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1 **Title**

2 Subthalamic nucleus mediates the modulation on cocaine self-administration induced by ultrasonic
3 vocalizations playback in rats

4 **Running Head:** STN and USV on cocaine intake

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1 **Abstract**

2 Drug intake is known to be under the influence of social context. We have recently shown that
3 presence of a peer influences drug intake in both rats and humans. Whether or not social acoustic
4 communications between the peers play a role during cocaine or sucrose self-administration (SA)
5 was investigated here, using playback of ultrasonic vocalizations (USV) at 50- and 22-kHz,
6 conveying respectively positive and negative internal affective states in adult rats. To assess the
7 neurobiological substrate of a potential USV influence on drug and food intake, we tested the
8 effects of subthalamic nucleus (STN) lesions, given its role in emotional and motivational
9 processes. In sham-control rats, playback of USV associated with positive affective states induced
10 long-term decreased cocaine consumption, while USV associated with negative affective states
11 induced short-term increase. Interestingly, no effect of USV playback was observed on sucrose
12 intake, whatever the frequency. STN lesions abolished the influence of USV on cocaine intake,
13 highlighting the influence of STN in emotional processes induced by USV emitted by a peer. These
14 results show how acoustic social communication is important to regulate drug intake in rats and
15 how STN modulation could interfere with addiction processes.

16 **Keywords:** addiction, basal ganglia, conditioned place aversion, conditioned place preference,
17 social influence, sucrose.

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1 **Introduction**

2 Drug use disorders are a major worldwide public health problem in our society (APA 2013).
3 Recently it was demonstrated that the so-called “proximal social factors”, that are the social factors
4 present at the time of drug exposure such as the presence and behavior of peers, play an important
5 role in both human drug consumption and in rats self-administering drugs (Strickland & Smith 2013
6 for review; Giorla et al. 2018). Epidemiologic studies have shown that people associated with peers
7 who use drugs have higher probabilities to use drugs than people not associated with drug users
8 (Bahr, Hoffmann, & Yang 2005). Moreover in a semi naturalistic bar setting (Larsen et al. 2009)
9 and in a real bar study (Larsen et al. 2012) it was shown that subjects in company of a heavy-
10 drinking peer consumed more alcohol than subjects in company of a light-drinking peer or an
11 abstinent peer. In the rat, the peer presence influences drug intake in a different manner if the peer is
12 abstinent or is also taking drugs (see Strickland & Smith 2013 and Discussion). Abuse of food,
13 particularly of highly palatable sweet food rich in sugars, is another serious burden of our western
14 countries (for reviews see Ahmed, Guillem, & Vandaele 2013 and DiNicolantonio, O’Keef, &
15 Wilson 2017). Although there is no explicit definition of “proximal social factors” in eating-related
16 behaviors, it is well-known that presence and behavior of peers influence also our eating behavior, a
17 daily activity often shared with others. The probability to become obese is higher if a friend, a
18 sibling or a spouse has become obese (Christakis & Fowler 2007). Moreover, in presence of a non-
19 eating observer, people eat less than when alone (Roth et al. 2001), and when people eat together
20 their intake differs from when alone and depends on the others intake (Herman 2015). The social
21 facilitation phenomenon of eating behavior is well-known in different animal species such as rats
22 (Harlow 1932), chickens (Tolman 1965), dogs (Ross & Ross 1949) and monkeys (Harlow & Yudin
23 1933). Gardner & Engel (1971) showed that a rat increases its lever-pressing behavior for standard
24 laboratory food in presence of a peer having access to food. No further studies evaluated the effect
25 of the presence and behavior of a peer on food self-administration (SA), especially palatable sweet

1 food that, like drugs, can induce a loss of control over consumption in both animals and humans
2 (Ahmed, Guillem, & Vandaele 2013; DiNicolantonio, O’Keef, & Wilson 2017). Given that the
3 influence of proximal social factors in animals has been only recently studied, a few questions
4 remain to be addressed, such as their influence on both drug and food SA and the ways of
5 communication used between peers to influence each other.

6 Adult rats use 50- and 22-kHz ultrasonic vocalizations (USV) to respectively communicate to peers
7 their positive and negative affective internal states (Brudzynski 2013). This acoustic communication
8 could thus play an important role in the effects mediated by the peer presence on drug and food
9 intake. Although recording USV of rats self-administering drugs and palatable food as sucrose is
10 common to assess the emotional status of the animals (Browning et al. 2011; Barker, Simmons, &
11 West 2015), no study has ever evaluated the effects of USV playback on drug and food intake,
12 albeit other recent studies have shown that USV playback in rats is an essential tool to investigate
13 different human brain disorders, especially those involving social communication deficits (Wöhr &
14 Schwarting 2017). Moreover there are no studies regarding the neurobiological basis of the
15 proximal social factor’s effects on drug and food consumption. However, one can hypothesize that
16 emotional and motivational processes may interact in the influence of proximal social factors on
17 goal-directed behaviors. Therefore, the subthalamic nucleus (STN), a cerebral structure belonging
18 to the basal ganglia, could represent an interesting candidate because it was previously shown
19 to modulate affective responses to emotional stimuli (Pelloux et al. 2014) and its inactivation
20 reduces motivation for cocaine while increasing that for food only in a Progressive Ratio schedule
21 of reinforcement but not in standard SA conditions (Baunez et al 2005; Rouaud et al 2010). In this
22 study we thus aimed at evaluating the effects of 50- and 22- kHz USV playback on both cocaine
23 and sucrose SA, and in both sham-control and STN-lesioned rats.

