing heparin (Heparin Sodium, Sanofi, 3g/L) and enroflorilexine (Baytril, Bayer, 8g/L) during all the experiment to maintain their patency and to reduce infection risk. Rats were then allowed 10 days for recovery.

At the end of the experiment or after a session in case of doubt, catheters were tested with propofol (Propovet, Abbott, 10 mg/ml) to confirm their patency.

1 - Behavioral experiment

Apparatus

Homemade self-administration chambers (60x30x35cm) divided into two compartments separated by a metallic grid were used in this experiment. The wall of one compartment per cage was equipped with two levers and a central light. All the chambers described above were controlled by a custom-built interface and associated software (built and written by Y. Pelloux).

Experimental procedure of cocaine self-administration

Before the start of the session, catheters of rats were connected to cocaine syringes positioned on motorized pumps (Razel Scientific Instruments, St-Albans, VT, USA), via infusion lines and liquid swivels. The cocaine injection was assigned to one of the two levers (“active lever”) and counterbalanced between rats. An FR1 schedule of reinforcement was used, implying that each lever pressing on the active lever delivered an intravenous infusion of cocaine (250µg per 90µl infusion in 5s) and switched ON the cue-light during cocaine delivery. Each injection was followed by a 20-s time-out period, during which any further lever press on the active lever was recorded as

perseveration but had no consequence. Pressing on the other lever ("inactive lever") was recorded but had no consequence.

Rats were first trained to self-administer cocaine for 2-h daily sessions. Once consumption became stable (<25% of variability in the number of cocaine injections for 5 consecutive days) – considered here as the baseline consumption - they were exposed to 6-h daily sessions of cocaine self-administration for 20 days. Starting at day 10, rats' optics fibers were connected to the optic coupler, with the laser OFF, during the 2 first hours of the sessions, for habituation to the cables.

After completion of the escalation, rats were exposed to 10 others 2-h sessions of cocaine self-administration, with laser stimulation (ON). This condition was composed of two blocks of 5 five consecutive days, during which rats would self-administer cocaine alone or in presence of an observer peer, placed in the other compartment of the cage. Because we already showed that STN optogenetic modulations alter social recognition memory (Vielle et al., unpublished), the observer peer was different every day. The orders of the conditions 'Without peer' vs 'With peer' and the presentation of the observers (5 individuals, in total) were counterbalanced between rats.

Optogenetic manipulation during behavioral testing

Homemade optic fibers (230µm, Thorlabs) were connected to a 200mW 532nm DPSS laser via an optic coupler (FCMM625-50A, Thorlabs). Light pulses were generated under the control of a signal generator (DS8000 Digital Stimulator, World Precision Instruments) at the following parameters: 2ms light pulse at 130Hz pulse train for STN HF-stimulation and 15s light pulse at 0.2Hz pulse train for STN inhibition. During the experiment, the light pulses were discontinuous, with 5-min bins of light ON, interleaved with 5-min bins of light OFF. Before experiments, light power at fiber tip was set, using a power meter (PM20A, Thorlabs), at 10mW for STN HF-stimulation and 5mW for inhibition. Control rats were randomly assigned to one light stimulation condition for the whole experiment. The effects of these light delivery parameters on the STN neurons firing pattern were tested in vitro and in anesthetized animals (see above).

2 – Intracellular *ex vivo* recordings of STN neuronal activity

Animals were anesthetized with intraperitoneal injection of ketamine (Imalgene, Merial, 200 mg/kg) and medetomidine (Domitor, Janssen 60 mg/kg) and perfused intra-cardiacally with ice-cold artificial cerebrospinal fluid (ACSF). 200 µm coronal slices containing the STN were prepared in ice-cold ACSF with a vibratome (1200S, Leica). After being cut, the slices were maintained for 10 minutes at 33ºC and then transferred to holding ACSF at room temperature. ACSF used for perfusion, cutting, and recovery contained NMDG as a sodium substitute and contained, in mM: 92 NMDG, 2.5 KCl, 1.25 NaH2PO4, 30 NaHCO3, 20 HEPES, 25 glucose, 2 thiourea, 5 Na-ascorbate, 3 Napyruvate, 0.5 CaCl2 and 10 MgSO4-7H2O (pH: 7.35). ACSF used for holding slices prior to recording was identical but contained 92 mM NaCl instead of NMDG and contained 1 mM MgCl and 2 mM CaCl2. ACSF used to perfuse slices during recording was maintained at 31ºC and contained, in mM, 125 NaCl, 2.5 KCl, 1.25 NaHPO4, 1 MgCl, 2.4 CaCl, 26 NaHCO3 and 11 glucose. All ACSF preparations were saturated with 95% O2 and 5% CO2. Cells were patched using glass pipets with resistance 3.5-5.0MΩ, filled with internal solution containing, in mM, 140 potassium gluconate, 5 NaCl, 2 MgCl2, 10 HEPES, 0.5 EGTA, 2 ATP, 0.4 GTP (pH: 7.35). Series resistance was monitored during experiment with 10mV hyperpolarizing pulses and maintained below 20MΩ, or cells were discarded. Firing pattern of

