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Research Articles

WAY-208466, a 5-HT₆ receptor agonist, increases food motivation in primates: A behavioural and PET imaging study opening perspectives in eating disorders

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ABSTRACT

The 5-HT₆ receptor (5-HT₆R) is highly expressed in the anterior striatum, a region involved in food-intake and anxiety-related behaviours that shows abnormal activity in *anorexia nervosa* (AN) patients. Indeed, 5-HT₆R antagonists decrease food motivation across species. Rodent studies have shown the acute anxiolytic properties of WAY-208466, a selective 5-HT₆R agonist. Thus, we hypothesized that this agent could increase food motivation and decrease anxiety-related behaviours by impacting cortico-striatal circuits. We administered acute (0.1 mg/kg) and subchronic (0.03 and 0.1 mg/kg) intramuscular injections to four macaques performing a food-choice task and expressing spontaneous behaviours. We used PET imaging with the [¹⁸F]2FNQ1P specific tracer to determine 5-HT₆R occupancy in several regions that could be linked with the induced behavioural changes. Acute and subchronic activation of 5-HT₆R transmission led to a reversible increase in food motivation. WAY-208466 also decreased anxiety-like behaviours when given acutely but not subchronically. However, in the subchronic protocol, the higher dose led to increased activity characterized by movements and object-related behaviour expression. PET imaging revealed that these behavioural changes could be sustained by cortico-striatal circuits involved in food intake. These findings demonstrate that this 5-HT₆R agonist subchronic treatment successfully increases food motivation but does not fully achieve the expected anxiolytic effect in monkeys. It suggests that targeting 5-HT₆R may be a promising approach for treating the restrictive subtype of AN, which, unlike anxiety, has not been shown to benefit from treatment with selective serotonin reuptake inhibitors.

1. Introduction

Serotonergic dysfunction in the striatum and cortico-basal ganglia circuits, which are involved in motivation, decision-making processes, and anxiety-related behaviours, may underlie several psychiatric disorders (Beaudoin-Gobert et al., 2015; Macpherson and Hikida, 2019; Tremblay et al., 2015). This neurotransmission system is well known as

being essential for feeding and mood regulation (Wurtman, 1993). Eating disorders are often comorbid with anxiety (Kask et al., 2016) and are thought to involve dysfunctional decision-making and food motivation processes that override autonomic nervous system signals (Compan et al., 2015). Although the neurobiological mechanisms behind these maladaptive choices are not fully elucidated, serotonergic dysfunctions are involved in the pathophysiology of these disorders, particularly *anorexia nervosa* (AN) (Galusca et al., 2008; Kaye et al.,

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Abbreviations

5-HT6R	5-HydroxyTryptamine Receptor 6
ACC	Anterior Cingulate cortex
AN	Anorexia Nervosa
AN-R	Anorexia Nervosa Restrictive type
CR	Completion Rate
GPe	Globus Pallidus external segment
mPFC	medial Prefrontal Cortex
NAc	Nucleus Accumbens
NaCl-pre	pre-treatment week
NaCl-post	post-treatment week
OFC	Orbitofrontal Cortex
PCC	Posterior Cingulate Cortex
PET	Positron Emission Tomography
PI	Preference Index
RT	Retrieval Time
SSRI	Selective Serotonin Reuptake Inhibitors
VS	Ventral Striatum
VFC	Ventral Frontal Cortex

