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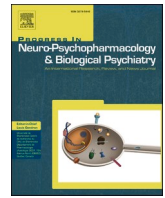
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# Progress in Neuropsychopharmacology & Biological Psychiatry

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## SB-258585 reduces food motivation while blocking 5-HT<sub>6</sub> receptors in the non-human primate striatum

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### ABSTRACT

The interest in new 5-HT<sub>6</sub> agents stems from their ability to modulate cognition processing, food motivation and anxiety-like behaviors. While these findings come primarily from rodent studies, no studies on primates have been published. Furthermore, our understanding of where and how they act in the brain remains limited. Although the striatum is involved in all of these processes and expresses the highest levels of 5-HT<sub>6</sub> receptors, few studies have focused on it. We thus hypothesized that 5-HT<sub>6</sub> receptor blockade would influence food motivation and modulate behavioral expression in non-human primates through striatal 5-HT<sub>6</sub> receptors. This study thus aimed to determine the effects of acute administration of the SB-258585 selective 5-HT<sub>6</sub> receptor antagonist on the feeding motivation and behaviors of six male macaques. Additionally, we investigated potential 5-HT<sub>6</sub> targets using PET imaging to measure 5-HT<sub>6</sub> receptor occupancy throughout the brain and striatal subregions. We used a food-choice task paired with spontaneous behavioral observations, checking 5-HT<sub>6</sub> receptor occupancy with the specific PET imaging [<sup>18</sup>F]2FNQ1P radioligand. We demonstrated, for the first time in non-human primates, that modulation of 5-HT<sub>6</sub> transmission, most likely through the striatum (the putamen and caudate nucleus), significantly reduces food motivation while exhibiting variable, weaker effects on behavior. While these results are consistent with the literature showing a decrease in food intake in rodents and proposing that 5-HT<sub>6</sub> receptor antagonists can be used in obesity treatment, they question the antagonists' anxiolytic potential.

### 1. Introduction

Clinical interest in novel 5-HT<sub>6</sub> receptor (5-HT<sub>6</sub>R) agents comes from rodent studies that have demonstrated their ability to modulate cognitive processes, food motivation, and anxiety-related behaviors. Their multi-activity profile could stem from their serotonergic modulation role in several neuronal networks, depending on the affected brain regions.

The properties of 5-HT<sub>6</sub>R antagonists in increasing cholinergic (Bentley et al., 1999; Riemer et al., 2003) and glutamatergic (Dawson, 2011) neurotransmission in rodents has led clinical research to focus primarily on the treatment of cognitive disorders in Alzheimer's disease and schizophrenia. Multiple candidates have revealed a safe profile yet exhibited disappointing efficacy at later stages (Nirogi et al., 2023).

At the same time, blocking 5-HT<sub>6</sub>R with Ro 04-6790 or down-

**Abbreviations:** 5-HT<sub>6</sub>R, 5-hydroxytryptamine receptor 6; AC, Anterior Commissure; Amyg, Amygdala; ANOVA, Analysis of Variance; BMI, Body Mass Index; BP<sub>ND</sub>, Non-Displaceable Binding Potential; CdN, Caudate Nucleus; DMS, Dorsomedial Striatum; fMRI, Functional Magnetic Resonance Imaging; Hippo, Hippocampus; IM, Intramuscular; NAc, Nucleus Accumbens; NHP, Non-Human Primate; PET, Positron Emission Tomography; PFC, Prefrontal Cortex; PMC, Pre-Motor Cortex; Put, Putamen; RT, Retrieval Time; SD, Standard Deviation; SEM, Standard Error of the Mean; SMA, Supplementary Motor Area; SRTM, Simplified Reference Tissue Model; Thal (DM), Dorsomedial Nucleus of the Thalamus; VLPF, Ventral Lateral PFC; VPut, Ventral Putamen; VS, Ventral Striatum.

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regulating their expression with antisense oligonucleotides has been reported to reduce food intake and body weight in non-obese rats (Woolley et al., 2001). Similarly, mice with non-functional 5-HT6Rs showed reduced weight gain on a high-fat diet (Frassetto et al., 2008). Multiple preclinical studies have confirmed these results in non-obese rats or in rodent obesity models (Bentley et al., 1999; Dudek et al., 2015; Heal et al., 2008; Kotańska et al., 2018; Woolley et al., 2004). Unfortunately, anti-obesity 5-HT6R antagonist candidates from pharmaceutical companies (PRX-07034, BVT.74316, MEM-68626, SUV-504, see review by (Heal et al., 2011)) have not passed Phase I clinical trials.

5-HT6Rs may also regulate mood, in particular anxiety. Pre-clinical studies investigated this aspect because 5-HT6Rs are expressed in limbic regions (Hamon et al., 1999; Kohen et al., 1996; Ruat et al., 1993) and some antidepressant and antipsychotic drugs show 5-HT6R-antagonistic activity (Monsma et al., 1993). However, drawing conclusions regarding the role of these receptors in anxiety and the possible impact of their inhibition is challenging due to disparities in research methods and findings. For instance, decreasing 5-HT6R expression showed anxiogenic effects in two rat anxiety models (Hamon et al., 1999; Otano et al., 1999), but did not in the conditioned-fear rat model (Yoshioka et al., 1998). 5-HT6R KO mouse models show both anxious-like (Tecott and Brennan, 2000) or unmodified phenotypes (Bonasera et al., 2006). Conversely, Wesolowska et al. found that peripheral injections of the antagonist SB-399885 (Wesolowska, 2008; Wesolowska and Nikiforuk, 2007) exerted an anxiolytic-like effect on rodent anxiety models, which injection of SB-742457 did not (Wesolowska et al., 2021). This was also the case when injecting SB-258585 into the rat hippocampus or amygdala (Sun et al., 2018; Wesolowska et al., 2007), whereas injecting another 5-HT6R antagonist (SB-271046) into the mouse dorsomedial prefrontal cortex produced anxiogenic effects (Geng et al., 2018). Thus, these effects are notably dependent on the brain regions targeted.

The lack of commercially available 5-HT6R drugs may be due to limited non-human primate (NHP) studies on the translational potential of some molecules from rodents to human pathology. Since 5-HT6R structure and distribution differ from those in humans and rats, murine models must be used with caution (Gérard et al., 1997; Hirst et al., 2003). To date, only one NHP behavioral study has reported results on SB-271046, a 5-HT6R antagonist that induced cognitive enhancement (Upton et al., 2008). Positron emission tomography (PET) imaging studies have tested the [<sup>18</sup>F]2FNQ1P radioligands' 5-HT6R specificity (Becker et al., 2015; Emery et al., 2020), showing that the macaque striatum had the highest 5-HT6R levels (Sgambato-Faure et al., 2017), similar to rodent (Ruat et al., 1993) and human brain distributions (Hirst et al., 2003; Kohen et al., 1996). Despite being a key structure in the cognitive and motivational processes behind goal-directed behaviors (Tremblay et al., 2009), especially in food motivation and anxiety-related behaviors (Saga et al., 2019; Sgambato-Faure et al., 2016; Worbe et al., 2009; Yang et al., 2012), few behavioral studies have examined 5-HT6R striatal modulation.

The SB-258585 5-HT6R antagonist has good pharmacodynamic properties (Hirst et al., 2000), and can modify [<sup>18</sup>F]2FNQ1P fixation in NHPs (Sgambato-Faure et al., 2017). Moreover, preclinical rodent studies have only examined the cognitive effects of peripheral SB-258585 injections (Gravius et al., 2011).

Therefore, our study investigated the effects of SB-258585 on food motivation and self-initiated behaviors in six macaques, while concurrently evaluating brain 5HT6R occupancies using PET imaging with [<sup>18</sup>F]2FNQ1P. Given the widespread distribution of 5HT6Rs throughout the striatum, we hypothesized that blocking 5-HT6Rs could: 1) decrease food motivation by targeting the ventral striatum (VS); 2) modulate anxiety-like behaviors also mediated via the VS; and 3) modify behavioral activity through the dorsal striatum.

## 2. Materials and methods

### 2.1. Animals and training

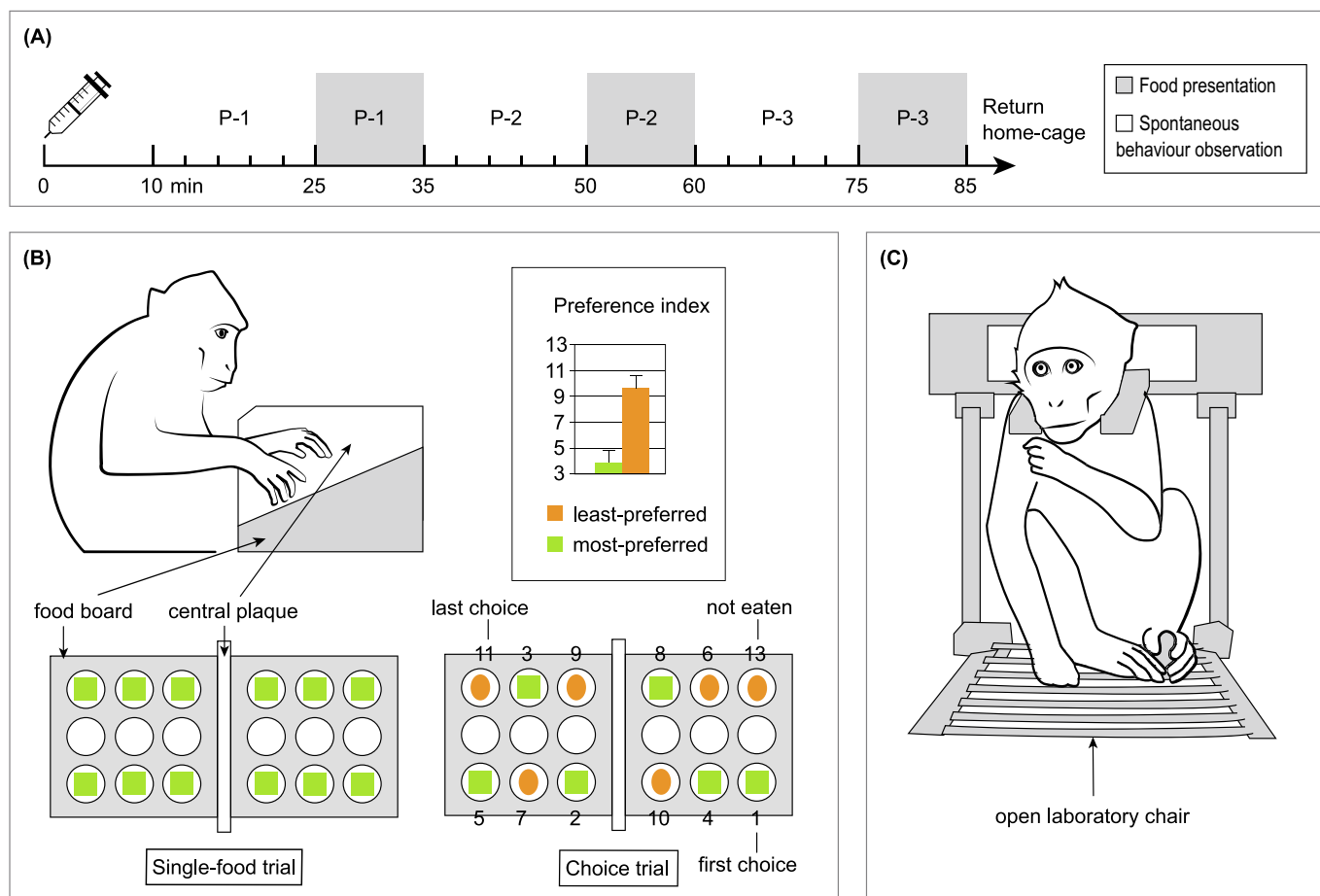
Six male *Macaca fascicularis* (MF1 to MF6; 3–8 kg; 2.5–8 years) were used for the behavioral study. For PET imaging, the data from four monkeys involved in a previous study (Sgambato-Faure et al., 2017) were supplemented with data from two other monkeys. Experiments, animal care and standard housing complied with the French National Research Council's Guide for the Care and Use of Laboratory Animals (1996), the recommendations of the European Communities Council Directive (2010/63/EU) and the ARRIVE guidelines. Procedures were authorized by the French National Committee (2013/113) (#991-2015063017055778 and #991-2015051213399473). To ensure stable performance before (starting after three to five months) and during the experimental period, the monkeys were trained five days a week with controlled access to water and pellets (schedule and quantity). They were neither food-deprived nor fasting.