STN neurons was determined by injecting depolarizing currents during 500ms with 20pA increment steps.

Cells were visualized on an upright microscope with infrared differential interference contrast and fluorescence microscopy (Olympus, BX51WI). Recordings were made with pClamp 10.3 software using a MultiClamp 700B amplifier set with 4 kHz low-pass Bessel filter and 10 kHz digitization (Molecular Devices, Sunnydale, CA). The light beam of the same laser was directed at the preparation using an 200µm optic fiber submerged into the bath with light beam aimed at the STN. Laser intensities were adjusted to match those used in behavioral experiments.

Cells were imposed a given rhythm through current injection. Cells expressing inhibitory opsins ARCHT3.0 were driven at 10Hz, with injected current intensity corresponding to rheobase + 20-130pA in order to induce stable and robust firing pattern. Cells expressing excitatory opsins hChR2 were driven at 10Hz, intensity set at +0-50pA to elicit a few action potentials in a stable pattern. Stability of the excitation was assessed trough a 2min baseline recording, then we applied the laser patterns used for behavioral experiments for 5min and the cells were monitored for another 5min with the laser OFF.

For experiments testing the influence of light intensity and pulse width, cells were held at -60mV in current clamp configuration and discrete light pulses were applied. When testing the influence of light intensity, pulse duration was set to 15s and 1s for inhibition and stimulation groups, respectively. Testing the influence of the pulse duration was performed at constant light intensity: 10mW (Stimulation) and 5mW (Inhibition).

3 - Extracellular *in vivo* recordings of STN neuronal activity

After at least 3 weeks of recovery following viral injection, *in vivo* electrophysiological recordings were performed in 11 rats (n = 4, ARCHT3.0 group and n = 7, hChR2) to assess the impact of STN optogenetic modulation on STN neurons. Briefly, rats were anesthetized with a mixture of ketamine/xylazine (100/10 mg/kg, *i.p.* supplemented as needed during the recording session) and mounted in a stereotaxic head frame (Horsley-Clarke apparatus; Unimécanique, Epinay-sur-Seine, France). Body temperature was maintained at 36.5°C with a homeothermic blanket controlled by a rectal probe (Harvard Apparatus, Holliston, MA). Single-unit activity of neurons in the STN was recorded extracellularly using glass micropipettes (25-35 MΩ) filled with a 0.5 M sodium chloride solution. Action potentials were recorded using the active bridge mode of an Axoclamp-2B amplifier (Molecular Devices, San Jose, CA), amplified, and filtered with an AC/DC amplifier (DAM 50; World Precision Instruments). Data were sampled on-line at 10 kHz rate on a computer connected to a CED 1401 interface and off-line analyzed using Spike2 software (Cambridge Electronic Design, Cambridge, UK). Optical fiber (230 µm-Thorlabs) was inserted with a 15° angle and the fiber tip was lowered just above the STN and connected to a a 200mW 532nm DPSS laser. The entry point had the following coordinates: AP: -3.7 mm, ML: +1.0 mm. The fiber tip was at a depth of 7.3 mm from the cortical surface.

The pattern of STN neurons activity was analyzed at least 20s before, 180s during and 20s after photo-modulation. The parameters of light stimulation were the same than those used in behavioral experiments: rats expressing ARCHT3.0 opsin were subjected to bins laser stimulation of 15s ON, followed by 5s OFF (corresponding to 9 consecutive bins for 180 s), with the light power at the

tip of optical fiber at 5 mW (±10%). Rats expressing hChR2 opsin were subjected to bins laser stimulation at 130 Hz, with 2 ms width, with the light power set up at 10mW (±10%).