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1 **Material and methods**

2 **Animals and Surgery**

3 A total of 37 male Lister Hooded rats (Charles River Laboratories, Saint-Germain-sur-l'Arbresle,
4 France) weighing ~290 g at their arrival were used. Animals were housed in pairs, maintained in
5 animal facility and subjected to intra-jugular implantation of catheters and/or STN ibotenic
6 excitotoxic lesions (rats underwent oral sucrose SA were only subjected to STN lesions) as
7 previously described (Baunez et al. 2005; Pelloux et al. 2014; see Supporting Information). All the
8 rats had free access to standard laboratory food pellets (Scientific Animal Food and Engineering,
9 Augy, France) and water, except during the experimental sessions. All animal procedures were
10 approved by local ethic committee and the University of Aix-Marseille (#3129.01), and were in
11 accordance with the European Community Regulations for Animal Use in Research (CEE N°
12 86/609) and the French regulation (Decree 2010-118).

13 **Experiments overview**

14 In total, 4 different experiments were performed: 1) evaluation of the effects of 50- and 22-kHz
15 USV playback on cocaine SA; 2) evaluation of the effects of 50- and 22-kHz USV playback on
16 sucrose SA; 3) assessment of the rewarding properties of the 50-kHz USV using the Conditioned
17 Place Preference (CPP) paradigm; 4) assessment of the aversive properties of the 22-kHz USV
18 using the Conditioned Place Aversion (CPA) paradigm. To limit the number of animals, the rats
19 that first underwent the cocaine SA procedure were also tested in the CPA paradigm, and the rats
20 tested in the CPP paradigm were subsequently used for the sucrose SA experiment (except 3 sham-
21 control rats used only for the CPP paradigm). Between the two experimental procedures all animals
22 were maintained for 2-3 months in standard housing conditions, without any exposure to cocaine,
23 sucrose or USV playback. The rats were randomly allocated into the different experimental groups
24 when the study was designed with the aim to use the smallest number of animals sufficient to

1 consider the results reliable (*cocaine SA/CPA*: sham-control rats, N=6; STN-lesioned rats,
2 N=9; *CPP*: sham-control rats, N=11; STN-lesioned rats, N=9; *sucrose SA*: sham-control rats, N=8;
3 STN-lesioned rats, N=9).

4 **Playback display of 50- and 22-kHz USV over cocaine and sucrose SA sessions**

5 *Apparatus*: four standard SA chambers (Camden Inst, 24- cm length, 25- cm width, and 26-cm
6 height) placed in sound- and light attenuating cubicles were used. Each chamber was equipped with
7 two retractable levers positioned on the right-hand wall, two white stimulus lights (one above each
8 lever), an extractable dipper for the delivery of liquids in the food magazine (placed between the 2
9 levers), and a white house light located in the ceiling. Acoustic stimuli were presented through
10 ultrasonic loudspeakers (Ultrasonic Dynamic Speaker Vifa, Avisoft Bioacoustics, Glienicke,
11 Germany) placed in an opening in the middle of the chambers ceiling (see Supporting Information).

12 *Procedures*: testing began 10 days after the surgery. Different animals were used to test the USV
13 playback on cocaine and sucrose SA. Daily 1h sessions took place during the dark phase, between
14 09:00 and 17:00 hours. Immediately before the start of sessions, catheters of rats that were self-
15 administering cocaine were connected – through infusion lines and liquid swivels - to cocaine
16 syringes positioned on motorized pumps (Razel Scientific Instruments, St. Albans, VT, USA)
17 placed outside of the chambers. At the start of each session a white house light was turned on for
18 the duration of the session and two lever were extended. Depending on the experiment, pressing the
19 active lever (counterbalanced between rats) delivered either an intravenous infusion of cocaine
20 (250 μ g/90 μ l/5s) or a 10% solution of sucrose accessible in the dipper (300 μ l/dipper/5s) and
21 switched on the white cue light above the active lever during the reward delivery; pressing the
22 inactive lever was recorded but had no consequence. A 20-s time-out period was imposed (and
23 maintained during all the phases of the experiment), during which any further lever press was
24 recorded as perseverative response but had no consequence. Few sessions were run under a
25 continuous schedule of reinforcement (FR1; every lever press on the active lever was reinforced).

1 Then progressively over days, the number of lever presses required to obtain a reward was
2 increased to FR2 and FR5 (the 2nd and the 5th lever press was respectively reinforced). When rats
3 reached a stable cocaine or sucrose SA (baseline condition: $\leq 20\%$ of variability in the number of
4 rewards for 5 consecutive days), they were exposed – for 3 different blocks of 5 consecutive days
5 and in the following order – to the playback of 50-/22-kHz USV, background noise, and 22-/50-kHz
6 USV, counterbalancing between rats the starting order of the USV (50- *versus* 22-kHz). During the
7 playback sessions, the first lever press of each FR5 ratio resulted in the playback of the appropriate
8 file (1.5 sec length) and the lever pressing counting was not interrupted during the playback (see
9 Acoustic Stimuli section and Supporting Information for further details on the USV used and the
10 playback procedure). The background noise was used as control stimulus of possible unspecific
11 effects not due to the communicative function of the USV. The chambers, pumps and loudspeakers
12 were controlled by a home-built interface and by a home-written PC-program (built and written by
13 Y. Pelloux).

14 **Assessment of USV emotional properties through the Conditioned Place Preference/Aversion** 15 **(CPP/CPA) paradigm**

16 The rewarding and aversive properties of the USV were assessed using the CPP/CPA paradigm (for
17 a review see Tzschentke 2007).

18 *Apparatus:* As previously described (Rouaud et al. 2010), a gray Perspex box (70 cm length x 30
19 cm width x 40 cm height) divided in two main lateral compartments (30 x 30 x 40 cm each) with a
20 small intermediate chamber (10 cm in length) was used for the experiments. The two lateral
21 compartments differed only in the spatial organization of two vertical columns (i.e. conditioned
22 stimulus). Acoustic stimuli were played back through the same ultrasonic loudspeakers used for the
23 SA sessions, placed above the middle of the lateral compartments of the apparatus. A camera (PNP-
24 90/TL, Swann Communications Ltd, Central Milton Keynes, UK) connected to a Computer Video

1 Interface (EzCAP USB Video Grabber, EZCAP.TV, Newton Stewart, UK) was mounted above the
2 apparatus for video recording.