### 2.2. Drug administration protocol

The selective 5-HT6R antagonist, SB-258585 hydrochloride, purchased from Tocris® (Lille, France), was dissolved in a DMSO vehicle. The solution was administered via intramuscular (IM) injection at 3 mg/kg 10 to 15 min before the experiment. This dose was twice as high as the 1.5 mg/kg intravenous dose previously used to displace the 5-HT6R-specific radioligand [<sup>18</sup>F]2FNQ1P (Sgambato-Faure et al., 2017), developed and produced as described (Becker et al., 2015; Colomb et al., 2014). Injections were spaced far enough apart (once-weekly administration) to assess only the acute effects of the molecule, and to avoid possible receptor down-regulation. However, they were grouped closely enough (for 4 to 5 weeks) to preserve each animal's consistent behavioral pattern, which might evolve over months. Thus, SB-258585 was administered once a week for 4 to 5 weeks. For each monkey, SB-258585 injection days were compared to control or vehicle days. As the NHP SB-258585 pharmacokinetic had never been characterized, we also analyzed four animals' post-injection days for long-lasting effects (+24 h).

### 2.3. Experimental design: spontaneous behavior and food intake task

Sessions were recorded and analyzed off-line using Behavioral Observation Research Interactive Software (Friard and Gamba, 2016). Ten minutes after the vehicle or SB-258585 injection in the cage, the animal was seated in a laboratory open chair for the 85 to 90 min session. Within this session, two contexts were used to investigate SB-258585's effects on spontaneous behavior and food intake. The injection time defined the main 3 alternating "P periods" of each context and the sub-periods of analysis called "T periods". Fig. 1A depicts this task sequence. The session included 15 min of resting without interaction, as illustrated in Fig. 1C (spontaneous behaviors are described in section 2.3.2). This was followed by an 8-trial presentation period (alternating single and choice trials), as shown in Fig. 1B (food parameters are defined in section 2.3.1). Briefly, each trial involved the experimenter presenting a board containing 12 food pieces that the animal could either consume or reject (totally or partially). This alternating sequence of observation and food presentation periods was repeated three times. The task design varied slightly for two monkeys (MF1 and MF4). They performed the task in 55 min, with 5 observational "P periods" of 6 min each, alternating with 5 food presentation "P periods" composed of 2 trials (a single food trial and a food choice trial). The task concluded with 7 consecutive "P" food presentation periods of 2 trials. Consequently, these two individuals had a total of  $n = 10$  T sub-periods of 180 s, while the other four had  $n = 15$  T observation sub-periods. Both design groups undertook the same number of food trials ( $n = 12$  trials of each type). For technical reasons, one had no recording for food preference



**Fig. 1.** Experimental design of the behavioral task assessing pharmacological effects on food intake and spontaneous behavior. **A** Daily experimental schedule for the task. SB-258585 or vehicle were administered via IM injection in the cage 10 min prior to video recording of the experiment in the laboratory. The task alternated between 3 behavioral observation and 3 food presentation phases, each lasting 15 and 10 min, respectively. These “P periods” were also analyzed in finer detail in “T” subperiods (i.e., 180-s periods for behavioral observations and trial-level periods for food presentation). **B** Food motivation task. The animal was seated in front of an inclined board containing 12 holes filled with food items, all being reachable simultaneously. In each trial (i.e., presentation of the board), the monkey could either retrieve and consume the desired number of pieces, or to reject them. There was no limitation on the duration of food retrieval. A trial ended when the last reward was consumed, or when the monkey gave up completing it after the same board was presented three more times. The right and left food boards illustrate the two types of trials. A total of 12 trials of each type were alternately proposed: single-food trials (with only pieces of apple) and choice trials between two items (6 pieces of apple and 6 raisins randomly positioned on the board). For the choice trial example (right board), showing a given food retrieval sequence, the grey panel shows the corresponding preference index for the trial. The rewards are scored from 1 to 12 based on the time sequence of choices. Any unconsumed reward is scored 13. In this example, with a mean score of 3.8, apple is the more-preferred item and raisins the less-preferred reward. **C** Illustration of a monkey resting during the spontaneous observation phase. The open laboratory chair held the animal's collar slightly, allowing limb and head movements, as well as body rotation and production of complex behaviors from its normal repertoire.

index and retrieval time (RT).

**2.3.1. Food-intake parameters**

For the food-intake context, the animals were trained to retrieve and eat the presented food from the board. The process is depicted in Fig. 1B. Performance was assessed using 3 parameters, the first two of which were food motivation markers (Pessiglione et al., 2004). (1) The trial completion rate was measured for each trial and item, depending on the context (single-food or food-choice trial). It was defined as the percentage of rewards taken out of all those made available. (2) The average RT was calculated as the trial duration (i.e., the total time required to consume all desired pieces of apple on a single board) divided by the number of rewards taken, indicating the average time to retrieve and eat one piece of apple. Only single-food trials with at least 50% completion were used to measure this variable. Only trial numbers (1 to 12) with sufficient RT data points in each condition to allow balanced comparisons between them were used. (3) The preference index between the two items was calculated during choice trials. The order of food retrieval assessed animal preferences and changes in the

motivational values of each option following pharmacological injections.

The distinction between these two contexts aimed to assess whether the drug could induce an anorexigenic effect by impacting motivation (completion rate and RT in single-food trials), decision-making processes, and/or both (completion rate and preference index in food-choice trials).

**2.3.2. Spontaneous behavior categories**

In the spontaneous behavior context, the animals were seated in an open primate chair, allowing them to express most of their spontaneous behavioral repertoire in a controlled environment. To better characterize the effect of SB-258585 on each behavior's duration and occurrence over time, observation “P periods” were then analyzed in 3-min fragments (“T periods”). Thus, the duration of each behavior can be expressed as a proportion of the total time (180 s). Behavioral patterns from previous studies were used to group behaviors into five categories after detailed, sequential counting (Worbe et al., 2009, 2011). These were: (1) Inactive (resting, drowsy, yawning); (2) Movements (non-goal

limb or mouth movements); (3) Object-directed behaviors (touching or cleaning the chair); (4) Anxiety-like behaviors (self-directed behaviors such as grooming, self-scratching, licking, biting body parts, or repetitive object scratching); and (5) Others (less frequent behaviors non-related to the previous categories). We added measures of overall and specific behavioral activity duration from T periods. (1) The duration of active behaviors was the sum of all behavior durations excluding inactive behaviors. (2) The average duration of an active behavior was the ratio between duration and occurrence of undifferentiated activity behaviors. (3) The percentage of anxiety-like behaviors among overall activity was the ratio between the duration of anxiety-like and active behavior.

#### 2.4. PET imaging acquisition

Before and during the experiment, the monkeys remained anesthetized and maintained in an acrylic stereotactic apparatus during data acquisition. A 1.5 T Siemens Magnetom scanner was used to collect a 3-dimensional anatomical T1-weighted MRI (TE = 2.89 ms, TR = 2160 ms, IT = 1100 ms, flip angle = 15°, FoV = 154 mm, matrix size = 256 × 256, 176 planes, voxel size = 0.6 × 0.6 × 0.6 mm). PET scans were acquired in 3-D mode on a Siemens Biograph mCT/S64 scanner (spatial transverse resolution: 4.4 mm), with a 90-min dynamic acquisition following the intravenous [<sup>18</sup>F]2FNQ1P injection (mean ± SD: 114.3 ± 30.7 in MBq). PET data were corrected for attenuation using a transmission scan and were reconstructed using the Siemens ultraHD PET algorithm (12 iterations, 8 subsets, zoom factor 21) on a 256 × 256 × 109 matrix (voxel size: 0.398 × 0.398 × 2.027 mm) in series of 28 frames (4 × 30s, 4 × 60s, 8 × 180 s, 12 × 300 s). For scans during the drug condition, SB-258585 was administered 13 ± 5 min (mean ± SD) before the radiotracer injection, 1.5 mg/kg intravenously in the four monkeys from the previous study, and 3 mg/kg IM in the two additional animals.

Individual PET dynamics were motion-corrected using an averaged PET image as a reference and were co-registered to T1-weighted MRI images. Individual MRIs were spatially normalized to a *Macaca fascicularis* template (voxel size: 0.6 × 0.6 × 0.6 mm) using a non-linear transformation matrix (Ballanger et al., 2013). Direct and inverse transformations were used to resample the template and atlases in the individuals' native space, or to reciprocally normalize PET data to the template space. PET studies were analyzed using voxel-based kinetic modeling with the simplified reference tissue model (SRTM) to compute non-displaceable binding potential (BP<sub>ND</sub>) parametric maps (Gunn et al., 1997). The cerebellar grey matter (excluding vermis) was used as the reference region, as previously used in several studies (Parker et al., 2012, 2015; Sgambato-Faure et al., 2017). Regions of interest were obtained from the *Macaca fascicularis* atlas (Ballanger et al., 2013). For a total of 18 scans, the six monkeys underwent from 1 to 3 averaged control scans (separated by a period ranging from 2 to 10 months) and 1 SB-258585 [<sup>18</sup>F]2FNQ1P PET scan (0.5 to 9 months from any control scan of the same animal).

#### 2.5. Statistical analysis

Because pharmacological effects vary between individuals and NHP studies cannot be conducted on large numbers of animals, these effects were investigated at the individual and group levels. At the individual level, the molecule's anorexigenic effect on food parameter trial means was verified using independent one-tailed permuted *t*-tests, as it would be indicated by a decrease in completion rate and an increase in RT. Independent two-tailed permuted *t*-tests compared trial means between conditions for the preference index, which could vary either inversely or similarly for each food item. Both the specific drug effect and its significant appearance over a given period of the task (kinetic effect) were tested. Thus, 2-way ANOVAs were used to investigate the Condition factor (F<sub>1</sub>: SB-258585 vs. control) effect on the completion rate and RT

parameters, and its interaction with the P- or T-Period factors (F<sub>2</sub>). If significant, Tukey's HSD post-hoc multiple comparison tests were performed on the interaction. For a given animal, behavioral category, measure (duration or occurrence), and condition, the behaviors belonging to the same category were summed up within sub-periods of analysis (T periods of 180 s). We used an exploratory statistical approach: conducting an independent two-tailed permuted *t*-test to compare the duration and occurrence of each behavioral category between conditions. All T sub-periods from different test days involving the same animal were treated as individual data points. Long-lasting effects were also tested on post-injection days. In the second level of analysis, drug effects on all task parameters were assessed for the group, using one or two-sided paired *t*-tests, depending on the hypothesis (anorexigenic effect or any behavioral variation). Python software (Van Rossum and Drake, 2009) was used and the results considered significant when *P* < 0.05.

Concerning PET imaging data analysis, two statistical approaches were used. First, the [<sup>18</sup>F]2FNQ1P BP<sub>ND</sub> means of each region of interest (ROI) were compared between control and SB-258585 conditions using one-sided paired *t*-tests with Benjamini-Yekutieli correction. Secondly, a voxel-based analysis was performed to compare the control and SB-258585 conditions using flexible-factorial design in SPM12 (Statistical Parametric Mapping, Wellcome Department for Cognitive Neuroscience, London, UK) software implemented in Matlab® 2019b (MathWorks Inc., Natick, Massachusetts, USA). A statistical threshold of uncorrected *P* < 0.001 was then applied at the voxel level (T-scores > 3.6).