Histology

At the end of the experiments, rats were deeply anesthetized via an injection (through their catheter for animals subjected to behavioral testing or *i.p.* for those subjected to in vivo electrophysiologic recordings), with ketamine (Imalgene, Merial, 200 mg/kg) and medetomidine (Domitor, Janssen 60 mg/kg). Then, they were perfused intra-cardiacally with 4% paraformaldehyde dissolved in PBS. Brains were extracted and after cryo-protection in 30% sucrose, they were frozen into liquid isopentane (-80°C), and finally cut in 40µm coronal slices with a cryostat.

In hChR2 rats, native fluorescence, while present, required immuno-staining to control the exact boundaries of viral expression. After PBS rehydration (3 x 5 min), brain sections underwent a 90 min permeation step (PBS, 1% bovine serum albumin (BSA) 2% normal goat serum (NGS), 0.4% TritonX-100), PBS washes (3 x 5 min) and incubation with primary antibody (mouse anti-GFP, A11120, Life technologies; 1:200, in PBS 1% BSA, 2% NGS, 0.2% TritonX-100) at 4°C overnight. Sections were then washed with PBS (3 x 5 min), followed by 2h incubation at room temperature with secondary antibody (Goat anti-mouse Alexa 488, A11011, Life technologies, 1:400 in PBS 1% BSA, 2% NGS) and finally washed with PBS (3 x 5 min).

Brain sections of all animals were then mounted on to glass slides with homemade mounting medium and examined with an epifluorescence microscope (Zeiss, Imager.z2). Animals with no fluorescence in the STN, or with fluorescence partially or totally out of the STN were excluded from the results, as well as rats presenting optic fibers misplacement (n=15 in tot al).
Representative correct optic fibers' location and fluorescence expression are illustrated in **Fig. 1A**.

Statistical analysis

All variables are expressed as mean number ± SEM, statistical tests were two-tailed, and the p-value threshold was set at α=0.05. Statistical tests and graph were performed using GraphPad Prism 6 (version 6.07).

1 - Behavioral analyses

Mixed 3- or 2-way ANOVA, followed by Tuckey's or Dunnett's post hoc, were used, when appropriate, with optogenetic groups (ARCHT3.0, hChR2 and EYFP-control) as between factors, and sessions or conditions (baseline, escalation: 'Esc1', 'Esc2', 'Esc3', 'Esc4', 'With peer' and in 'Without peer') as within factors. For the cocaine escalation only, we compared the mean number of cocaine injections during 6h sessions. To compare cocaine consumption between blocks of escalation and others blocks, we used the number of drug injections received during the two first hours of escalation, since cocaine escalation has an effect from the beginning of drug long access sessions *(34)*.

2 – *ex vivo* and *in vivo* electrophysiological data analyses

Electrophysiological data were analyzed with Friedman test followed by Dunn's post-hoc test within groups. Variations in cells properties were analyzed with Kruskall-Wallis test or mixed ANOVA.

Supplementary Materials

We controlled the effects of the escalation on other EYFP-control (n=7) and ARCHT3.0 (n=6) animals, subjected to the same protocol, except for the 20 days of escalation procedure, during which they were maintained in short access (2h) sessions (results are illustrated in **Fig. S1**). We compared their baseline consumption and cocaine intake during the two first hours of the following 20 sessions using 3-way mixed ANOVA, with access duration ('long access'; 6h and 'short access'; 2h) and optogenetic groups (ARCHT3.0 and EYFP-control) as between factors and sessions as within factor. Results of the 3-way ANOVA for the baseline and escalation are respectively shown in **Table S1** and **Table S2**. Then, we compared cocaine intake in rats maintained in short access sessions, using mixed 3- or 2-way ANOVA, followed by Tuckey's or Sidak's post hoc, with optogenetic groups (ARCHT3.0, hChR2 and EYFP-control) as between factors, and sessions or conditions (baseline, 'Esc4', 'With peer' and 'Without peer') as within factors. Results of the three-way analysis are shown in **Table S4**.