2009). However, unlike depression and anxiety disorders, SSRI treatments have shown inconsistent results and limited efficacy in underweight acute-phase AN patients (Marvanova and Gramith, 2018), especially in the restrictive subtype of AN (AN-R). Further research is therefore needed to address this pharmacological need. Beyond reducing side effects, the use of more specific targets with a post-synaptic action should offset any potential deficit in the serotonin precursor tryptophan, due to restrictive feeding. In this regard, the 5-HydroxyTryptamine Receptor 6 (5-HT6R) is an interesting target. First, it is highly expressed in the primate antero-posterior striatum (Sgambato-Faure et al., 2017), a region exhibiting functional abnormalities in AN patients (Foerde et al., 2015; Wierenga et al., 2015). Second, local perturbations induced in the non-human primate (NHP) anterior striatum mimicked the major AN disorders, i.e., a loss of food motivation, anxiety-related behaviours, and maladaptive choices (Worbe et al., 2009; Sgambato-Faure et al., 2016; Saga et al., 2019; Martinez et al., 2020). In recent years, 5-HT6R antagonists have been clinically tested for several conditions, but less attention has been devoted to agonists, especially with regard to their potential on food intake and anxiety. To date, only preclinical rodent studies have demonstrated the antidepressant and anxiolytic properties of systemic administration of 5-HT6R agonists (Carr et al., 2011; Pereira et al., 2015; Schechter et al., 2008; Svenningsson et al., 2007; Wesolowska et al., 2021) and partial agonists, such as EMD-386088 (Jastrzębska-Więsek et al., 2014, 2015). Although 5-HT6R antagonists are well known to reduce food intake (Heal et al., 2011; Kotańska et al., 2018; Pitoy et al., 2024), few studies have proposed that 5-HT6R activation has orexigenic properties, without direct testing in AN models. EMD-386088 has been shown to enhance intake of palatable food in rats both through acute subcutaneous administration (da Silva et al., 2018) or local injections in the Nucleus Accumbens (NAc). In this striatal region, Pratt's team showed an increase in appetitive and consummatory food motivation (Anderson et al., 2023; Pratt et al., 2009, 2012). Conversely, when used systemically in rat obesity and overeating models, the same drug reduced body weight and calorie intake (Kotańska et al., 2017). In a similar model, another partial agonist, E-6837, showed similar results with chronic administration (Fisas et al., 2006). However, EMD-386088 has been shown to have dual action by simultaneously blocking the dopamine transporter (Jastrzębska-Więsek et al., 2016). Thus, its antidepressant, anti-obesity or motivational effects could have been mediated by a dopamine enhancement rather than a specific action on 5-HT6Rs (Jastrzębska-Więsek et al., 2016; Kotańska et al., 2017). Using a selective, full agonist (Schechter

et al., 2008) in primates (WAY-208466), our study aimed to clarify what can be expected from 5-HT6R activation. This compound has only been tested for its anxiolytic effects in schedule-induced polydipsia (Schechter et al., 2008), the defensive burying test, and novelty-induced hypophagia without affecting normal food intake in the latter case (Carr et al., 2011). In the absence of more specific studies focusing on food intake, much less is known about the effects of WAY-208466 on feeding behaviour and its underlying mechanisms.

This preclinical study carried out in healthy macaques aimed: (1) to determine the effects of acute and subchronic intramuscular injections of WAY-208466 on food-intake and spontaneous behaviours, with a focus on anxiety-related behaviours, and (2) to identify the brain regions directly impacted by this agent. For the first purpose, four monkeys performed a food-intake task combined with spontaneous behavioural observations. For the second, we used PET imaging with the 5-HT6R specific radioligand [¹⁸F]2FNQ1P. Using the same paradigm, we have recently shown that acute systemic administration of the 5-HT6R antagonist SB-258585 reduces food motivation, probably by acting in the striatum, with few changes in behaviour (Pitoy et al., 2024). Conversely, we hypothesized that the 5-HT6R agonist should increase food motivation and decrease anxiety-related behaviours, using a dose of 0.1 mg/kg in an acute protocol, and that these beneficial effects could be reproduced in a subchronic protocol using a lower (0.03 mg/kg) or similar (0.1 mg/kg) dose. Finally, we postulated that the striatum might again be the main structure underlying these effects.

Eating disorders are a group of disorders that affect a large proportion of the population. Among them, AN is a major public health concern, given its lifetime prevalence (0.3–2.2% in women and 0.24–0.3% in men) (Galmiche et al., 2019), high mortality rate among young women (Arcelus et al., 2011; Steinhausen, 2002), and lack of pharmacological treatment. In this context, NHPs may facilitate the translation of rodent 5-HT6R agonist research to human therapeutic applications.

2. Material and methods

2.1. Animals and training

Four male *Macaca fascicularis* from our previous study (Pitoy et al., 2024) with a 5-HT6R antagonist (MF2, MF3, MF5, and MF6; age 3–9.5 years; weight 3–10 kg) were used for both PET imaging and behavioural aspects of this study. The experiments, animal care and standard housing complied with the French National Research Council's Guide for the Care and Use of Laboratory Animals (1996) and the recommendations of the European Communities Council Directive (2010/63/EU). Procedures were approved under French National Committee (2013/113) reference number #990–2015063017055778. Several months before and during data recording, the animals were trained to perform the task 5 days a week. Additionally, they were given a consistent daily supply of water and pellets to meet their needs.