### 3. Results

#### 3.1. SB-258585 reduced food motivation

In the control condition, daily completion rates for all trials ranged from 55.2 ± 47.9% to 97.9 ± 5.7% (mean ± SD) across the animals, a scope that allowed variations to be detected. At the individual level, in five out of the six animals, SB-258585 significantly reduced the trial completion rate (Fig. 2A; one-tailed permuted *t*-test; MF3: *t* = 2.881 *P* = 0.0013, MF6: *t* = 0.795 *P* = 0.221, MF2: *t* = 4.575 *P* < 0.001, MF1: *t* = 3.325 *P* < 0.001, MF5: *t* = 2.184 *P* = 0.016, MF4: *t* = 2.393 *P* = 0.0096).

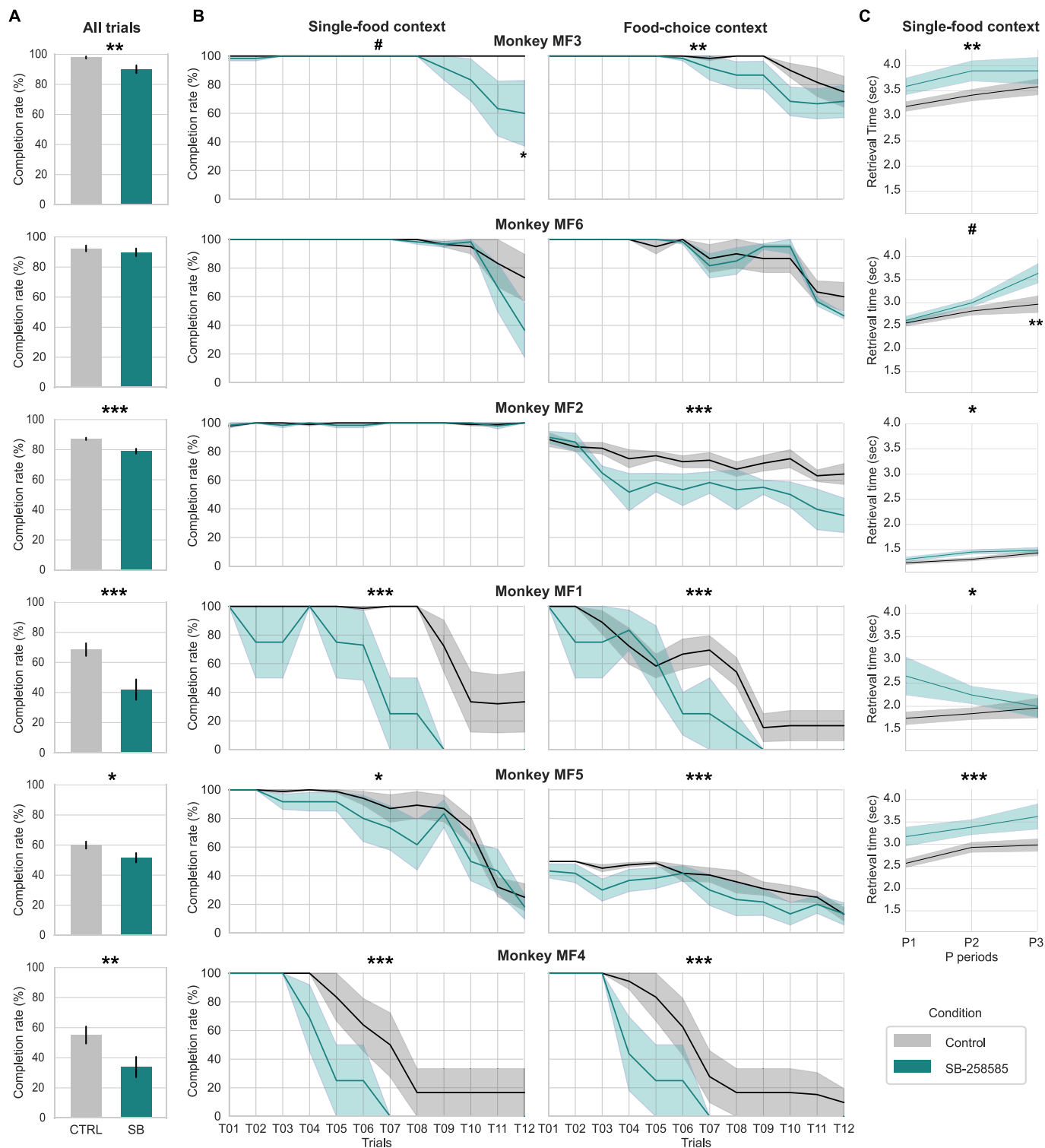
At the individual level, kinetic effects across the trial completion rate were sought. In both trial contexts, the drug acted broadly across the task, independently of the period (Fig. 2B). SB-258585 decreased completion rates in single-food trials in three animals (MF1: F<sub>1(1,96)</sub> = 36.505 *P* < 0.001, MF5: F<sub>1(1120)</sub> = 5.463 *P* = 0.021, MF4: F<sub>1(1,96)</sub> = 13.613 *P* < 0.001). Additionally, five animals exhibited decreased completion rates in choice trials (MF3: F<sub>1(1,95)</sub> = 7.623 *P* = 0.007, MF2: F<sub>1(1128)</sub> = 33.873 *P* < 0.001, MF1: F<sub>1(1,96)</sub> = 13.376 *P* < 0.001, MF5: F<sub>1(1120)</sub> = 12.563 *P* < 0.001, MF4: F<sub>1(1,96)</sub> = 13.440 *P* < 0.001). With the exception of a late drug effect at T12 in monkey MF3 in the single-food context (F<sub>1x2(11,95)</sub> = 2.077 *P* = 0.029; Tukey's HSD post-hoc test; T12: *P*-adj = 0.045), drug-period interactions were not significant.

To assess the impact of SB-258585 on the animals' motivation to retrieve pieces of food from the plate and a potential kinetic effect, the average RT at each P period of the task was evaluated (Fig. 2C). In 4 out of 5 animals, a global drug effect was found across the task (Fig. 2C; MF3: F<sub>1(1109)</sub> = 8.509 *P* = 0.004, MF2: F<sub>1(1146)</sub> = 5.259 *P* = 0.023, MF1: F<sub>1(1,42)</sub> = 5.811 *P* = 0.021, MF5: F<sub>1(1103)</sub> = 19.841 *P* < 0.001). For the fifth animal, the drug effect depended on the P3 period (MF6; F<sub>1x2(2102)</sub> = 3.507 *P* = 0.034; post-hoc test; P3: *P*-adj = 0.006).

Four animals performed the task 24 h later. Compared to control days, in each animal, daily completion rates and/or RTs were still significantly impacted on post-injection days (Table. S1).

The same parameters were analyzed at the group level. The average completion rate also decreased significantly, by 16.2% (Fig. S1; one-tailed paired *t*-test; *t*<sub>(5)</sub> = 3.289, *P* = 0.011). In the control condition, independently of their food preference, the monkeys performed better in the single-food trials than in the choice trials (85.8% versus 67.9%





**Fig. 2.** Individual effects of SB-258585 on food parameters. For panels A, B and C, each animal is shown in line from top to bottom. **A** Daily completion rate in all trials. Performance measured for each trial type ( $n = 48$  to  $94$  trials per condition and animal) was averaged over the entire task (mean  $\pm$  SEM) separately for each drug condition (control vs SB-258585) and each monkey (two-tailed permuted  $t$ -test,  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ ). **B** Evolution in completion rate across trials within the given context. The two panels illustrate the evolution of food intake performance in single-food (left) and choice (right) trial types for each monkey and drug condition. Solid lines show the mean completion rate ( $n = 4$  to  $8$  data points per condition, animal and T period); shaded areas show the SEM. Large stars indicate an overall effect of the drug condition factor ( $F_1$ ) and the hash indicates an interaction between drug condition and T period factors ( $F_1 \times F_2$ ); small stars indicate the significant effect of the drug on a specific T period (two-way ANOVA, Tukey's HSD post-hoc test,  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ ). **C** Retrieval time in single-food trials across P periods. The average time to retrieve a food reward was measured across task P periods for each monkey and drug condition ( $n = 5$  to  $32$  data points per condition, animal and P period); Statistical comparisons were similar to those in B (with  $F_1$ : drug condition,  $F_2$ : P period;  $F_1 \times F_2$  interaction). In summary, administration of SB-258585 at  $3$  mg/kg decreased food motivation individually across all monkeys, without exhibiting substantial kinetic effects (i.e., significance of the  $F_1 \times F_2$  interaction). CTRL: control; SB: SB-258585 ( $3$  mg/kg).

completion rate; two-tailed paired  $t$ -test;  $t_{(5)} = 2.714$   $P = 0.042$ ). SB-258585 decreased the completion rate to a greater extent in choice trials ( $-17.5\%$ ; Fig. S1; one-tailed paired  $t$ -test;  $t_{(5)} = 3.752$   $P = 0.007$ ) than in single-food trials ( $-15.2\%$ ;  $t_{(5)} = 2.463$ ,  $P = 0.028$ ).

In single-food trials, SB-258585 increased average RT at both the individual (Fig. 2C) and group (Fig. S1; one-tailed paired  $t$ -test;  $t_{(4)} = -3.557$   $P = 0.012$ ) levels.

In choice trials, we calculated the animals' relative preference between two food items. SB-258585 was hypothesized to decrease motivation to retrieve food rewards (i.e., to increase the preference index), in particular for the less preferred item. However, due to inter-individual variability, the group analysis ( $n = 5$  animals) showed SB-258585 had no significant effects on this parameter (Fig. S2; two-tailed paired  $t$ -test).

On post-injection days, the daily completion rate significantly decreased by 9.6% (Table. S1; one-tailed paired  $t$ -test;  $t_{(3)} = 4.902$   $P = 0.008$ ), and the average RT increased by 15.4% ( $t_{(3)} = -3.029$   $P = 0.028$ ).

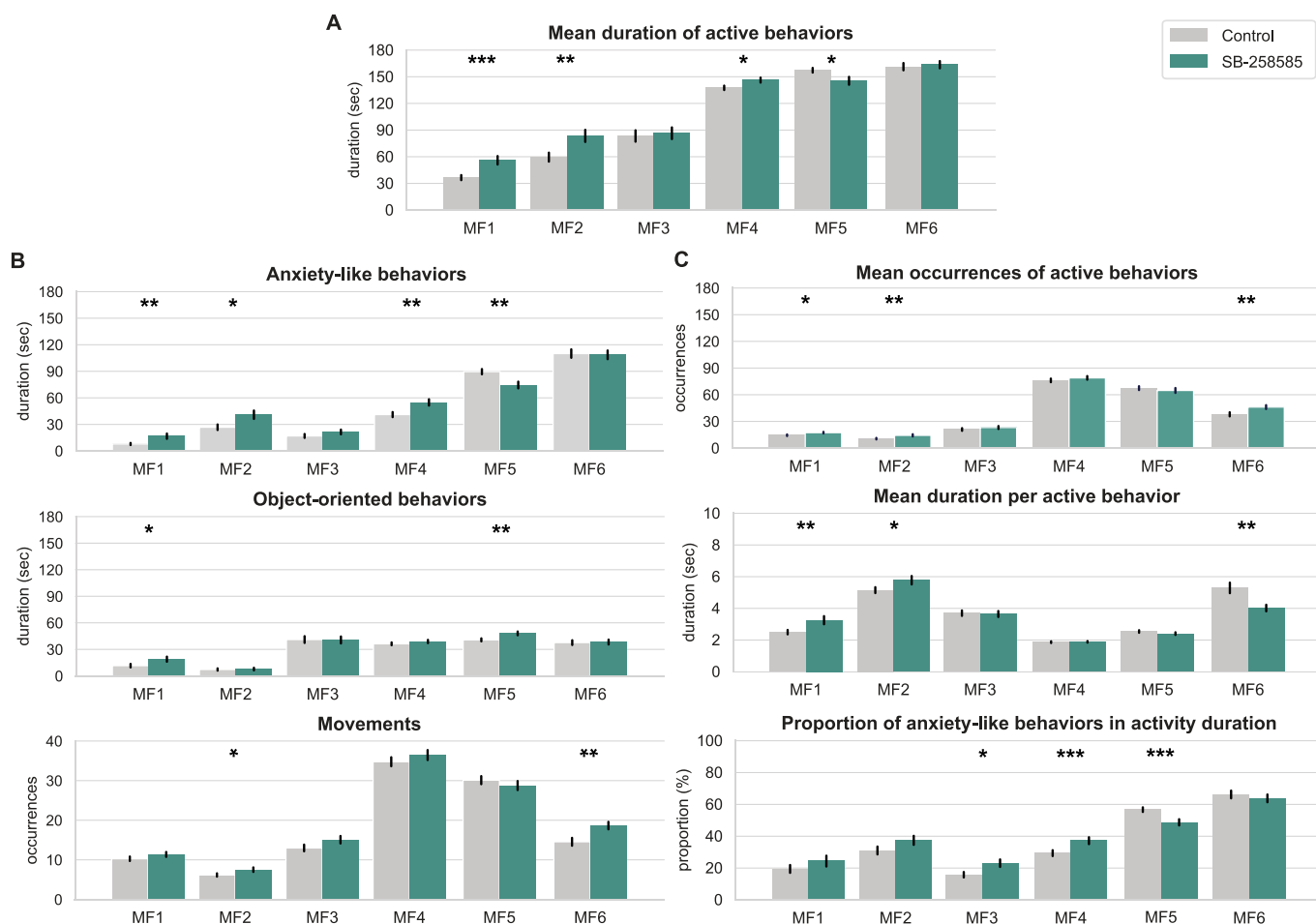
### 3.2. SB-258585 slightly and differently affected spontaneous behaviors