To check whether or not the presence of a peer and the optogenetic modulation of the STN specifically modulate responses towards the drug, with limited side-effects, we analyzed the number of perseverations (*i.e.* presses on the active lever during the 20-s time-out period; reflective of impulsive behavior) and errors (*i.e.* presses on the inactive lever; reflecting a possible aspecific general hyperactivity that is not goal directed towards the drug) performed by the animals during the main behavioral experiment (see **Fig. S2**) using mixed 3-way ANOVA, with conditions (baseline, 'Esc4', 'With peer' and 'Without peer') and sessions as within factors, and optogenetic groups as between factors. Results of the statistical analysis for the perseverations and errors are respectively shown in **Table S5** and **Table S6**.

Supplementary figures

Fig. S1. Peer's presence reduces recreational cocaine intake; an effect suppressed by STN photo-inhibition.

(**A**) Mean number of cocaine injections received during the two first hours of the baseline and the twenty long (6h; n=9 ARCHT3.0; plain red dots and n=9 EYFP-control; plain blue dots) or short (2h; n=6 ARCHT3.0; red empty dots and n=7 EYFP-control; blue empty dots) access sessions access to the drug. (**B**) Cocaine injections received during the two hours of the baseline, 'Esc4', and with the laser activated (in green): 'With peer' and 'Without peer'. (**C**,**D**) Mean number of cocaine injections received during the two hours of the sessions in 5-sessions blocks (baseline, 'Esc4', 'With peer', 'Without Peer') respectively in EYFP-control and ARCHT3.0 animals. (**E**) Mean number of cocaine injections received during the two first hours of the sessions with the laser activated 'With peer' and 'Without peer' in EYFP-control and ARCHT3.0 rats. Each dot represents individual mean per block of 5 sessions in **C**,**D**,**E**. Green area: laser ON. *£££: p<0.01 compared with long access group.*:p<0.05, **:p<0.01, ***:p<0.001.*