3 *Procedures:* different animals were used for the CPP and the CPA experiment. Rats that underwent
4 the CPP experiment were exposed to the 50-kHz USV, while the ones that underwent the CPA
5 experiment were exposed to the 22-kHz USV. Briefly, on the preconditioning day, rats explored the
6 whole apparatus for 15 min and the time (in sec) spent in each compartment was measured via the
7 video recording. From the following day and for 8 consecutive days (conditioning phase: 30
8 min/session/day), animals were confined - on alternate days- one day in one of the two lateral
9 compartments and exposed to the USV (50- or 22- kHz USV), and the day after in the other lateral
10 compartment and exposed to the background noise [counterbalancing between rats the lateral
11 compartment paired with the USV and the starting order of the acoustic stimuli (USV vs
12 background noise)]. The same acoustic files (1.5 sec length) played back during the SA sessions
13 were displayed during each conditioning session, every 5 minutes for a total of 5 playbacks/session
14 (see Acoustic Stimuli section and Supporting Information for further details on the USV used and
15 the playback procedure). The day after the end of the conditioning, on the testing day, rats were
16 again let free to explore the apparatus for 15 min without any playback and the time spent in each
17 compartment was measured via the video recording, as in the pre-conditioning day. The rewarding
18 or aversive properties of the USV were inferred by the measurement of the time spent during the
19 test in the compartment previously paired with the USV in comparison with the time spent in the
20 same compartment in the preconditioning day.

21 **Acoustic Stimuli**

22 Animals were tested for their auditory capacity before the beginning of the experiments, after
23 recovery from surgery; all rats showed an excellent startle response after a sudden sound
24 stimulation and then no rat was excluded from the experiments due to hearing deficits. The USV
25 displayed during the experiments were recorded from 2 adult male Lister Hooded rats, unfamiliar to

1 the tested rats and not included in any experimental procedure. A long (1.5s) 22-kHz USV call was
2 recorded from a rat that received an electric foot shock (0.5 mA for 2s) in a SA chamber before the
3 recording. For playback a 50-kHz USV, the length had to be identical to the 22-kHz USV. Since
4 such long 50-kHz USV do not exist, a series of 50-kHz calls (total file length: 1.5s; total calling
5 time: 0.45s) was recorded from a rat during the exploration of a SA chamber containing food, water
6 and scents from its cage mate after depriving him overnight from all of the above reported
7 stimuli (see Supporting Information for further details on the USV used).

8 A Condenser Ultrasound Microphone (CM16/CMPA-48, Avisoft Bioacoustics) and its Computer
9 Audio Interface (QUAD-CAPTURE USB 2.0 Audio Capture, Roland Corporation, Los Angeles,
10 CA, U.S) were used to record the USV with a sampling rate of 192 kHz in 16-bit format using
11 Avisoft RECORDER (Version 4.2, Avisoft Bioacoustics).

12 **Histology**

13 See Supporting Information

14 **Statistical analysis**

15 The statistical analyses have been performed using SPSS 20 software and all variables were
16 expressed as mean number \pm SEM.

17 *Playback display of 50- and 22-kHz USV over cocaine and sucrose SA sessions:* in both sham-
18 control and STN-lesioned rats the number of rewards reached during the sessions was analyzed via
19 a two-way repeated measures ANOVA with playback condition (4 levels: baseline, background
20 noise, 50- and 22- kHz USV) and session (5 levels: five days for each playback condition) as
21 within-subject factors. The average of the baseline condition was also compared with the number of
22 rewards obtained during the 1st day of the other playback conditions, through a one-way repeated
23 measure ANOVA. Significant ANOVA effects ($p \leq 0.05$) were followed by post hoc Fisher's LSD
24 tests. No statistical methods were used to predetermine sample size, but the samples are comparable

1 to those reported in previous studies. However, power analysis (1-group, 2-tails and equal variance
2 model) was performed, using the Free Online Power and Sample Size Calculator available at
3 powerandsamplesize.com, to confirm that our sample sizes were sufficient to detect reliable
4 changes in cocaine intake under USV playback influence. Comparisons between groups on the
5 number of cocaine/sucrose rewards reached by rats during the 1st day of playback and for the
6 average of each 5-day block, were made using independent t-test (2-tails).

7 *Assessment of the USV emotional properties through the Conditioned Place Preference/Aversion*
8 *(CPP/CPA) paradigm*: we calculated the preference/avoidance score as the difference between the
9 time (in sec) spent on the testing day in the compartment previously associated with the USV and
10 the time spent in the same compartment on the preconditioning day. A positive score indicates a
11 preference, while a negative score an avoidance, for the compartment previously paired with the
12 USV. The conditioning effect was evaluated within each group via the non-parametric Wilcoxon
13 signed-rank test for paired samples, while differences between groups were assessed through the
14 non-parametric Mann-Whitney U test for independent samples.

15 **Results**

16 **Histology**

17 Five rats (2 and 3 from the cocaine SA/CPA and sucrose SA/CPP experiments respectively) were
18 excluded from the STN-lesioned groups results and statistical analyses due to lesions either
19 unilateral, too restricted or outside the STN. The sites and extent of lesions were characterized by a
20 neuronal loss and associated gliosis (see Fig. 1 for a representative intact and lesioned STN).