In six macaques, the duration and occurrences of five behavioral

categories were quantified (see Table. S2 for details). In the control condition, the animals exhibited diverse behavioral profiles (Fig. 3). The three least active animals (MF1, MF2, and MF3) were inactive over 50% of the time. Conversely, three animals exhibited higher levels of activity (Fig. 3A), distributed among (Fig. 3B): time spent touching or cleaning objects (MF5: 22.7%, MF6: 21.0%, MF4: 20.2%), and longer duration of anxiety-like behaviors (MF6: 61.1%, MF5: 49.8%, MF4: 22.6%), associated with greater frequency of movement in two of these three animals (MF4: 34.8, MF5: 30.1).

First, our investigation focused on SB-258585 behavioral modulation at the individual level (Fig. 3). Anxiety-like behaviors were the most frequently modified behaviors (Table. S2). Because these behaviors are usually long-lasting, we focused on the 5-HT6R antagonist effect on the duration of anxiety-like behaviors. This increased in half of the animals (Fig. 3B; two-tailed permuted  $t$ -test; MF1:  $t = -2.796$   $P = 0.006$ , MF2:  $t = -2.535$   $P = 0.012$ , MF4:  $t = -3.046$   $P = 0.003$ ) and decreased in one of the most anxious animals (MF5:  $t = 3.232$   $P = 0.002$ ).

Furthermore, SB-258585 produced hyperactivity in four animals, either by increasing activity duration in three animals (Fig. 3A; MF1:  $+53.4\%$ ,  $t = -3.562$   $P < 0.001$ , MF2:  $+40.4\%$   $t = -2.891$   $P = 0.004$ , MF4:  $+6.3\%$   $t = -2.333$   $P = 0.022$ ) and/or by increasing



**Fig. 3.** Individual effects of SB-258585 on spontaneous behavior. For panels A, B and C, behavioral measurements on T periods of analysis ( $n = 39$  to 120 per animal and drug condition) were averaged over the entire task (mean  $\pm$  SEM) separately for each drug condition (control vs SB-258585) and each monkey (two-tailed permuted  $t$ -test,  $*P < 0.05$ ,  $**P < 0.01$ ,  $***P < 0.001$ ). **A** Effects on overall duration of activity. The animals' different profiles are shown from least active (MF1) to most active (MF6). **B** Effects on different spontaneous behavioral types. Averaged measurements of behavioral categories are presented in line from top to bottom as follows: duration of anxiety-like behaviors, duration of object-oriented behaviors, and non-goal movement occurrences. **C** Verification of observed changes in activity and anxiety-like behaviors. Calculated measurements of behaviors are presented in line from top to bottom as follows: the average total number of active behaviors per 3-min period shows behavioral switching between active behaviors; the mean duration of a given active behavioral type shows the animal's capacity to maintain an initiated behavior; the proportion of anxiety-like behavior duration among the overall activity duration shows the extent to which anxiety-like behaviors contribute to changes in activity duration and checks the relative variation in these anxiety-like behavior changes.

behavioral switching (i.e., the total number of occurrences per period) in three animals (Table. S2; MF6:  $t = -2.780$   $P = 0.006$ , MF1:  $t = -2.280$   $P = 0.025$ , MF2:  $t = -2.070$   $P = 0.041$ ). This was accompanied by increases in the duration of object-oriented behavior (Fig. 3B; MF1: +62.4%  $t = -2.216$   $P = 0.029$ ) or movement occurrences (MF6: +28.1%  $t = -3.016$   $P = 0.003$ ; MF2: +21.0%  $t = -1.985$   $P = 0.049$ ). Conversely, in the drug condition, MF5 simultaneously showed decreased activity ( $-7.6\%$   $t = 2.334$   $P = 0.021$ ) and decreased duration of anxiety-like behaviors ( $-16.9\%$   $t = 3.231$   $P = 0.002$ ) (mainly finger licking-biting) in favor of object directed-behaviors (mainly object cleaning) (+18.1%  $t = -2.792$   $P = 0.006$ ). Finally, the molecule did not affect monkey MF3's behavior. We therefore wanted to know whether animals with increased activity could still remain engaged in the same behavioral type by calculating the mean duration of an active behavior (Fig. 3C). Two of these four animals had higher behavioral maintenance (MF1: +32.0%  $t = -2.764$   $P = 0.006$ ; MF2: +13.7%  $t = -2.012$   $P = 0.045$ ), MF4 showed no difference, and only MF6 displayed reduced duration of engagement in a given behavior ( $-24.5\%$   $t = 3.274$   $P = 0.001$ ).

In addition, we investigated whether these SB-258585-induced variations in behavioral activity could be linked to anxiety-like behavioral changes, by examining the variation in the proportion of anxiety-like behaviors within the overall duration of behavioral activity (Fig. 3C). Among the four animals displaying increased activity, MF2, MF1 and MF6 non-specifically showed increased anxiety-like behaviors along with increased overall activity, whereas in MF4, these anxiety-like behaviors increased non-proportionally along with overall activity ( $t = -2.749$   $P = 0.007$ ). The animal with decreased behavioral activity under the effects of SB-258585 showed a non-proportional decrease in the duration of anxiety-like behaviors (MF5:  $t = 3.396$   $P < 0.001$ ).

We also tested whether the antagonist affected the animals' wakefulness by measuring the duration of drowsy behavior (classified as inactive) in both conditions. We found a significant decrease, from  $37.0 \pm 5.1$  to  $14.3 \pm 4.4$  s over 180 s, only in monkey MF2 (two-sided permuted  $t$ -test;  $t = 3.346$   $P = 0.001$ ). The other animals did not express this behavior sufficiently to detect any change.

In four animals, we compared spontaneous behaviors between post-injection and control days (Table. S2). This revealed no significant group differences (results not shown), while some individual changes persisted. MF5 maintained reduced duration of anxiety-like behaviors ( $t = 2.571$   $P = 0.011$ ) and increased duration of inactivity ( $t = -3.031$   $P = 0.003$ ). MF6's movement occurrences remained high ( $t = -2.271$   $P = 0.025$ ), and this effect also appeared in MF3 ( $t = -2.652$   $P = 0.004$ ), despite the absence of any detectable acute effects in this monkey. In these two animals, the total number of occurrences in 180 s increased (MF6:  $t = -2.394$   $P = 0.018$ , MF3:  $t = -2.184$   $P = 0.031$ ).

Focusing on the group-level effects of SB-258585, we wanted to determine whether the molecule had specific effects on behavioral types despite inter-individual variability in behavioral expression. Occurrences of object-oriented behavior increased by 7.67% (Table. 1; two-tailed paired  $t$ -test;  $t_{(5)} = -3.229$   $P = 0.023$ ). No other behavioral category was significantly affected at the group level.

### 3.3. SB-258585 significantly reduced 5HT6R occupancy in the striatum

The radiotracer [ $^{18}\text{F}$ ]2FNQ1P evidenced the presence of 5-HT6Rs in various brain regions (Fig. 4). In the baseline condition, high [ $^{18}\text{F}$ ]2FNQ1P binding levels were found in the anterior and posterior territories of the striatum, i.e., the putamen, the caudate nucleus (CdN), and the VS, as well as in the ventrolateral prefrontal cortex (VLPF), the premotor cortex, the insula and the dorsomedial (DM) nucleus of the thalamus (Fig. 4A). The  $\text{BP}_{\text{ND}}$  of [ $^{18}\text{F}$ ]2FNQ1P within the raphe nucleus, insula and amygdala was lower ( $\text{BP}_{\text{ND}} < 0.3$ ) than in the above-mentioned cerebral structures.

We then compared [ $^{18}\text{F}$ ]2FNQ1P  $\text{BP}_{\text{ND}}$  in control and SB-258585 scans to determine the molecule's target region (Fig. 4B, C). ROI

**Table 1**

Acute behavioral effects induced by SB-258585 in the group ( $n = 6$  animals).

Type of behavioral measure	Condition		p-value
	Control	SB-258585	
<b>Duration (s)</b>			
Inactive	74.1 (41.1%)	66.5 (36.9%)	0.21
Movements	19.7 (10.9%)	21.5 (11.9%)	0.32
Object-oriented behaviors	29.2 (16.2%)	32.2 (17.9%)	0.09
Anxiety-like behaviors	48.7 (27.1%)	52.9 (29.4%)	0.40
Other	8.3 (4.6%)	6.9 (3.9%)	0.29
<b>Occurrence</b>			
Inactive	7.2 ± 1.5	7.5 ± 1.4	0.52
Movements	18.2 ± 4.7	19.7 ± 4.5	0.09
Object-oriented behaviors	7.6 ± 2.4	8.2 ± 2.5	0.02 *
Anxiety-like behaviors	8.8 ± 3.1	9.8 ± 3.0	0.10
Other	3.3 ± 2.2	3.1 ± 1.9	0.71
<b>Total occurrences</b>	45.1 ± 11.4	48.3 ± 10.8	0.06
<b>Duration of an active behavior (s)</b>	3.5 ± 1.3	3.5 ± 1.2	0.98
<b>Anxiety-like behaviors in overall activity (%)</b>	36.3 ± 8.3	39.0 ± 6.3	0.36

Mean (percentage of 180-s period); Mean ± SEM;

\* $P < 0.05$ , \*\* $P < 0.01$ , \*\*\* $P < 0.001$  (two-tailed paired  $t$ -test).

analysis across striatal regions revealed  $\text{BP}_{\text{ND}}$  decreases in the anterior ( $t_{(11)} = 2.391$   $P = 0.0179$ ) and posterior putamen ( $t_{(11)} = 3.531$   $P = 0.0024$ ), and in the anterior CdN ( $t_{(11)} = 2.099$   $P = 0.0298$ ), but these effects survived multiple-region testing correction only in the posterior putamen (Fig. 4A; one-sided paired  $t$ -test with Benjamini-Yekutieli correction). A non-significant decrease in  $\text{BP}_{\text{ND}}$  was observed in the posterior CdN ( $t_{(11)} = 1.075$   $P = 0.153$ ) and VS ( $t_{(11)} = 1.129$   $P = 0.142$ ). SPM analysis confirmed the drug's significant effects in the antero-posterior dorsal striatum (Fig. 4D). The clusters were bilateral in the putamen and unilateral in the CdN (cluster peak coordinates for the left hemisphere:  $-7.0, 1.4, 7.3$  and right hemisphere:  $14.0, -5.2, 0.7$ ;  $k_{\text{L}} = 963$  and  $k_{\text{R}} = 449$  voxels; uncorrected p-value  $p_{\text{L}} = 0.006$  and  $p_{\text{R}} = 0.046$ ).