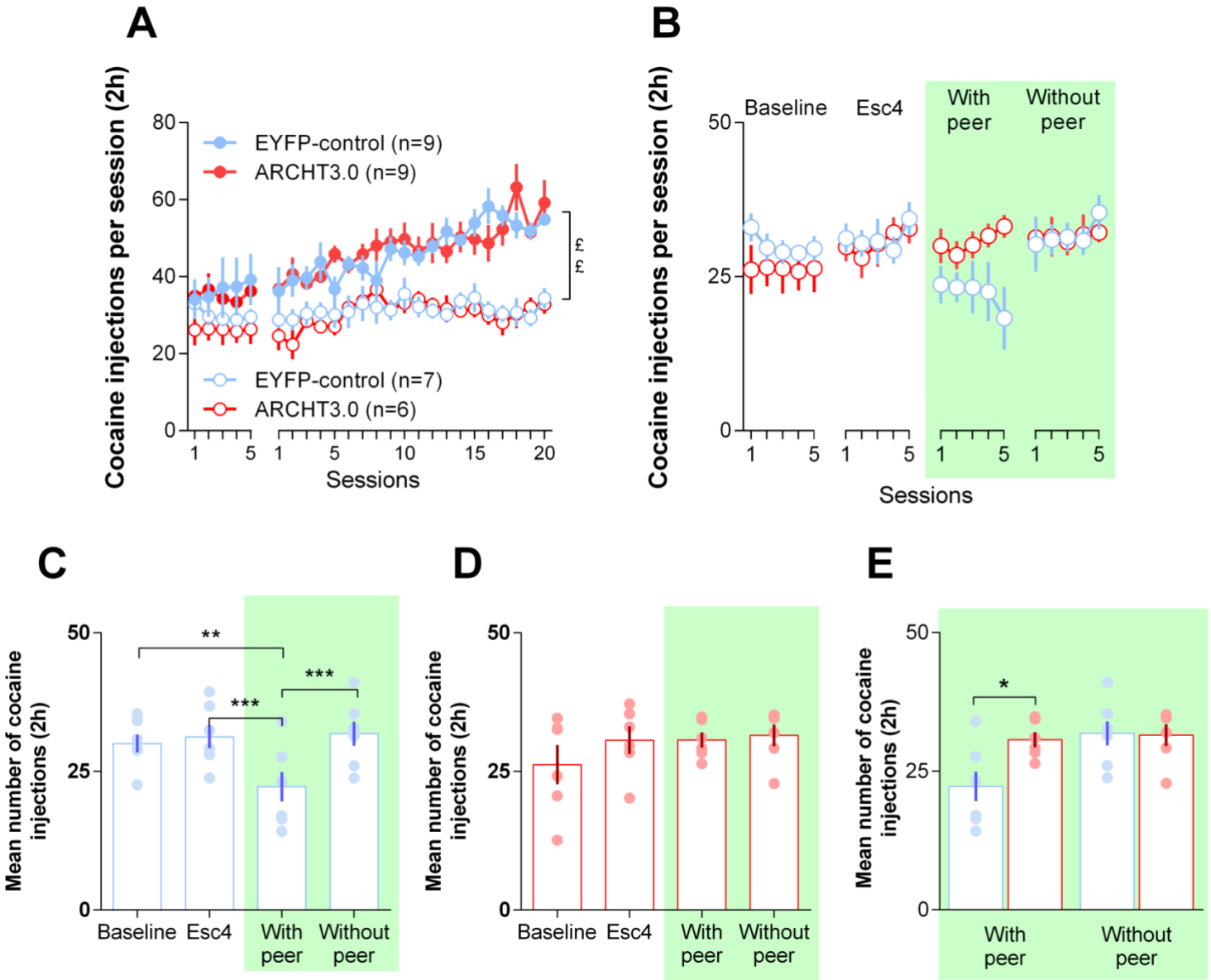


Fig. S2. Peer's presence and STN optogenetic modulations modulated neither errors nor perseverations.

Number of perseverations (**A**) and errors (**B**) performed by EYFP-control (n=9, blue), ARCHT3.0 (n=9, red) and hChR2 (n=9, yellow) rats within the two first hours of sessions during the baseline, the end of the escalation ('Esc4'), and during laser activation (in green) 'With peer' and 'Without peer'.