2.2. Drugs and administration protocols

The specific radioligand [¹⁸F]2FNQ1P (CERMEP, Lyon, France) was used for 5-HT6R imaging (Colomb et al., 2014). This radiotracer had been already used in NHPs in prior studies (Becker et al., 2015; Sgambato-Faure et al., 2017; Pitoy et al., 2024). The drug tested in this study, 3-[(3-Fluorophenyl)sulfonyl]-N,N-dimethyl-1H-pyrrolo[2,3-b]pyridine-1-ethanamine hydrochloride (WAY-208466), a potent full agonist (EC₅₀ = 7.3 nM; E_{max} = 100%) with high affinity (K_i = 4.8 nM) and good selectivity for the 5-HT₆ human receptor (Schechter et al., 2008), was purchased from Sigma-Aldrich® (Saint Quentin Fallavier, France). WAY-208466 powder was dissolved in an NaCl vehicle and administered intramuscularly. The acute protocol aimed to detail the agent's individual and group effects in the task and to explore the processes and brain regions involved. After observing the kinetics of behavioural

effects and a preliminary dose-finding study in MF2 (Fig. S1), a consistent 0.1 mg/kg dose was used 2 h prior to both the acute PET-imaging scan (one injection) and the acute behavioural protocol (repeated acute injections, once a week for 4–5 weeks). We tested whether acute effects can be replicated at group level in a subchronic protocol of four consecutive weeks. The first and fourth weeks consisted of daily vehicle injections (NaCl-pre: pre-treatment and NaCl-post: post-treatment) and the second and third weeks consisted of daily drug injections at doses of 0.033 and 0.1 mg/kg for five days each week.

2.3. Experimental task design

2.3.1. Experimental task sequence

Animals were injected with either the vehicle or WAY-208466 2 h prior to the experiment. Animals were then positioned on a laboratory primate open chair for the whole 85–95 min session for a task previously described more extensively (Pitoy et al., 2024). Briefly, each session was divided into two main alternating contexts: spontaneous behaviours and food-intake task. Each context had three alternating main phases, with further analysis conducted on subperiods termed "T periods". Based on the context, these T periods featured either 3 min of spontaneous behavioural observation (with 15 T periods per session), or food board presentation (12 T periods per session).

2.3.2. Food intake parameters

During the food-intake task, animals could retrieve and consume presented food items from a 12-hole board. Two types of trials were performed: single-food trials using 12 apple pieces, and choice trials between two food items (6 apple pieces and 6 raisins). The parameters evaluated were: (1) trial completion rate (CR), *i.e.*, the percentage of rewards retrieved from a board; (2) average retrieval time (RT) per food piece, *i.e.*, the trial duration divided by the number of rewards taken in the single-food context; and (3) preference index (PI), calculated to evaluate the animal's relative preference for each food item during choice trials. Trials with at least 50% completion rate were selected for measuring RT. Only trials from T01 to T12 that were balanced between conditions were selected for comparisons. Contrasting the two types of trials aimed to investigate how the treatment could induce an orexigenic effect, either by increasing appetitive motivation, as assessed by the first two parameters (Pessiglione et al., 2004), or by also affecting decision-making processes (CR and PI in food-choice trials).

2.3.3. Spontaneous behaviour observation

In the spontaneous behaviour context, animals in the open chair were left undisturbed to freely express most of their usual behavioural repertoire. Sessions were recorded and analysed offline using Behavioural Observation Research Interactive Software (Friard and Gamba, 2016). Behavioural patterns identified in prior research (Worbe et al., 2009, 2011; Sgambato-Faure et al., 2016; Galineau et al., 2017; Saga et al., 2022; Pitoy et al., 2024) were used to categorize behaviours into five groups: inactivity, movements, object-oriented behaviours, anxiety-like behaviours, and others. Each behaviour's duration and occurrence were assessed, with duration being expressed as a percentage of the overall time (3-min T period).

2.4. PET-MRI acquisition

WAY-208466 was administered intramuscularly at 0.1 mg/kg, 2.0 ± 0.3 h prior to [¹⁸F]2FNQ1P radiotracer injection for scans in the treatment condition. Each animal underwent one control scan and one WAY-treated scan, with at least 2 weeks between scans.