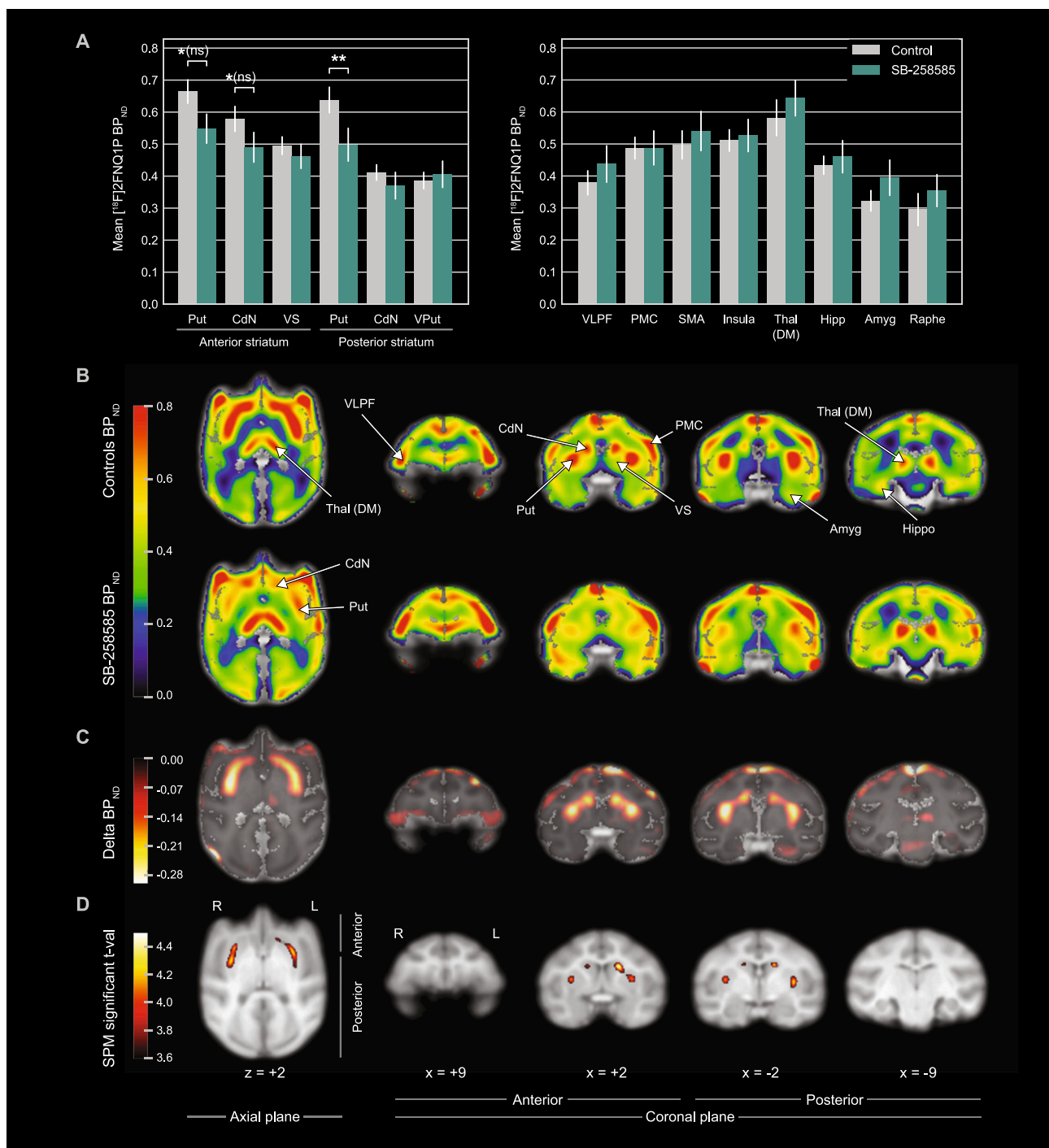
## 4. Discussion

We investigated the impact of a competitive 5-HT6R antagonist on self-initiated and food-intake behaviors in six macaques. This 5-HT6R neuromodulation significantly impacted food motivation. However, with the exception of object-oriented behaviors, other behavioral effects, such as anxiety-related behaviors, were variable and individual-dependent. Additionally, we sought to identify the antagonists' primary sites of action using 5-HT6R PET imaging. These effects appear to be mediated by striatal 5-HT6R modulation, since the molecule selectively displaced the radiotracer in this subcortical nucleus. Overall, SB-258585 was well tolerated in primates.

### 4.1. SB-258585, a selective 5-HT6R antagonist, reduces food motivation in primates

SB-258585 consistently affected food intake and motivation parameters both at the individual and group levels of analysis. These effects remained significant at least 24 h post-injection. While the effects of 5-HT6R antagonists on feeding are generally consistent across various studies (Bentley et al., 1999; Garfield et al., 2014; Heal et al., 2008, 2011; Sargent and Moore, 2009; Woolley et al., 2001, 2004), and now between rodents and NHPs, its underlying mechanisms are less clear. The drug may reduce feeding: 1) by reducing hunger, motivation, satiety breakpoint, or nausea threshold; 2) by modifying the hedonic value of food; or 3) by expressing behaviors interfering with feeding. Our task





**Fig. 4.** Effect of SB-258585 pre-injection on  $[^{18}\text{F}]2\text{FNQ1P BP}_{\text{ND}}$  **A** Comparison of  $[^{18}\text{F}]2\text{FNQ1P BP}_{\text{ND}}$  in ROIs (regions of interest) in control versus SB-258585 conditions. Each bar shows the  $[^{18}\text{F}]2\text{FNQ1P BP}_{\text{ND}}$ , averaged over six macaques ( $n = 12$  hemispheres per region,  $n = 6$  for the raphe), in SB-258585 scans versus averaged control scans. The left panel shows the striatal ROI analysis (paired  $t$ -test between SB-258585 and averaged baseline  $\text{BP}_{\text{ND}}$  with Benjamini-Yekutieli correction for multiple comparisons in 6 ROIs). Stars indicate uncorrected significant  $p$ -values (one-tailed paired  $t$ -test;  $*P < 0.05$ ,  $**P < 0.01$ ), and “ns” identifies the regions that did not survive correction. Overall, acute SB-258585 pre-injections significantly reduced only the posterior putamen  $[^{18}\text{F}]2\text{FNQ1P BP}_{\text{ND}}$  in the ROI analysis. The right panel illustrates the  $[^{18}\text{F}]2\text{FNQ1P BP}_{\text{ND}}$  level of other cerebral regions presenting high  $\text{BP}_{\text{ND}}$  baseline levels or involved in 5-HT<sub>6</sub>R studies. **B**  $[^{18}\text{F}]2\text{FNQ1P BP}_{\text{ND}}$  maps in control and SB-258585 conditions. In the six monkeys, the  $[^{18}\text{F}]2\text{FNQ1P BP}_{\text{ND}}$  parametric maps were averaged in the baseline (first row) and SB-258585 (second row) conditions. One axial slice ( $\text{AC } z = +2$ ) and four coronal slices ( $\text{AC } x = +9, +2, -2, -9$ ) are shown. Anteroposterior indications refer to the anterior commissure (AC). **C** Averaged  $[^{18}\text{F}]2\text{FNQ1P BP}_{\text{ND}}$  deltas. Each delta (SB-258585 minus control) image resulting from averaged  $[^{18}\text{F}]2\text{FNQ1P BP}_{\text{ND}}$  parametric maps shows a large decrease in  $[^{18}\text{F}]2\text{FNQ1P BP}_{\text{ND}}$ . The Put and CdN display large variations both at the anterior ( $x = \text{AC} + 2$ ) and posterior ( $x = \text{AC} - 2$ ) levels of the striatum. **D** Whole-brain analysis of SB-258585's effect on voxel  $[^{18}\text{F}]2\text{FNQ1P BP}_{\text{ND}}$ . SPM analysis shows each significant voxel, with a T-score value higher than the 3.6 threshold (uncorrected  $p$ -value  $\geq 0.001$ ). Significant voxel clusters were localized in the antero-posterior CdN and Put striatal regions. Abbreviations:  $\text{BP}_{\text{ND}}$ : non-displaceable binding potential; CdN: caudate nucleus; Put: putamen; VS: ventral striatum; VPut: ventral putamen; VLPF: ventral lateral prefrontal cortex; PMC: pre-motor cortex; SMA: supplementary motor area; Thal (DM): dorsomedial nucleus of the thalamus; Hippo: hippocampus; Amyg: amygdala.

allowed animals to express pure food-intake behavior (single-food trials) and decision-making processes (food RT, choice processes, and food preference), which are controlled by different striatal territories. Therefore, this experimental paradigm may help in determining functional processes and neuronal networks that may regulate feeding behaviors. A step has been taken in this direction. Higgs et al. argued in favor of differentiating the mechanisms reducing food intake (i.e., palatability or hedonic value vs. satiety) by evaluating the 5-HT<sub>6</sub> receptor antagonist SB-742457 in a microstructural analysis of glucose licking patterns, concluding on a change in satiety (Higgs et al., 2016). Heal et al. found that systemic injections of BVT.5182 or PRX-07034 5-HT<sub>6</sub>R antagonists affected meal termination (time spent feeding), but not initiation (latency to eat) in rats (Heal et al., 2008, 2011). This reflected action on satiety rather than on appetite. They excluded other interacting causes such as gastro-intestinal disturbance, stereotypy, hyperactivity, or sedation. This is consistent with the primates' results. SB-258585 did not prevent food intake but rather reduced trial completion. Moreover, this could not be attributed to any disruptive behaviors or sedation during trial performance. However, for most of the animals, the drug effect was evenly distributed throughout the trials. Conversely, Pratt's et al. found that 5-HT<sub>6</sub>R in the medial nucleus Accumbens (NAc) are implicated in both the consummatory and appetitive motivational aspects of food intake (Pratt et al., 2012) by conjointly measuring normal feeding and food reward effort (Anderson et al., 2023). In our study, animals under the effects of SB-258585 were less willing to retrieve the food from the first periods of the daily task, as shown by the increase in RT, which reflects their motivation. Moreover, the single-food versus dual-food choice also provides valuable insights into food-intake behavior processes. The animals exhibited a more marked decrease in completion rate in dual-choice trials, which require preference-based decisions. This suggests that SB-258585-induced hypophagia may arise from the disturbance of preferred-based decision-making and motivational processes. These mechanisms depend on anterior striatal territories (Benningfield et al., 2014; Martinez et al., 2020; Tremblay et al., 2009), where the drug occupied 5-HT<sub>6</sub>R. Food motivation certainly involves several interacting cognitive, hedonic, and interoceptive processes (Berthoud, 2011). These processes are governed by different brain structures, including cortico-striatal circuits that 5-HT<sub>6</sub>R agents may target to produce beneficial effects on eating disorders, such as obesity or binge eating disorder.

#### 4.2. SB-258585 variably affects individual behaviors, but affects object-directed behaviors more generally

Although our group-level analysis revealed little change in the different behavioral categories, occurrences of object-directed behavior increased significantly. Associated with the increased arousal detectable in only one animal, this could possibly reflect a nootropic (i.e., neurostimulating) effect of SB-258585, increasing the level of attention towards the environment. On the other hand, behaviors driven by stimuli or objects may be associated with the anterior CdN and putamen, respectively (Worbe et al., 2009, 2013), the regions exhibiting the highest levels of 5-HT<sub>6</sub>R occupancy when subjected to SB-258585 in this study. Similar increases in touching behavior, limb movements and frequent behavior changes have been linked to induced hyperactivity in NHP studies (Galineau et al., 2017; Grabli et al., 2004; Worbe et al., 2009). This state was produced both by micro-stimulation (Worbe et al., 2011) and by local injection of a GABAergic antagonist or a non-selective 5-HT receptor antagonist (mianserin) (Neumane et al., 2012; Worbe et al., 2009) into the dorsal part of the anterior striatum. To a lesser extent, we observed similar changes in half of the individuals. With the exception of one animal, SB-258585 did not induce hyperactivity-like pathological features, as animals were still capable of maintaining and completing the initiated behaviors while showing increased activity. Additionally, the relative proportion of anxiety-like behavior duration among the overall duration of activity was

maintained in these three animals, indicating that the increase in behavioral activity is likely a specific effect rather than a modification of the affective state.