21 **Effects of 50- and 22-kHz USV playback on cocaine SA sessions**

22 Thirteen rats were included in the analysis (sham-control group: N=6; STN-lesioned group: n=7).

1 *Sham-control rats*: the analysis of the effects of the USV playback across all sessions revealed a
2 significant effect of playback condition [ANOVA: $F(3,15)=6.711$, $p=0.020$], but not of session
3 [ANOVA: $F(4, 20)=3.365$, $p=0.060$] nor playback condition \times session interaction [ANOVA:
4 $F(12,60)=2.269$; $p=0.118$]. Post hoc comparisons between the different playback conditions,
5 illustrated in Fig. 2a and in Fig. 2b as averaged by condition, indicated that the playback of
6 background noise did not affect cocaine intake compared with baseline ($p=0.240$), whereas the
7 playback of the 50-kHz USV induced a significant decrease in the number of cocaine injections
8 compared to baseline ($p=0.048$, power=0.99), background noise ($p=0.034$, power=0.99) and 22-
9 kHz USV ($p=0.028$, power=1), and the 22-kHz USV had no significant effect when compared to
10 baseline and background noise ($p=0.244$; $p=0.094$ respectively). Interestingly, when the analysis
11 only included the 1st day of each playback condition, as illustrated in Fig 2c, there was a significant
12 playback effect [ANOVA: $F(3,15)=9.802$, $p=0.001$] and post-hoc comparisons revealed that
13 baseline and background noise did not affect cocaine intake differently ($p=0.723$), and that both 50-
14 and 22- kHz USV had a significant effect, the first decreasing cocaine intake ($p=0.046$ with
15 power=0.60 and $p=0.041$ with power=0.71, from baseline and background respectively), while the
16 second increased it ($p=0.034$ with power=0.92 and $p=0.024$ with power=0.94, from baseline and
17 background respectively), leading to a significant difference between 50- and 22 kHz USV
18 ($p=0.019$, power=1).

19 *STN-lesioned rats*: in this group the number of cocaine injections was not affected by the different
20 playback conditions (Fig. 2d-f). As shown in Fig. 2d and in Fig. 2e as averaged by condition, the
21 analysis across the sessions did not indicate a significant effect of playback condition [ANOVA:
22 $F(3,18)=0.800$, $p=0.468$], session [ANOVA: $F(4,24)=0.371$, $p=0.772$] or playback condition \times
23 session interaction [ANOVA: $F(12,72)=0.600$, $p=0.624$]. As shown in Fig. 2f, no playback
24 condition effect was further found on the 1st day of playback [ANOVA: $F(3,18)=0.653$, $p=0.513$].

1 Comparisons between groups showed that sham-control rats, in comparison to STN-lesioned rats,
2 took a higher number of cocaine injections during the 1st day of the 22-kHz USV playback
3 [t(11)=2.322; p=0.42], and a lower number of injections during the 50-kHz 5-day block [t(11)=
4 2.837; p=0.021].

5 **Effects of 50- and 22-kHz USV playback on sucrose SA sessions**

6 Fourteen rats were included in the analysis (sham-control group: N=8; STN-lesioned group: N=6).

7 *Sham-control rats*: the number of sucrose rewards obtained was stable regardless of playback
8 condition and session (Fig. 3a-c). The analysis of the number of sucrose rewards earned across all
9 sessions did not indicate any significant effect of playback condition [ANOVA: F(3,21)=0.898,
10 p=0.433], session [ANOVA: F(4,28)=1.315, p=0.289] or playback condition x session interaction
11 [F(12,84)=0.898, p=0.530], as shown in Fig. 3a and in Fig. 3b as averaged by condition. As shown
12 in Fig. 3c, no significant playback condition effect was further found in the analysis of the 1st day of
13 playback [ANOVA: F(3,21)=1.376, p=0.284].

14 *STN-lesioned rats*: the number of sucrose rewards in STN-lesioned rats was not modulated by the
15 different acoustic stimuli played back (Fig. 3d-f). As shown in Fig. 3d and in Fig. 3e as averaged by
16 condition, no significant effect of playback condition [ANOVA: F(3,15)=1.802, p=0.190], session
17 [ANOVA: F(4,20)=1.065, p=0.383] or playback condition x session interaction [ANOVA:
18 F(12,60)=0.913, p=0.458] was found. As illustrated in Fig. 3f, the absence of a significant playback
19 condition effect was confirmed in the analysis of the first 1st day of playback [ANOVA:
20 F(3,15)=1.246, p=0.328].

21 **Assessment of the rewarding and aversive properties of the 50- and 22-kHz USV**

22 For the CPP and CPA experiments, 17 sham-control (N=11 and 6 respectively) and 13 STN-
23 lesioned rats (N=6 and 7 respectively) were included in the analysis. As shown in Fig. 4, only in the
24 sham-control group, playback of the 50- and 22-kHz USV respectively induced a preference and an

1 avoidance towards the environment previously paired with the USV. As shown in Fig. 4a, the
2 playback of the 50-kHz USV induced a conditioning effect for the compartment paired with the
3 USV in sham-control but not in STN-lesioned rats (Wilcoxon signed-rank test: $p=0.026$ and
4 $p=0.400$, respectively), leading to a significant difference between the two groups (Mann-Whitney
5 U test: $p=0.027$). Similarly, and as shown in Fig. 4b, the playback of the 22-kHz USV induced a
6 conditioning effect for the compartment paired with the call in sham-control but not in STN-
7 lesioned rats (Wilcoxon signed-rank test: $p=0.028$ and $p=1.000$, respectively), leading again to a
8 significant difference between the two groups (Mann-Whitney U test: $p=0.022$).

9 **Discussion**

10 *Affective meaning of USV and consequences on cocaine intake*

11 We have shown here that in sham-control rats the playback of 50-kHz USV induced a decreased
12 intake of cocaine, while the playback of 22-kHz USV increased it transiently. The 50-kHz calls may
13 have acted as an alternative reward able to reduce cocaine intake, as it was previously reported for
14 other rewards. It was shown indeed that four regular food pellets placed in the self-administration
15 chambers reduced heroin intake in rats fed *ad libitum* (Lenoir & Ahmed 2008). Moreover in a
16 mutually exclusive choice procedure, majority of rats fed *ad libitum* choose to self-administer sugar
17 solutions over cocaine and other drugs (see Ahmed, Guillem, & Vandaele 2013 for a review). The
18 presence of a peer has also been shown to be rewarding for rats, given that they develop a social
19 interaction-induced CPP (Calcagnetti & Schechter 1992; Giorla et al. 2018) and learn to press a bar
20 for a physical contact with another rat or only its view (Angermeier 1960). Interestingly, in the SA
21 studies that evaluated the effects of peers on drug intake, rats tested with a non-self-administrating
22 peer took less cocaine than rats tested with a peer also taking cocaine or rats tested alone (Smith
23 2012; Robinson et al. 2016; Giorla et al. 2018). Here we have shown that the playback of 50-kHz
24 calls induced a CPP, confirming the positive meaning of the 50-kHz USV (Brudzynski 2013). This
25 is particularly in line with the highest positive affective states conveyed by the frequency-