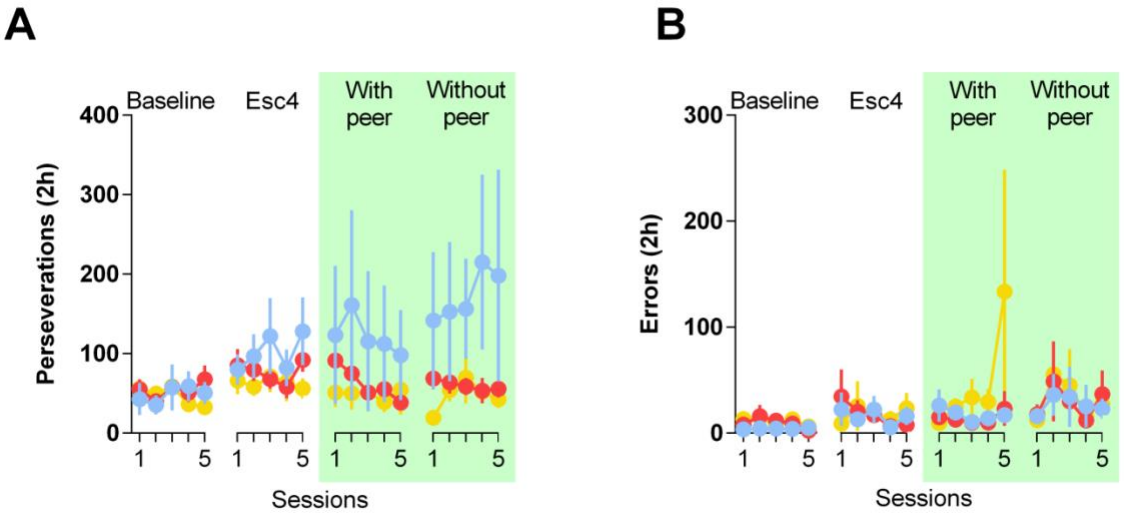
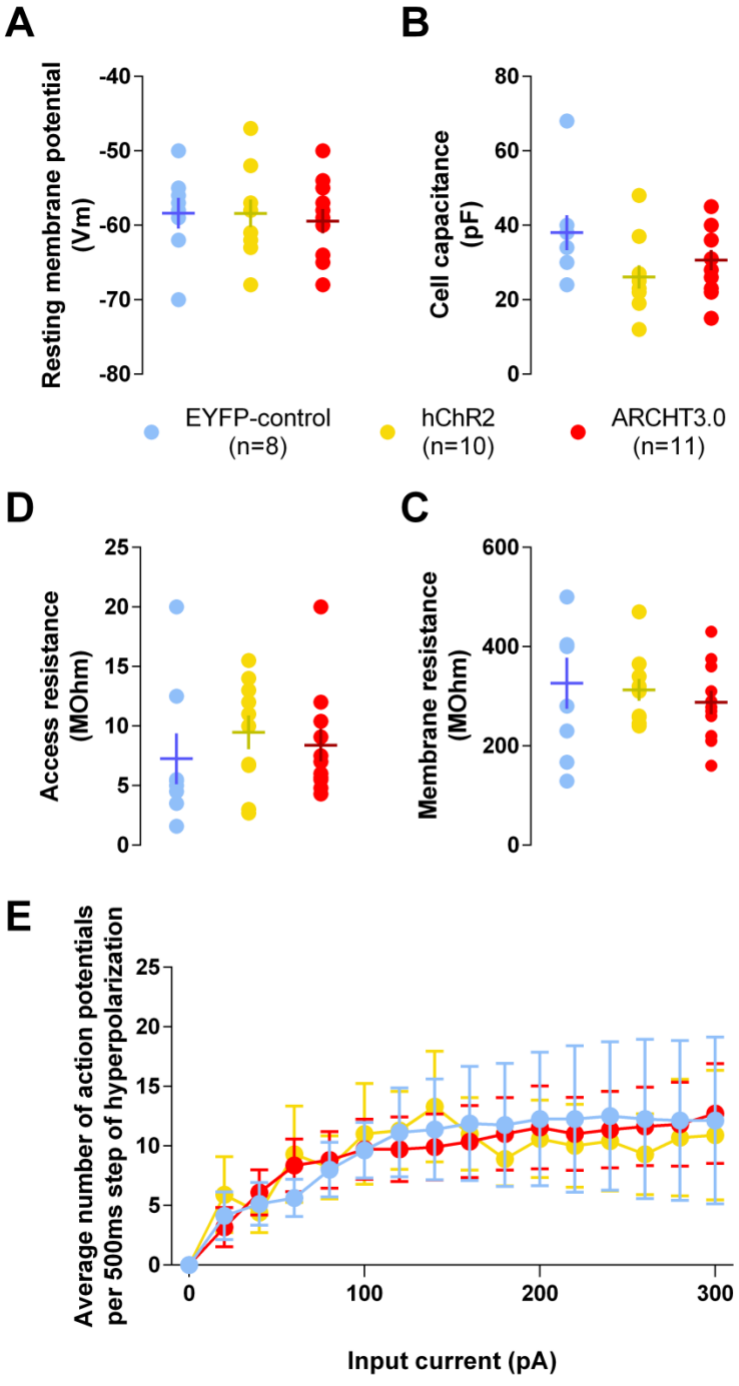