Our NHP results provide little evidence that the 5-HT<sub>6</sub>R regulates anxiety-related behaviors. SB-258585 variably modified the duration of anxiety-like behaviors in some individuals. All but two of these variations could be non-specifically attributed to variations in overall activity. Beyond inter-individual variability, these remained unchanged in the group analysis, in line with some rodent studies (Bonasera et al., 2006; Yoshioka et al., 1998). However, it is surprising that our results do not reflect the anxiolytic-like effect observed when using peripheral injections of 5-HT<sub>6</sub>R antagonist (Wesołowska, 2008; Wesołowska and Nikiforuk, 2007), although the same team has recently showed that neither acute nor chronic intraperitoneal injection of SB-742457 at 3 mg/kg produced anxiolytic effects by itself (Wesołowska et al., 2021). Our results in NHPs indicate that these discrepancies in rodent research might also be attributed to inter-individual differences. These could potentially arise from differences in 5-HT<sub>6</sub>R levels among animals, as well as across striatal sub-territories. This emphasizes the need to consider animal behavioral profiles in future research endeavors.

#### 4.3. [<sup>18</sup>F]2FNQ1P PET imaging highlights the striatum as the anatomical substrate of SB-258585 behavioral effects in primates

Despite our initial hypothesis, SB-258585 did not significantly reduce [<sup>18</sup>F]2FNQ1P BP<sub>ND</sub> in the VS but did so in the antero-posterior CdN and putamen territories of the striatum. Several conclusions can be drawn from this result. First, 5-HT<sub>6</sub>R located in the VS, or the NAc in rodents, may be involved in the pathophysiology of anxiety-related behaviors (Otano et al., 1999; Saga et al., 2019; Saga and Tremblay, 2017). In our previous studies, stereotyped behaviors such as grooming and finger biting were generated by blocking the GABAergic neurons in the medial part of the VS (Sgambato-Faure et al., 2016; Worbe et al., 2009, 2013). Thus, the fact that SB-258585 did not induce a significant anxiety-related change in the group analysis is consistent with the absence of a statistically significant reduction in [<sup>18</sup>F]2FNQ1P BP<sub>ND</sub> in the VS. Its lower 5-HT<sub>6</sub>R level, compared to that in the CdN and putamen, may explain the limited changes in anxiety-like behaviors. Further studies with a larger number of monkeys could correlate functional imaging and PET data with behavioral changes to determine whether the 5-HT<sub>6</sub>R level within the VS is associated with anxiety-like behaviors.

Secondly, these results support the idea that SB-258585 may affect food intake via the anterior CdN and putamen. Indeed, all functional territories of the striatum are involved in the complex decision whether or not to direct action towards a food reward. The VS is known to play a central role in motivational processes guiding goal-directed behaviors by anticipating and detecting the predictable goal or reward (Saga and Tremblay, 2017; Tremblay et al., 2009). The CdN is involved in detecting environmental stimuli that meet an individual's goals (Hollerman et al., 1998; Kim and Hikosaka, 2015). Regarding food intake, CdN neurons encode representations of preferred and non-preferred food stimuli (Cromwell et al., 2018; Hassani et al., 2001) and guide attention and decision-making processes towards the approach or avoidance of these stimuli (Martinez et al., 2020; Saga and Tremblay, 2017). The anterior putamen is involved in the goal-directed behavior process by selecting and initiating the approach movement towards the object or food appropriate to the individual's goal (Romo et al., 1992; Vicente et al., 2012). Beyond the NAc, broader examples can be found regarding the implication of more dorsal parts of the striatum in mediating 5-HT<sub>6</sub>R alimentary effects. One study found that rats subjected to a low-protein diet during perinatal life had increased c-fos neuronal response to 5-HT<sub>6</sub>R activation and palatable food in all striatal territories and the amygdala, compared to controls (da Silva et al., 2018). We previously found that local disruption of GABAergic transmission in the ventral anterior putamen reduced food intake in macaques (Worbe et al., 2009).

Finally, food intake behaviors depend not only on context-adapted voluntary decisions involving anterior cortico-striatal circuits but also on food habits (Compan, 2013) and automatic learned behaviors, which are controlled by posterior cortico-striatal circuits (Ashby et al., 2010; Graybiel, 2008; Kim and Hikosaka, 2015; Smith and Graybiel, 2016). Therefore, some SB-258585 effects may arise from its actions on the posterior striatal territories, specifically on the putamen, where it significantly reduced the radiotracer's 5-HT6R occupancy. It is very likely that increased object-oriented movements also result from SB-258585 action on the posterior putamen. Interestingly, SB-258585 only significantly occupied 5-HT6Rs inside the striatum, despite high levels in other brain structures. Thus, the diversity of striatum functional domains and the wide distribution of 5-HT6Rs in this subcortical structure in primates offer broad therapeutic prospects.

#### 4.4. An experimental paradigm to test the translational potential of 5-HT6R ligands from rodents to human pathology via the NHP

Eating disorders typically arise from an imbalance between rewarding and interoceptive or cognitive systems, for example when food seeking surpasses physiological energy needs. Obese individuals are often more sensitive to food cues and thus have greater motivation to approach and eat food (Dagher, 2012). The main regions drawn from fMRI studies in humans as appetitive circuitry for food are the VS, VMPF, orbitofrontal cortex, insula, amygdala, and hippocampus (Dagher, 2012). However, studies in overweight individuals generally highlight activity in the dorsal striatum. The dorsal striatum response to high-calorie food images differed between obese and control women, and the signal change correlated positively with BMI (Rothmund et al., 2007). In another study, functional connectivity between the CdN and the somatosensory cortex, which encodes the valence of gustatory stimulus, was also correlated with food craving and future weight gain in obese participants (Contreras-Rodríguez et al., 2017). The propensity for food addiction as assessed using the Yale-Brown scale has been linked with increased activation of the CdN during food reward expectancy (Gearhardt et al., 2011). All of these authors have emphasized similarities between food seeking in obesity and addiction (Gearhardt et al., 2011; Lipton et al., 2019).

The 5-HT6R was also studied in rodents in the context of addiction, as its modulation in the dorsomedial striatum (DMS) could reduce habit and automaticity in compulsive and addictive behaviors, favoring behavioral flexibility (Eskenazi et al., 2015; Lipton et al., 2019; Mohler et al., 2012). As we have shown in NHPs, SB-258585 preferentially acts in the dorsal striatum and thus may be of particular therapeutic interest in obesity and addictive disorders. Reducing habits and automatic behaviors might facilitate the reevaluation of stimuli that drive reward-seeking and goal-directed behaviors. We can also expect this predominantly central therapeutic target to avoid the peripheral side effects of non-selective anti-obesity drugs. Thus far, 5-HT6R antagonists have displayed a safe profile in both the present experiment and previous clinical trials (Nirogi et al., 2023).

A few limitations should be considered before generalizing the present data. This study used only male monkeys to avoid hormonal changes that could have been confounding factors in the behavioral experiment. Moreover, it was not designed to create an obesity model, as acute injection protocols are not suitable for weight loss in healthy animals. Instead, this study proposes an experimental paradigm in NHPs to study the specific 5-HT6R modulation of feeding behaviors.

## 5. Conclusions

This study demonstrated that SB-258585 preferentially decreased food motivation, and highlighted the striatum as a central structure in these effects. These findings underscore interest in SB-258585 as an anti-obesity treatment but not as an anxiolytic treatment. This is the first NHP experiment to explore in vivo both the brain distribution and the

behavioral effects of a 5-HT6R antagonist, identifying mechanisms and brain structures that sustain these behavioral changes towards food. This will pave the way to performing local injections of 5-HT6R agents directly into each striatal sub-territory to determine their specific involvement in food intake behaviors and motivation subject to 5-HT6R modulation. This paradigm can also allow the testing of 5-HT6R agonists to increase food intake. Such a finding would be relevant for therapeutic applications in Anorexia nervosa, an SSRI-resistant eating disorder.

All authors have read the final version and accept its submission.

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## CRedit authorship contribution statement

**Mathilde Pitoy:** Writing – review & editing, Writing – original draft, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation. **Lisa Gauthier:** Writing – review & editing, Investigation. **Justine Debatisse:** Writing – review & editing, Writing – original draft, Visualization, Software, Formal analysis, Data curation. **Julie Maulavé:** Writing – review & editing, Investigation. **Elise Météreau:** Writing – review & editing, Visualization, Software, Formal analysis, Data curation. **Maude Beaudoin:** Writing – review & editing, Investigation. **Karine Portier:** Writing – review & editing, Resources, Investigation. **Véronique Sgambato:** Writing – review & editing, Methodology, Investigation. **Thierry Billard:** Writing – review & editing, Resources, Investigation. **Luc Zimmer:** Writing – review & editing, Supervision, Resources, Funding acquisition. **Sophie Lancelot:** Writing – review & editing, Resources, Investigation. **Léon Tremblay:** Writing – review & editing, Writing – original draft, Supervision, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

## Declaration of competing interest

The authors declare no competing interests.

## Data availability

Data will be made available on request.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.pnpbp.2024.110970>.

## References

- Anderson, G.E., Sharp, A., Pratt, W.E., 2023. Stimulation of nucleus accumbens 5-HT6 receptors increases both appetitive and consummatory motivation in an effort-based