1 modulated (FM) and trills calls (Burgdord et al. 2008; Burgdorf & Moskal 2010), of which our file
2 is in majority composed (see Acoustic Stimuli section and Supporting Information for further
3 details on the USV used). Our CPP result is also in agreement with the fact that rats can self-
4 administer playback of 50-kHz USV (Burgdorf et al. 2008), and that 50-kHz USV playback induces
5 dopamine release (Willuhn et al. 2014) and neuronal activation (Sadananda, Wöhr, & Schwarting
6 2008) in the nucleus accumbens, these latter results being interpreted as a stimulation of the reward
7 system. If we then consider the apparently rewarding properties of the 50-kHz USV and the ability
8 of other rewards to reduce cocaine intake, it is not surprising that also the 50-kHz calls were able to
9 do so. It was also well documented that 50-kHz USV serve an important affiliative pro-social
10 function (Wöhr et al. 2016; Wöhr & Schwarting 2017). Consequently it could be possible that rats
11 decreased their cocaine intake because they were approaching the loudspeaker and looking for a
12 peer. However, given that in a previous report rats showed an approach behavior towards the source
13 of the 50-kHz USV playback only during the first USV exposure (Wöhr & Schwarting 2012), we
14 should not expect it to last as long as 5 consecutive SA sessions. Moreover, our data obtained with
15 the CPP (a classical and well established paradigm to study the rewarding properties of drug and
16 non-drug stimuli; see Tzschentke 2007 for a review), showed that rats, during the test day, kept
17 approaching the compartment associated with the USV in absence of their playback. Considering
18 the 50-kHz USV are uttered during – mostly positive – various social interactions (Burgdorf et al.
19 2008), but also after exposure to food and drugs (Browning et al. 2011; Barker, Simmons, & West
20 2015), or in mildly aversive situations as a short social isolation (Wöhr et al. 2007), we can
21 reasonably suppose that the social and rewarding aspects of the 50-kHz USV could overlap in some
22 circumstances, while in other situations one aspect could prevail on the other. Further research is
23 necessary to investigate this point.

24 In another hand, the playback of the 22-kHz USV probably increased cocaine intake through the
25 induction of an aversive state, in line with a series of results indicating higher levels of cocaine

1 intake after exposure to stressful stimuli in rats (Goeders & Guerin 1994). In support of this
2 hypothesis, the long 22-kHz call used here induced a CPA, confirming the aversive meaning of the
3 22-kHz USV, in particular of the long ones (Brudzynski et al. 1993; Brudzynski 2013; see Acoustic
4 Stimuli section and Supporting Information for further details on the USV used). Moreover
5 previous findings indicated that rats avoid SA of 22-kHz USV playback (Burgdorf et al. 2008), and
6 that their playback induces neuronal activation in areas correlated to fear regulation such as
7 amygdala and periaqueductal grey area (Sadananda, Wohr, & Schwarting 2008). Interestingly,
8 starting from the 2nd day of playback, the 22-kHz USV had no effect on cocaine intake, suggesting
9 that rats could have habituated to the aversive properties of the calls (Rankin et al. 2009). However
10 none was observed with the 50-kHz USV, and repeated 22-kHz USV playback was able to induce –
11 in the same animals - a CPA after 2-3 months from the end of the cocaine SA experiment, ruling out
12 the hypothesis of an habituation process. Alternatively, the 22-kHz USV could be less negative than
13 50-kHz USV were rewarding, albeit in both cases we used highly affective stimuli (see above and
14 Supporting Information). Taken all together, our results seem to suggest that the aversive properties
15 of the 22-kHz USV were apparently maintained along the SA sessions, without however interfere
16 with cocaine consumption, possibly because animals have quickly understood the absence of any
17 physical threat.

18 *The USV-induced modulation is specific to cocaine*

19 Our results also indicate that the USV ability to affect cocaine intake in sham-control rats is specific
20 to the drug-related behavior, as it was not observed when animals had access to sucrose, suggesting
21 that the rewarding properties of sucrose overcame the emotive properties of the USV. Interestingly,
22 although the rats were fed *ad libitum*, the number of sucrose rewards was much higher than the
23 number of cocaine infusions (122.91 ± 5.95 sucrose dippers *versus* 8.57 ± 0.95 cocaine infusions).
24 Even if it can be argued that the rewarding value of a sucrose reward could be lower than a cocaine
25 infusion, there is a large literature showing that sugar can be addictive, inducing binge-like behavior

1 and withdrawal symptoms in rodents (DiNicolantonio, O’Keef, & Wilson 2017), and that addictive
2 properties of sugar can be indeed stronger than those of cocaine and other drugs such as nicotine and
3 heroin (Lenoir et al. 2007; Vandaele et al. 2016; Huynh et al. 2017). This has been recently
4 explained by the “*delay drug reward hypothesis*” (Ahmed 2017), suggesting that the delay between
5 an intravenous drug injection and its actions on the brain reward systems diminishes “apparently”
6 its rewarding efficacy, as opposed to the immediate rewarding effect of sugar. In another hand it
7 could be argued that the sucrose experiment was conducted 3 months after the rats were exposed to
8 50-kHz USV in the CPP experiment, exposure that may prevent the playback of USV to affect
9 sucrose SA. However, if a former exposure to USV was sufficient to prevent them to modulate
10 behavior, the exposure to 22- kHz USV in the cocaine SA experiment should have prevented the
11 development of a CPA induced by 22- kHz USV playback tested 3 months later. Since CPA was
12 observed, we can rule this hypothesis out.