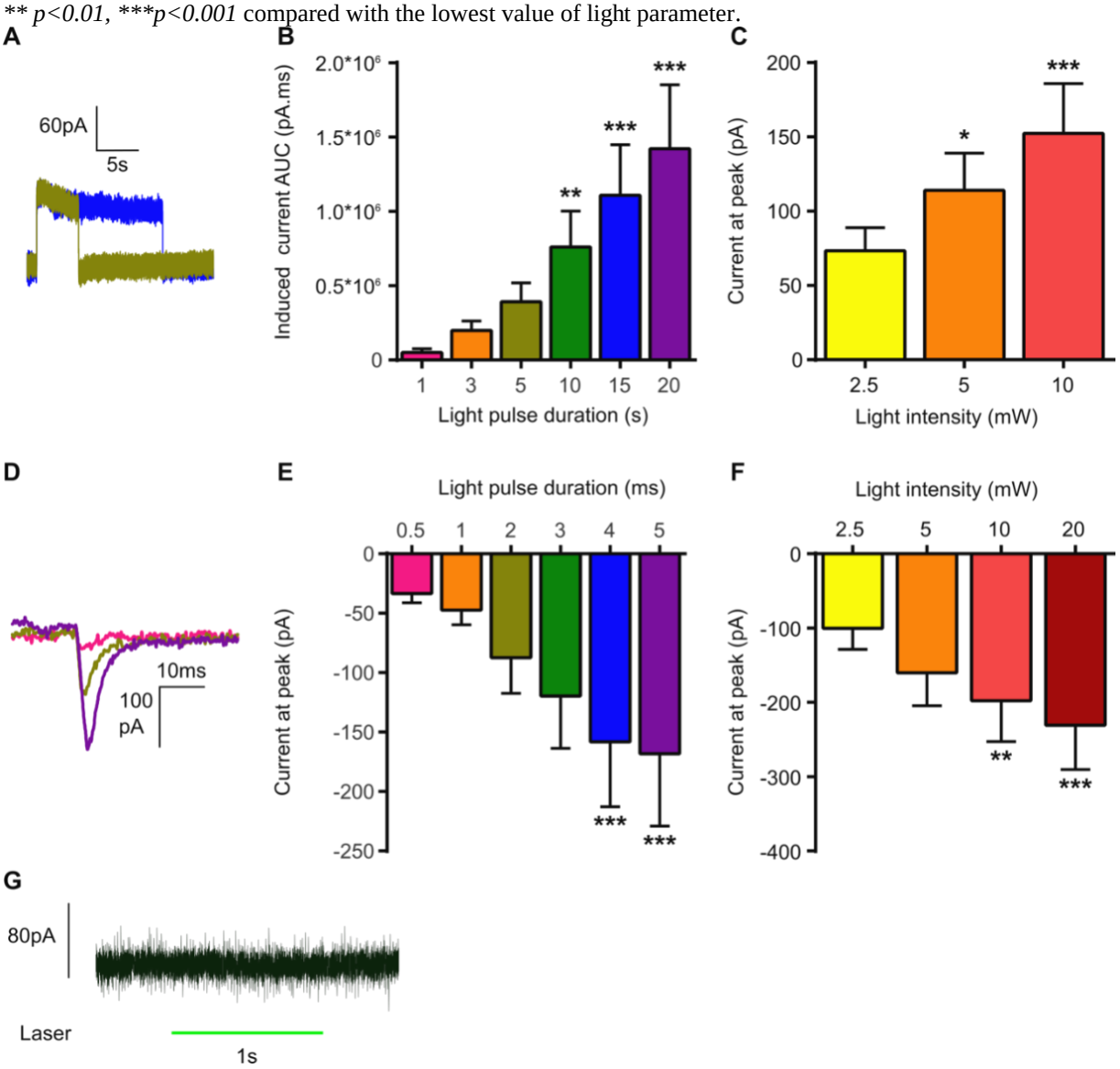
Fig. S3. Cells properties of STN neurons after viral infection recorded in whole cell configuration.

Cell resting membrane potential (**A**), cell capacitance (**B**), membrane resistance (**C**), access resistance (**D**) and average number of action potentials (APs) elicited by a 500ms step of hyperpolarization (**E**) for controls EYFP-control (n=8, blue), hChR2 (n=10, yellow) and ARCHT3.0 (n=11, red) cells.



627 **Fig. S4. Evaluation of light stimulation parameters in STN neurons after viral infection.**

628 Example of light induced inhibitory currents in one STN neuron expressing ARCHT3.0 by a 5s (brown) and 15s (blue)
629 pulse (**A**). Average area under the curve (AUC) calculated for different durations of light pulses with intensity set at
630 5mW in ARCHT3.0 cells (n=11) (**B**). Average current induced in ARCHT3.0 cells by a single 15s pulse applied with
631 different light intensities (**C**). Example of induced depolarizing current in hChR2 cells induced by a 0.5ms (pink), 2ms
632 (brown) or 5ms (purple) light pulse (**D**). Average induced current in hChR2 cells (n=10) depending on the duration of
633 the light pulse with intensity set at 10mW (**E**). Induced current in hChR2 cells by a 1s light pulse as a function of light
634 intensity (**F**). Typical response of a neuron expressing EYFP-control facing a 1s laser pulse at 20mW (**G**).* *p<0.05*,
635 ** *p<0.01*, ***p<0.001* compared with the lowest value of light parameter.



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Supplementary tables:

Table S1. Results of the mixed 3-way ANOVA on the number of cocaine injections during baseline consumption with duration of access to the drug (long; 6h and short 2h access) and optogenetic groups (ARCHT3.0 and EYFP-control) as between factors and sessions as within factors.