- choice task. *Neuroreport* 34, 116–121. <https://doi.org/10.1097/WNR.0000000000001868>.
- Ashby, F.G., Turner, B.O., Horvitz, J.C., 2010. Cortical and basal ganglia contributions to habit learning and automaticity. *Trends Cogn. Sci.* 14, 208–215. <https://doi.org/10.1016/j.tics.2010.02.001>.
- Ballanger, B., Tremblay, L., Sgambato-Faure, V., Beaudoin-Gobert, M., Lavenne, F., Le Bars, D., Costes, N., 2013. A multi-atlas based method for automated anatomical Macaca fascicularis brain MRI segmentation and PET kinetic extraction. *NeuroImage* 77, 26–43. <https://doi.org/10.1016/j.neuroimage.2013.03.029>.
- Becker, G., Colomb, J., Sgambato-Faure, V., Tremblay, L., Billard, T., Zimmer, L., 2015. Preclinical evaluation of [18F]2FNQ1P as the first fluorinated serotonin 5-HT<sub>6</sub> radioligand for PET imaging. *Eur. J. Nucl. Med. Mol. Imaging* 42, 495–502. <https://doi.org/10.1007/s00259-014-2936-y>.
- Benningfield, M.M., Blackford, J.U., Ellsworth, M.E., Samanez-Larkin, G.R., Martin, P.R., Cowan, R.L., Zald, D.H., 2014. Caudate responses to reward anticipation associated with delay discounting behavior in healthy youth. *Dev. Cogn. Neurosci.* 7, 43–52. <https://doi.org/10.1016/j.dcn.2013.10.009>.
- Bentley, J.C., Bourson, A., Boess, F.G., Fone, K.C.F., Marsden, C.A., Petit, N., Sleight, A.J., 1999. Investigation of stretching behaviour induced by the selective 5-HT<sub>6</sub> receptor antagonist, Ro 04-6790, in rats. *Br. J. Pharmacol.* 126, 1537–1542. <https://doi.org/10.1038/sj.bjp.0702445>.
- Berthoud, H.-R., 2011. Metabolic and hedonic drives in the neural control of appetite: who is the boss? *Curr. Opin. Neurobiol.* 21, 888–896. <https://doi.org/10.1016/j.conb.2011.09.004>.
- Bonasar, S.J., Chu, H.-M., Brennan, T.J., Tecott, L.H., 2006. A null mutation of the serotonin 6 receptor alters acute responses to ethanol. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 31, 1801–1813. <https://doi.org/10.1038/sj.npp.1301030>.
- Colomb, J., Becker, G., Fieux, S., Zimmer, L., Billard, T., 2014. Syntheses, radiolabelings, and in vitro evaluations of fluorinated PET radioligands of 5-HT<sub>6</sub> serotoninergic receptors. *J. Med. Chem.* 57, 3884–3890. <https://doi.org/10.1021/jm500372e>.
- Compan, V., 2013. Under- to over-eating: how do serotonin receptors contribute? *Future Neurol.* 8, 701–714. <https://doi.org/10.2217/fnl.13.54>.
- Contreras-Rodríguez, O., Martín-Pérez, C., Vilar-López, R., Verdejo-García, A., 2017. Ventral and dorsal striatum networks in obesity: link to food craving and weight gain. *Biol. Psychiatry* 81, 789–796. <https://doi.org/10.1016/j.biopsych.2015.11.020>.
- Cromwell, H.C., Tremblay, L., Schultz, W., 2018. Neural encoding of choice during a delayed response task in primate striatum and orbitofrontal cortex. *Exp. Brain Res.* 236, 1679–1688. <https://doi.org/10.1007/s00221-018-5253-z>.
- da Silva, A.A.M., Oliveira, M.M., Cavalcante, T.C.F., do Amaral Almeida, L.C., Cruz, P.L.M., de Souza, S.L., 2018. Undernutrition during pregnancy and lactation increases the number of fos-cells in the reward system in response to a 5-HT<sub>6</sub> receptor agonist in male adolescent rats. *Int. J. Food Sci. Nutr.* 69, 488–493. <https://doi.org/10.1080/09637486.2017.1382455>.
- Dagher, A., 2012. Functional brain imaging of appetite. *Trends Endocrinol Metab* 23, 250–260. <https://doi.org/10.1016/j.tem.2012.02.009>.
- Dawson, L.A., 2011. The central role of 5-HT<sub>6</sub> receptors in modulating brain neurochemistry. *Int. Rev. Neurobiol.* 96, 1–26. <https://doi.org/10.1016/B978-0-12-385902-0.00001-2>.
- Dudek, M., Marcinkowska, M., Bucki, A., Olczyk, A., Kolaczowski, M., 2015. Idalopirdine – a small molecule antagonist of 5-HT<sub>6</sub> with therapeutic potential against obesity. *Metab. Brain Dis.* 30, 1487–1494. <https://doi.org/10.1007/s11011-015-9736-3>.
- Emery, S., Fieux, S., Vidal, B., Courault, P., Bouvard, S., Tourville, C., Iecker, T., Billard, T., Zimmer, L., Lancelot, S., 2020. Preclinical validation of [18F]2FNQ1P as a specific PET radiotracer of 5-HT<sub>6</sub> receptors in rat, pig, non-human primate and human brain tissue. *Nucl. Med. Biol.* 82–83, 57–63. <https://doi.org/10.1016/j.nucmedbio.2020.01.006>.
- Eskenazi, D., Brodsky, M., Neumaier, J.F., 2015. Deconstructing 5-HT<sub>6</sub> receptor effects on striatal circuit function. *Neuroscience* 299, 97–106. <https://doi.org/10.1016/j.neuroscience.2015.04.046>.
- Frassetto, A., Zhang, J., Lao, J.Z., White, A., Metzger, J.M., Fong, T.M., Chen, R.Z., 2008. Reduced sensitivity to diet-induced obesity in mice carrying a mutant 5-HT<sub>6</sub> receptor. *Brain Res.* 1236, 140–144. <https://doi.org/10.1016/j.brainres.2008.08.012>.
- Friard, O., Gamba, M., 2016. BORIS: a free, versatile open-source event-logging software for video/audio coding and live observations. *Methods Ecol. Evol.* 7, 1325–1330. <https://doi.org/10.1111/2041-210X.12584>.
- Galineau, L., Kas, A., Worbe, Y., Chaigneau, M., Herard, A.-S., Guillemier, M., Delzescaux, T., Féger, J., Hantraye, P., Tremblay, L., 2017. Cortical areas involved in behavioral expression of external pallidum dysfunctions: a PET imaging study in non-human primates. *NeuroImage* 146, 1025–1037. <https://doi.org/10.1016/j.neuroimage.2016.10.039>.
- Garfield, A.S., Burke, L.K., Shaw, J., Evans, M.L., Heisler, L.K., 2014. Distribution of cells responsive to 5-HT<sub>6</sub> receptor antagonist-induced hypophagia. *Behav. Brain Res.* 266, 201–206. <https://doi.org/10.1016/j.bbr.2014.02.018>.
- Gearhardt, A.N., Yokum, S., Orr, P.T., Stice, E., Corbin, W.R., Brownell, K.D., 2011. Neural correlates of food addiction. *Arch. Gen. Psychiatry* 68, 808–816. <https://doi.org/10.1001/archgenpsychiatry.2011.32>.
- Geng, F., Tian, J., Wu, J., Luo, Y., Zou, W.-J., Peng, C., Lu, G.-F., 2018. Dorsomedial prefrontal cortex 5-HT<sub>6</sub> receptors regulate anxiety-like behavior. *Cogn. Affect. Behav. Neurosci.* 18, 58–67. <https://doi.org/10.3758/s13415-017-0552-6>.
- Gérard, C., Martres, M.P., Lefèvre, K., Miquel, M.C., Vergé, D., Lanfumey, L., Doucet, E., Hamon, M., el Mestikawy, S., 1997. Immuno-localization of serotonin 5-HT<sub>6</sub> receptor-like material in the rat central nervous system. *Brain Res.* 746, 207–219. [https://doi.org/10.1016/S0006-8993\(96\)01224-3](https://doi.org/10.1016/S0006-8993(96)01224-3).
- Grabli, D., McCairn, K., Hirsch, E.C., Agid, Y., Féger, J., François, C., Tremblay, L., 2004. Behavioural disorders induced by external globus pallidus dysfunction in primates: I. Behavioural study. *Brain* 127, 2039–2054. <https://doi.org/10.1093/brain/awh220>.
- Gravias, A., Laszy, J., Pietraszek, M., Sághy, K., Nagel, J., Chambon, C., Wegener, N., Valastro, B., Danysz, W., Gyertyán, I., 2011. Effects of 5-HT<sub>6</sub> antagonists, Ro-4368554 and SB-258585, in tests used for the detection of cognitive enhancement and antipsychotic-like activity. *Behav. Pharmacol.* 22, 122–135. <https://doi.org/10.1097/FBP.0b013e328343d804>.
- Graybiel, A.M., 2008. Habits, rituals, and the evaluative brain. *Annu. Rev. Neurosci.* 31, 359–387. <https://doi.org/10.1146/annurev.neuro.29.051605.112851>.
- Gunn, R.N., Lammertsma, A.A., Hume, S.P., Cunningham, V.J., 1997. Parametric imaging of ligand-receptor binding in PET using a simplified reference region model. *NeuroImage* 6, 279–287. <https://doi.org/10.1006/nimg.1997.0303>.
- Hamon, M., Doucet, E., Lefèvre, K., Miquel, M.C., Lanfumey, L., Insausti, R., Frechilla, D., Del Rio, J., Vergé, D., 1999. Antibodies and antisense oligonucleotide for probing the distribution and putative functions of central 5-HT<sub>6</sub> receptors. *Neuropsychopharmacol. Off. Publ. Am. Coll. Neuropsychopharmacol.* 21, 68S–76S. [https://doi.org/10.1016/S0893-133X\(99\)00044-5](https://doi.org/10.1016/S0893-133X(99)00044-5).
- Hassani, O.K., Cromwell, H.C., Schultz, W., 2001. Influence of expectation of different rewards on behavior-related neuronal activity in the striatum. *J. Neurophysiol.* 85, 2477–2489. <https://doi.org/10.1152/jn.2001.85.6.2477>.
- Heal, D.J., Smith, S.L., Fisas, A., Codony, X., Buschmann, H., 2008. Selective 5-HT<sub>6</sub> receptor ligands: progress in the development of a novel pharmacological approach to the treatment of obesity and related metabolic disorders. *Pharmacol. Ther.* 117, 207–231. <https://doi.org/10.1016/j.pharmthera.2007.08.006>.
- Heal, D., Gosden, J., Smith, S., 2011. The 5-HT<sub>6</sub> receptor as a target for developing novel antiobesity drugs. In: Borsini, F. (Ed.), *International Review of Neurobiology, Pharmacology of 5-HT<sub>6</sub> Receptors*, Part II. Academic Press, pp. 73–109. <https://doi.org/10.1016/B978-0-12-385902-0.00004-8>.
- Higgs, S., Cooper, A.J., Barnes, N.M., 2016. The 5-HT<sub>6</sub> receptor agonist, lorcaserin, and the 5-HT<sub>6</sub> receptor antagonist, SB-742457, promote satiety; a microstructural analysis of feeding behaviour. *Psychopharmacology* 233, 417–424. <https://doi.org/10.1007/s00213-015-4112-x>.
- Hirst, W.D., Minton, J.A.L., Bromidge, S.M., Moss, S.F., Latter, A.J., Riley, G., Routledge, C., Middlemiss, D.N., Price, G.W., 2000. Characterization of [125I]-SB-258585 binding to human recombinant and native 5-HT<sub>6</sub> receptors in rat, pig and human brain tissue. *Br. J. Pharmacol.* 130, 1597–1605. <https://doi.org/10.1038/sj.bjp.0703458>.
- Hirst, W.D., Abrahamsen, B., Blaney, F.E., Calver, A.R., Aloj, L., Price, G.W., Medhurst, A.D., 2003. Differences in the central nervous system distribution and pharmacology of the mouse 5-hydroxytryptamine-6 receptor compared with rat and human receptors investigated by radioligand binding, site-directed mutagenesis, and molecular modeling. *Mol. Pharmacol.* 64, 1295–1308. <https://doi.org/10.1124/mol.64.6.1295>.
- Hollerman, J.R., Tremblay, L., Schultz, W., 1998. Influence of reward expectation on behavior-related neuronal activity in primate striatum. *J. Neurophysiol.* 80, 947–963. <https://doi.org/10.1152/jn.1998.80.2.947>.
- Kim, H.F., Hikosaka, O., 2015. Parallel basal ganglia circuits for voluntary and automatic behaviour to reach rewards. *Brain J. Neurol.* 138, 1776–1800. <https://doi.org/10.1093/brain/awv134>.
- Kohen, R., Metcalf, M.A., Khan, N., Druck, T., Huebner, K., Lachowicz, J.E., Meltzer, H.Y., Sibley, D.R., Roth, B.L., Hamblin, M.W., 1996. Cloning, characterization, and chromosomal localization of a human 5-HT<sub>6</sub> serotonin receptor. *J. Neurochem.* 66, 47–56. <https://doi.org/10.1046/j.1471-4159.1996.66010047.x>.
- Kotańska, M., Lustyk, K., Bucki, A., Marcinkowska, M., Śniecikowska, J., Kolaczowski, M., 2018. Idalopirdine, a selective 5-HT<sub>6</sub> receptor antagonist, reduces food intake and body weight in a model of excessive eating. *Metab. Brain Dis.* 33, 733–740. <https://doi.org/10.1007/s11011-017-0175-1>.
- Lipton, D.M., Gonzales, B.J., Citri, A., 2019. Dorsal striatal circuits for habits, compulsions and addictions. *Front. Syst. Neurosci.* 13.
- Martinez, E., Pasquereau, B., Saga, Y., Météreau, É., Tremblay, L., 2020. The anterior caudate nucleus supports impulsive choices triggered by pramipexole. *Mov. Disord.* 35, 296–305. <https://doi.org/10.1002/mds.27898>.
- Mohler, E.G., Baker, P.M., Gannon, K.S., Jones, S.S., Shacham, S., Sweeney, J.A., Ragozzino, M.E., 2012. The effects of PRX-07034, a novel 5-HT<sub>6</sub> antagonist, on cognitive flexibility and working memory in rats. *Psychopharmacology* 220, 687–696. <https://doi.org/10.1007/s00213-011-2518-7>.
- Monsma, F.J., Shen, Y., Ward, R.P., Hamblin, M.W., Sibley, D.R., 1993. Cloning and expression of a novel serotonin receptor with high affinity for tricyclic psychotropic drugs. *Mol. Pharmacol.* 43, 320–327.
- Neumane, S., Mounayar, S., Jan, C., Epinat, J., Ballanger, B., Costes, N., Féger, J., Thobois, S., François, C., Sgambato-Faure, V., Tremblay, L., 2012. Effects of dopamine and serotonin antagonist injections into the striatopallidal complex of asymptomatic MPTP-treated monkeys. *Neurobiol. Dis.* 48, 27–39. <https://doi.org/10.1016/j.nbd.2012.06.002>.
- Nirogi, R., Jayarajan, P., Shinde, A., Mohammed, A.R., Grandhi, V.R., Benade, V., Goyal, V.K., Abraham, R., Jasti, V., Cummings, J., 2023. Progress in investigational agents targeting Serotonin-6 receptors for the treatment of brain disorders. *Biomolecules* 13, 309. <https://doi.org/10.3390/biom13020309>.
- Otano, A., Frechilla, D., Cobreros, A., Cruz-Orive, L.M., Insausti, A., Insausti, R., Hamon, M., Del Rio, J., 1999. Anxiogenic-like effects and reduced stereological counting of immunolabelled 5-hydroxytryptamine-6 receptors in rat nucleus accumbens by antisense oligonucleotides. *Neuroscience* 92, 1001–1009. [https://doi.org/10.1016/S0306-4522\(99\)00066-4](https://doi.org/10.1016/S0306-4522(99)00066-4).