13 *The neurobiological substrate for such modulation requires intact STN*

14 The last noteworthy finding of our research is the inability of the 50- and 22-kHz USV playback to
15 affect both cocaine and sucrose SA in STN-lesioned rats. It could be due to the fact that STN-
16 lesioned rats were not able to decode the emotional properties of the USV. In fact STN-lesioned rats
17 did not develop a preference or an aversion for the CPP/CPA compartment respectively paired with
18 the 50- and 22-kHz USV, in agreement with their blunted emotional responses to both positive and
19 negative stimuli previously reported (Pelloux et al. 2014). The STN has long been considered a
20 motor structure and is the target of the surgical treatment for patients with Parkinson's disease using
21 the High Frequency Stimulation (HFS), also called Deep Brain Stimulation (DBS) (Limousin et
22 al. 1995). Although the action mechanisms of the DBS are still not fully understood, they seem to
23 partly mimic the effects of lesions of the targeted area (Gubellini et al. 2009). Interestingly STN
24 DBS of Parkinsonian patients alters the processing of emotive information regardless the positive or
25 negative stimulus valence, and regardless the visual or auditory sensory modality (for a review see

1 Péron et al. 2013). Regarding the acoustic sensory modality, Parkinson's disease patients under STN
2 DBS show deficits in identifying different vocal emotions (Péron et al. 2010) and in contrast,
3 decoding of emotional vocal expressions in healthy subjects involves an activation of the STN in
4 functional magnetic resonance imaging (fMRI) studies (Péron et al. 2016). Taken all together, these
5 human discoveries and our findings in STN-lesioned rats suggest that STN is a crucial node through
6 which the emotional context modulates motivational processes, but also highlight possible side-
7 effects in PD patients that should be monitored carefully. These results are also relevant for other
8 pathologies for which STN can be a surgical target, such as Obsessive Compulsive Disorders
9 (OCD; Mallet et al. 2008) or cocaine addiction (Pelloux & Baunez 2013). Indeed, highlighting the
10 possible risk to impair emotions in patients treated with STN DBS should lead to a better
11 monitoring of these patients. In another hand, this decrease in emotional functioning might explain
12 the beneficial effects of STN DBS in OCD patients by possibly reducing a pathological over-
13 emotionality.

14 *Conclusions*

15 In conclusion we found that, in sham-control rats, 50- and 22-kHz USV are respectively rewarding
16 and aversive. Playback of the rewarding USV leads to decreased cocaine intake, while playback of
17 the aversive one increased it transiently. Interestingly these effects are specific to the drug-related
18 behaviors as they were not observed when animals had access to sucrose. The neurobiological
19 substrate for these behavioral observations seems to involve the STN since STN-lesioned rats were
20 not able to decode the USV emotional content, as expressed by the lack of any effect in both the
21 CPP/CPA and the cocaine SA experiments. These results demonstrate how social acoustic
22 communication and positive contexts created either by USV or presence of a peer are important in
23 rats to diminish drug intake and how STN modulation could help preventing detrimental effect of
24 negative emotional and motivational contexts. These results can translate to the influence of
25 proximal social factors on drug intake described in human drug users (Giorla et al. 2018) and could

1 thus help improving risk reduction strategies. They are also critical for all patients that could be
2 subjected to STN lesions or DBS for PD, OCD or even addiction, who should be monitored
3 carefully regarding their emotional state.

4 Supporting Information is available online at Addict Biol's website.

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11 (ANR). All the authors declare no competing interests.

12 **Author contribution**

13 CM designed and performed the research, analyzed data and wrote the paper; EG performed
14 experiments; YP designed the research and contributed to analytic tools; CB designed and
15 supervised the research, performed surgery and wrote the paper.

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4 **Figure Legends**

5 **Figure 1. Frontal STN sections stained with cresyl violet**

6 *Top panel:* coronal section of the rat brain at the level of the STN (left STN indicated by the arrow),
7 from Paxinos & Watson 2005. *Bottom panels:* the STN is delineated by the dashed lines in a
8 representative sham-operated rat (A) and a representative STN-lesioned rat (B). The lesions were
9 characterized by neuronal loss, gliosis and calcifications. CP: cerebral peduncle; STN: subthalamic
10 nucleus.

11 **Figure 2. Effects of 50-kHz USV, 22-kHz USV and background noise playback on the mean** 12 **number of cocaine injections obtained**

13 The graphs represent the mean number (\pm SEM) of cocaine injections (250 μ g/90 μ l/5s) taken from
14 sham-control (N=6; *a-c*) and STN-lesioned rats (N=7; *d-f*) under the various conditions of playback
15 during all the sessions (*a,d*), during each 5-day block (*b,e*) and during the 1st day of playback (*c,f*).
16 *: $p \leq 0.05$ compared with baseline; #: $p \leq 0.05$ compared with background noise; \$: $p \leq 0.05$
17 compared with 22-kHz USV.

18 **Figure 3. Effect of 50-kHz USV, 22-kHz USV and background noise playback on the mean** 19 **number of sucrose rewards obtained**

20 The graphs represent the mean number (\pm SEM) of dipper activations giving access to a 10% liquid
21 solution of sucrose (300 μ l/dipper/5s) taken from sham-control (N=8; *a-c*) and STN-lesioned rats
22 (N=6; *d-f*) under the various conditions of playback during all the sessions (*a,d*), during each 5-day
23 block (*b,e*) and during the 1st day of playback (*c,f*).