Effect	F(DFn,DFd)	p-value
optogenetic groups	$F_{(1,27)}$=0.136	0.716
access duration	$F_{(1,27)}$=3.512	0.088
sessions	$F_{(1.62, 43.61)}$=0.490	0.576
optogenetic groups x access duration	$F_{(1,27)}$=0.558	0.462
optogenetic groups x sessions	$F_{(1.62, 43.61)}$=0.736	0.457
access duration x sessions	$F_{(1.62, 43.61)}$=3.37	0.812
optogenetic groups x access duration x sessions	$F_{(1.62, 43.61)}$=0.415	0.620

Table S2. Results of the mixed 3-way ANOVA on the number of cocaine injections during the 20 following sessions with duration of access to the drug (long; 6h and short 2h access) and optogenetic groups (ARCHT3.0 and EYFP-control) as between factors and sessions as within factors.

Effect	F(DFn,DFd)	p-value
optogenetic groups	$F_{(1,27)}$=0.0001	0.993
access duration	$F_{(1,27)}$=62.638	<0.0001
sessions	$F_{(4.91, 132.65)}$=6.22	<0.0001
optogenetic groups x access duration	$F_{(1,27)}$=0.199	0.659
optogenetic groups x sessions	$F_{(4.91, 132.65)}$=1.038	0.398
access duration x sessions	$F_{(4.91, 132.65)}$=3.37	0.007
optogenetic groups x access duration x sessions	$F_{(4.91, 132.65)}$=0.768	0.572

Table S3. Results of the mixed 3-way ANOVA on the number of cocaine injections with conditions (baseline, 'Esc4', 'With peer', 'Without peer') and sessions as within factors and optogenetic groups as between factors.

Effect	F(DFn,DFd)	p-value
optogenetic groups	$F_{(2,24)}$=2.605	0.095
conditions	$F_{(3,72)}$=31.582	<0.0001
sessions	$F_{(4,96)}$=1.062	0.380
optogenetic groups x conditions	$F_{(6,72)}$=2.524	0.028
optogenetic groups x sessions	$F_{(8,96)}$=0.549	0.817
conditions x sessions	$F_{(12,288)}$=1.258	0.283
optogenetic groups x conditions x sessions	$F_{(24,288)}$=1.317	0.220

Table S4. Results of the mixed 3-way ANOVA on the number of cocaine injections in rats maintained in short access with conditions (baseline, 'Esc4', 'With peer', 'Without peer') and sessions as within factors and optogenetic groups as between factors.

Effect	F(DFn,DFd)	p-value
optogenetic groups	$F_{(1,11)}$=0.127	0.728
conditions	$F_{(3,33)}$=0.698	0.006
sessions	$F_{(4, 44)}$=0.553	0.698
optogenetic groups x conditions	$F_{(3,33)}$=5.781	0.003
optogenetic groups x sessions	$F_{(4, 44)}$=0.460	0.765
conditions x sessions	$F_{(12, 132)}$=0.568	0.864
optogenetic groups x conditions x sessions	$F_{(12, 62)}$=0.777	0.674

Table S5. Results of the mixed 3-way ANOVA on the number of perseverations with conditions (baseline, 'Esc4', 'With peer', 'Without peer') and sessions as within factors and optogenetic groups as between factors.

Effect	F(DFn,DFd)	p-value
optogenetic groups	$F_{(2,24)}$=1.155	0.332
conditions	$F_{(3,72)}$=1.165	0.300
sessions	$F_{(4,96)}$=0.168	0.905
optogenetic groups x conditions	$F_{(6,72)}$=1.028	0.382
optogenetic groups x sessions	$F_{(8,96)}$=0.996	0.431
conditions x sessions	$F_{(12,288)}$=1.427	0.241
optogenetic groups x conditions x sessions	$F_{(24,288)}$=0.889	0.511

Table S6. Results of the mixed 3-way ANOVA on the number of errors with conditions (baseline, 'Esc4', 'With peer', 'Without peer') and sessions as within factors and optogenetic groups as between factors.

Effect	F(DFn,DFd)	p-value
optogenetic groups	$F_{(2,24)}$=0.536	0.592
conditions	$F_{(3,72)}$=3.119	0.050
sessions	$F_{(4,96)}$=1.145	0.319
optogenetic groups x conditions	$F_{(6,72)}$=0.780	0.549
optogenetic groups x sessions	$F_{(8,96)}$=0.760	0.532
conditions x sessions	$F_{(12,288)}$=1.214	0.307
optogenetic groups x conditions x sessions	$F_{(24,288)}$=0.680	0.615

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