- Parker, C.A., Gunn, R.N., Rabiner, E.A., Slifstein, M., Comley, R., Salinas, C., Johnson, C.N., Jakobsen, S., Houle, S., Laruelle, M., Cunningham, V.J., Martarello, L., 2012. Radiosynthesis and characterization of 11C-GSK215083 as a PET radioligand for the 5-HT6 receptor. *J. Nucl. Med.* 53, 295–303. <https://doi.org/10.2967/jnumed.111.093419>.
- Parker, C.A., Rabiner, E.A., Gunn, R.N., Searle, G., Martarello, L., Comley, R.A., Davy, M., Wilson, A.A., Houle, S., Mizrahi, R., Laruelle, M., Cunningham, V.J., 2015. Human kinetic modeling of the 5HT6 PET radioligand 11C-GSK215083 and its utility for determining occupancy at both 5HT6 and 5HT2A receptors by SB742457 as a potential therapeutic mechanism of action in Alzheimer disease. *J. Nucl. Med. Off. Publ. Soc. Nucl. Med.* 56, 1901–1909. <https://doi.org/10.2967/jnumed.115.162743>.
- Pessiglione, M., Guehl, D., Jan, C., François, C., Hirsch, E.C., Féger, J., Tremblay, L., 2004. Disruption of self-organized actions in monkeys with progressive MPTP-induced parkinsonism: II. Effects of reward preference. *Eur. J. Neurosci.* 19, 437–446. <https://doi.org/10.1111/j.0953-816x.2003.03089.x>.
- Pratt, W.E., Schall, M.A., Choi, E., 2012. Selective serotonin receptor stimulation of the medial nucleus accumbens differentially affects appetitive motivation for food on a progressive ratio schedule of reinforcement. *Neurosci. Lett.* 511, 84–88. <https://doi.org/10.1016/j.neulet.2012.01.038>.
- Riemer, C., Borroni, E., Levet-Trafit, B., Martin, J.R., Poli, S., Porter, R.H.P., Bös, M., 2003. Influence of the 5-HT6 receptor on acetylcholine release in the cortex: pharmacological characterization of 4-(2-Bromo-6-pyrrolidin-1-ylpyridine-4-sulfonyl)phenylamine, a potent and selective 5-HT6 receptor antagonist. *J. Med. Chem.* 46, 1273–1276. <https://doi.org/10.1021/jm021085c>.
- Romo, R., Scarnati, E., Schultz, W., 1992. Role of primate basal ganglia and frontal cortex in the internal generation of movements. II. Movement-related activity in the anterior striatum. *Exp. Brain Res.* 91, 385–395. <https://doi.org/10.1007/BF00227835>.
- Rothmund, Y., Preuschhof, C., Bohner, G., Bauknecht, H.-C., Klingebiel, R., Flor, H., Klapp, B.F., 2007. Differential activation of the dorsal striatum by high-calorie visual food stimuli in obese individuals. *NeuroImage* 37, 410–421. <https://doi.org/10.1016/j.neuroimage.2007.05.008>.
- Ruat, M., Traiffort, E., Arrang, J.M., Tardivel-Lacombe, J., Diaz, J., Leurs, R., Schwartz, J.C., 1993. A novel rat serotonin (5-HT6) receptor: molecular cloning, localization and stimulation of cAMP accumulation. *Biochem. Biophys. Res. Commun.* 193, 268–276. <https://doi.org/10.1006/bbrc.1993.1619>.
- Saga, Y., Tremblay, L., 2017. Chapter 4 - ventral Striatopallidal pathways involved in appetitive and aversive motivational processes. In: Dreher, J.-C., Tremblay, Léon (Eds.), *Decision Neuroscience*. Academic Press, San Diego, pp. 47–58. <https://doi.org/10.1016/B978-0-12-805308-9.00004-X>.
- Saga, Y., Ruff, C.C., Tremblay, L., 2019. Disturbance of approach-avoidance behaviors in non-human primates by stimulation of the limbic territories of basal ganglia and anterior insula. *Eur. J. Neurosci.* 49, 687–700. <https://doi.org/10.1111/ejn.14201>.
- Sargent, B.J., Moore, N.A., 2009. New central targets for the treatment of obesity. *Br. J. Clin. Pharmacol.* 68, 852–860. <https://doi.org/10.1111/j.1365-2125.2009.03550.x>.
- Sgambato-Faure, V., Worbe, Y., Epinat, J., Féger, J., Tremblay, L., 2016. Cortico-basal ganglia circuits involved in different motivation disorders in non-human primates. *Brain Struct. Funct.* 221, 345–364. <https://doi.org/10.1007/s00429-014-0911-9>.
- Sgambato-Faure, V., Billard, T., Météreau, E., Duperrier, S., Fieux, S., Costes, N., Tremblay, L., Zimmer, L., 2017. Characterization and reliability of [18F]2FNQ1P in Cynomolgus Monkeys as a PET radiotracer for serotonin 5-HT6 receptors. *Front. Pharmacol.* 8.
- Smith, K.S., Graybiel, A.M., 2016. Habit formation. *Dialogues Clin. Neurosci.* 18, 33–43. <https://doi.org/10.31887/DCNS.2016.18.1/ksmith>.
- Sun, Y.-N., Yao, L., Li, L.-B., Wang, Y., Du, C.-X., Guo, Y., Liu, J., 2018. Activation and blockade of basolateral amygdala 5-HT6 receptor produce anxiolytic-like behaviors in an experimental model of Parkinson's disease. *Neuropharmacology* 137, 275–285. <https://doi.org/10.1016/j.neuropharm.2018.05.016>.
- Tecott, L.H., Brennan, T.J., 2000. Serotonin 5-HT6 receptor knockout mouse. 6060642.
- Tremblay, L., Worbe, Y., Hollerman, J.R., 2009. Chapter 3 - the ventral striatum: A heterogeneous structure involved in reward processing, motivation, and decision-making. In: Dreher, J.-C., Tremblay, L. (Eds.), *Handbook of Reward and Decision Making*. Academic Press, New York, pp. 51–77. <https://doi.org/10.1016/B978-0-12-374620-7.00003-0>.
- Upton, N., Chuang, T.T., Hunter, A.J., Virley, D.J., 2008. 5-HT6 receptor antagonists as novel cognitive enhancing agents for Alzheimer's disease. *Neurotherapeutics* 5, 458–469. <https://doi.org/10.1016/j.nurt.2008.05.008>.
- Van Rossum, G., Drake, F.L., 2009. *Python 3 Reference Manual*. CreateSpace, Scotts Valley, CA.
- Vicente, A.F., Bermudez, M.A., del Romero, M.C., Perez, R., Gonzalez, F., 2012. Putamen neurons process both sensory and motor information during a complex task. *Brain Res.* 1466, 70–81. <https://doi.org/10.1016/j.brainres.2012.05.037>.
- Wesolowska, A., 2008. The anxiolytic-like effect of the selective 5-HT6 receptor antagonist SB-399885: the impact of benzodiazepine receptors. *Eur. J. Pharmacol.* 580, 355–360. <https://doi.org/10.1016/j.ejphar.2007.11.022>.
- Wesolowska, A., Nikiforuk, A., 2007. Effects of the brain-penetrant and selective 5-HT6 receptor antagonist SB-399885 in animal models of anxiety and depression. *Neuropharmacology* 52, 1274–1283. <https://doi.org/10.1016/j.neuropharm.2007.01.007>.
- Wesolowska, A., Nikiforuk, A., Stachowicz, K., 2007. Anxiolytic-like and antidepressant-like effects produced by the selective 5-HT6 receptor antagonist SB-258585 after intrahippocampal administration to rats. *Behav. Pharmacol.* 18, 439–446. <https://doi.org/10.1097/FBP.0b013e3282d28f9c>.
- Wesolowska, A., Rychtyk, J., Gdula-Argasińska, J., Górecka, K., Wilczyńska-Zawal, N., Jastrzębska-Więsek, M., Partyka, A., 2021. Effect of 5-HT6 receptor ligands combined with haloperidol or risperidone on antidepressant-/anxiolytic-like behavior and BDNF regulation in hippocampus and prefrontal cortex of rats. *Neuropsychiatr. Dis. Treat.* 17, 2105–2127. <https://doi.org/10.2147/NDT.S309818>.
- Woolley, M.L., Bentley, J.C., Sleight, A.J., Marsden, C.A., Fone, K.C., 2001. A role for 5-HT6 receptors in retention of spatial learning in the Morris water maze. *Neuropharmacology* 41, 210–219. [https://doi.org/10.1016/S0028-3908\(01\)00056-9](https://doi.org/10.1016/S0028-3908(01)00056-9).
- Woolley, M.L., Marsden, C.A., Fone, K.C.F., 2004. 5-HT6 receptors. *Curr. Drug Targets CNS Neurol. Disord.* 3, 59–79. <https://doi.org/10.2174/1568007043482561>.
- Worbe, Y., Baup, N., Grabli, D., Chaigneau, M., Mounayar, S., McCairn, K., Féger, J., Tremblay, L., 2009. Behavioral and movement disorders induced by local inhibitory dysfunction in primate striatum. *Cereb. Cortex N. Y. N 1991* (19), 1844–1856. <https://doi.org/10.1093/cercor/bhn214>.
- Worbe, Y., Epinat, J., Féger, J., Tremblay, L., 2011. Discontinuous long-train stimulation in the anterior striatum in monkeys induces abnormal behavioral states. *Cereb. Cortex* 21, 2733–2741. <https://doi.org/10.1093/cercor/bhr063>.
- Worbe, Y., Sgambato-Faure, V., Epinat, J., Chaigneau, M., Tandé, D., François, C., Féger, J., Tremblay, L., 2013. Towards a primate model of Gilles de la Tourette syndrome: anatomo-behavioural correlation of disorders induced by striatal dysfunction. *Cortex J. Devoted Study Nerv. Syst. Behav.* 49, 1126–1140. <https://doi.org/10.1016/j.cortex.2012.08.020>.
- Yang, H., Spence, J.S., Devous, M.D., Briggs, R.W., Goyal, A., Xiao, H., Yadav, H., Adinoff, B., 2012. Striatal-limbic activation is associated with intensity of anticipatory anxiety. *Psychiatry Res.* 204, 123–131. <https://doi.org/10.1016/j.psychres.2012.10.001>.
- Yoshioka, M., Matsumoto, M., Togashi, H., Mori, K., Saito, H., 1998. Central distribution and function of 5-HT6 receptor subtype in the rat brain. *Life Sci.* 62, 1473–1477. [https://doi.org/10.1016/S0024-3205\(98\)00092-7](https://doi.org/10.1016/S0024-3205(98)00092-7).