1 **Figure 4. Conditioned Place Preference and Conditioned Place Aversion for the compartment**
2 **respectively paired with the 50- and 22-kHz USV**

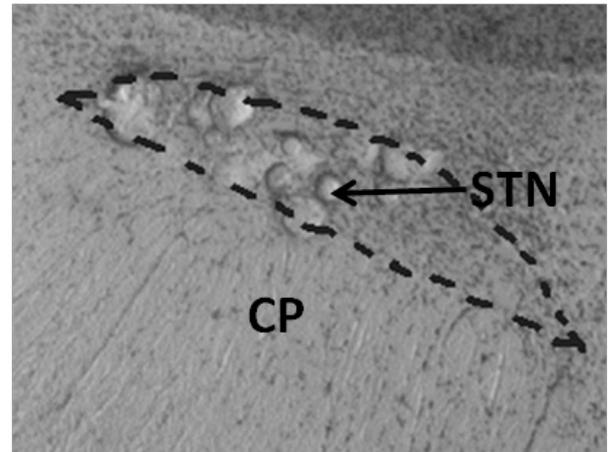
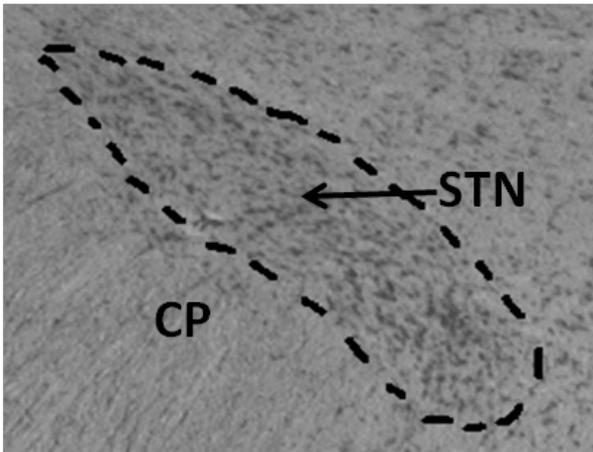
3 The figure shows the mean (\pm SEM) preference and avoidance score, respectively for the 50- and
4 22-kHz USV, calculated as the time (sec) spent during the test day in the compartment previously
5 associated with the USV minus the time spent in the same compartment on the preconditioning
6 day. *a*: preference score for the compartment paired with the 50-kHz USV in both sham-control
7 (N=11) and STN-lesioned rats (N=6) [conditioning effect: *, $p \leq 0.05$; group effect: #, $p \leq$
8 0.05]. *b*: avoidance score for the compartment paired with the 22-kHz USV in both sham-control
9 (N=6) and STN-lesioned rats (N=7) [conditioning effect: *, $p \leq 0.05$; group effect: #, $p \leq 0.05$].

10



A

B

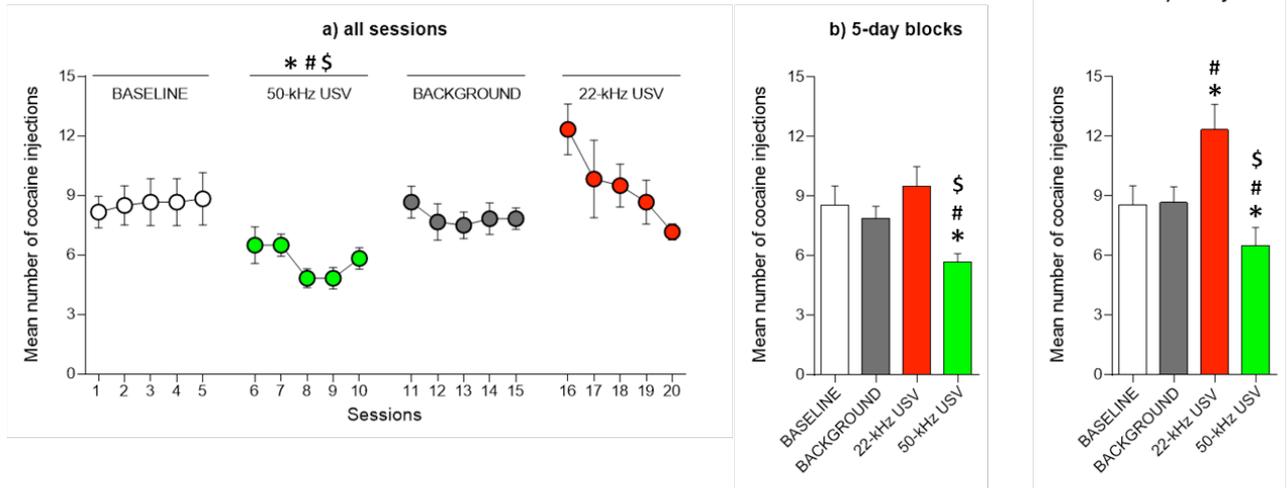


1

2 Figure 1

3

SHAM GROUP (n=6)

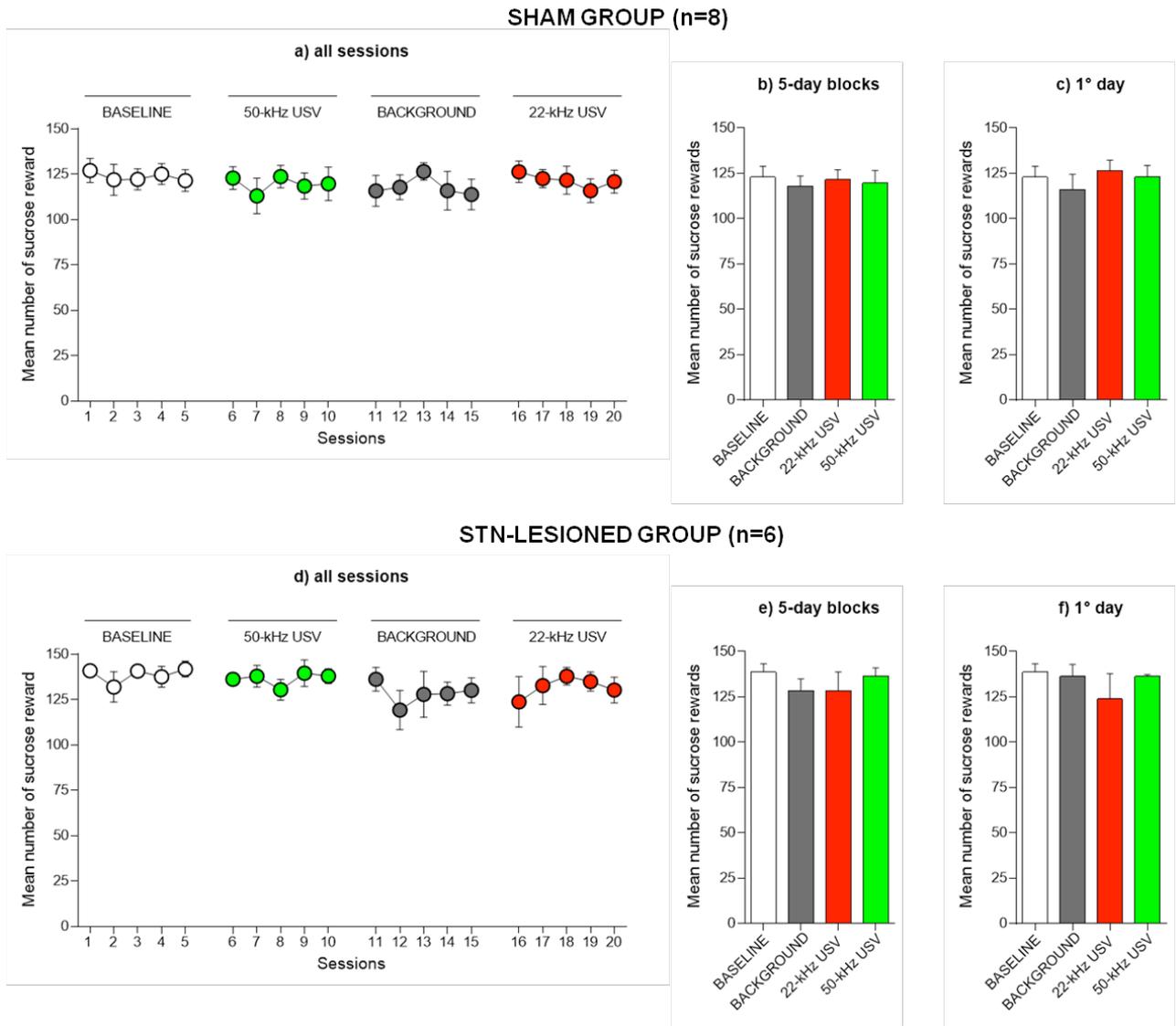


STN-LESIONED GROUP (n=7)

1

2 Figure 2

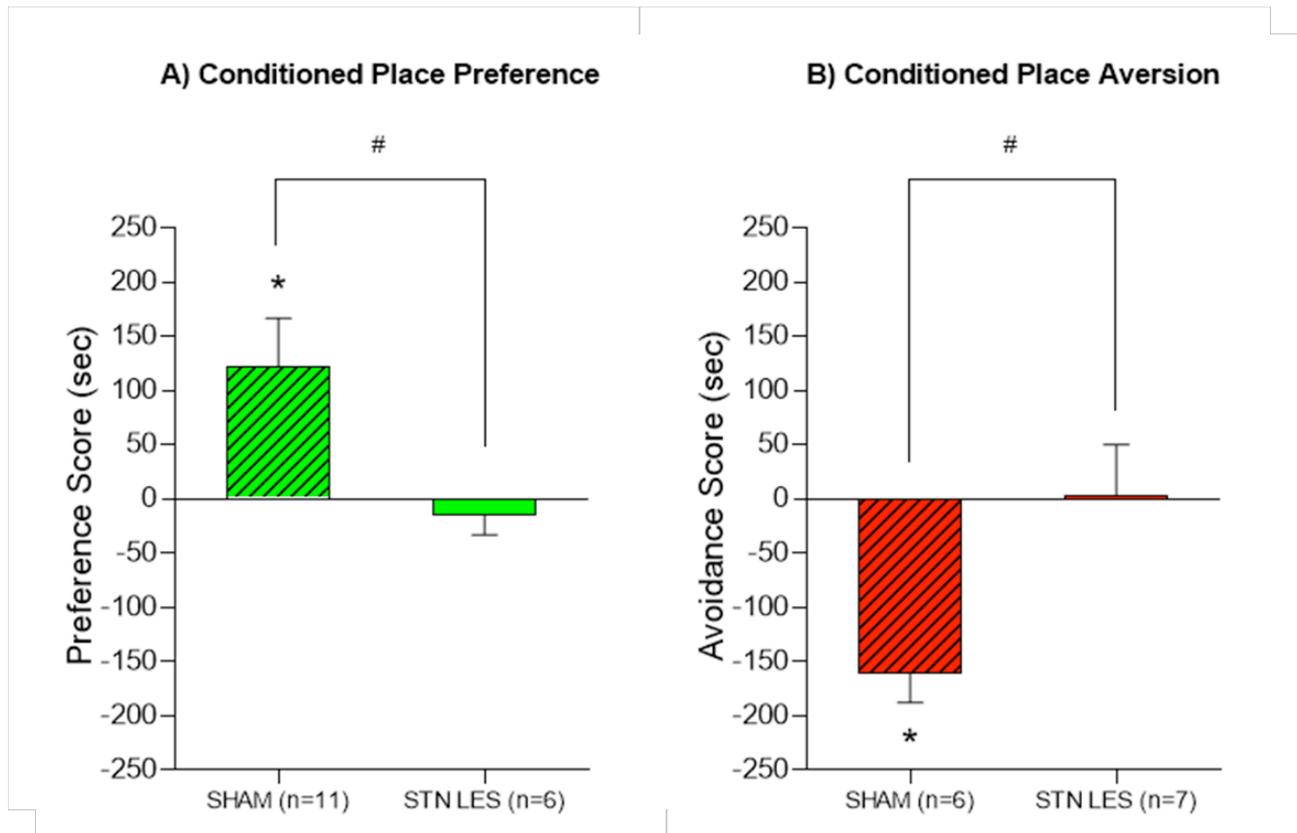
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1

2 Figure 3

3



1

2 Figure 4