During imaging sessions, animals received alfaxalone induction and remained under propofol anaesthesia. They were intubated and their vital constants carefully monitored. Their head was placed in a stereotactic apparatus. A 3T Siemens Biograph mMR simultaneous PET-MRI scanner collected a 3-dimensional anatomical T1-weighted MRI (TE =

4.1 ms, TR = 3400 ms, IT = 1050 ms, flip angle = 9°, FoV = 135 mm, matrix size = 192 × 192, 160 planes, voxel size = 0.7 × 0.7 × 0.7 mm³). 3-D PET-scans were acquired via 90-min dynamic acquisition following intravenous [¹⁸F]2FNQ1P injection (mean dose per scan ± SD: 80.5 ± 30.1 MBq). PET data were corrected for attenuation, scatters, randoms and normalization and reconstructed using the Siemens OP-OSEM HD PET algorithm (8 iterations, 21 subsets) with a zoom factor 3 on a 256 × 256 × 109 matrix (voxel size: 0.4 × 0.4 × 2.0 mm) in series of 30 frames (4 × 30 s, 6 × 60 s, 9 × 180 s, 11 × 300 s).

Each individual dynamic PET scan was motion corrected by aligning each temporal frame to the averaged reference PET image. T1-weighted MRI images were then realigned to the averaged reference PET image. Each MRI scan was spatially normalized to a *Macaca fascicularis* template (isotropic voxel: 0.6 mm) using a non-linear transformation matrix (Ballanger et al., 2013), with both direct and inverse transformations. We analysed PET images using voxel-based kinetic modelling with the simplified reference tissue model (SRTM) to generate non-displaceable binding potential (BP_{ND}) maps (Gunn et al., 1997). The cerebellar gray matter served as the reference region, consistent with previous studies (Parker et al., 2012, 2015; Sgambato-Faure et al., 2017). Regions of interest (ROIs) were defined from the *Macaca fascicularis* atlas (Ballanger et al., 2013).

2.5. Statistical analysis

For the acute protocol, we conducted a group analysis on the three food task parameters with and without WAY-208466 injection. We used one-sided (completion rate, RT) or two-sided (preference index) Wilcoxon signed rank tests (Wilcoxon T test; data paired on T periods and animals) to test if the drug induces a significant increase in food motivation. The same test was used at the individual level (Wilcoxon T test; data paired on T periods; n = 12) to compare means between conditions, as the injections days (sessions) could not be paired.

For the subchronic protocol, at both group and individual analysis levels, and following significant Friedman tests, we performed pairwise two-sided comparisons between the four conditions using *Conover post-hoc* tests for unreplicated blocked data with Holm's adjustment. This enabled better control for variability due to T periods, days of the week, and individuals (blocks), with each trial data point represented as a pair in every condition (n = 240 per condition).

For the RT parameter, trials with completion rates below 50% were considered as missing data. This involved adjusting the number of T periods analysed in relation to animal or group performance by either reducing the data points or increasing the grouping for paired tests.

For PET imaging analysis, we used one-sided paired *t*-tests (paired by animal and hemisphere with a Benjamini-Yekutieli correction) for each ROI to compare radioligand BP_{ND} means between control and WAY-208466 scans.

Statistical analyses were conducted using Seaborn v0.12.2, SciPy v1.11.3, and Scikit-posthocs v0.7.0.

3. Results

3.1. WAY-208466 increased food motivation in both acute and subchronic protocols

3.1.1. Acute protocol

Acute WAY-208466 injections in the four animals generally affected all food-intake parameters (Fig. 1). The drug significantly increased completion rates at group level in all trials (T = 74.5, p < 0.001, Fig. 1A) as well as in both types of trials (single-food: T = 85.5, p < 0.001; food-choice: T = 101.5, p < 0.001). At the individual level, completion rates increased in three out of four animals (all trials; MF2: T = 0.0, p < 0.001, MF6: T = 1.5, p = 0.002, MF5: T = 0.5, p = 0.001), and in each trial type (single-food: MF2: T = 5.0, p = 0.004, MF6: T = 1.5, p = 0.002, MF5: T = 1.5, p = 0.002; food-choice: MF2: T = 0.0, p < 0.001, MF6: T = 3.0, p

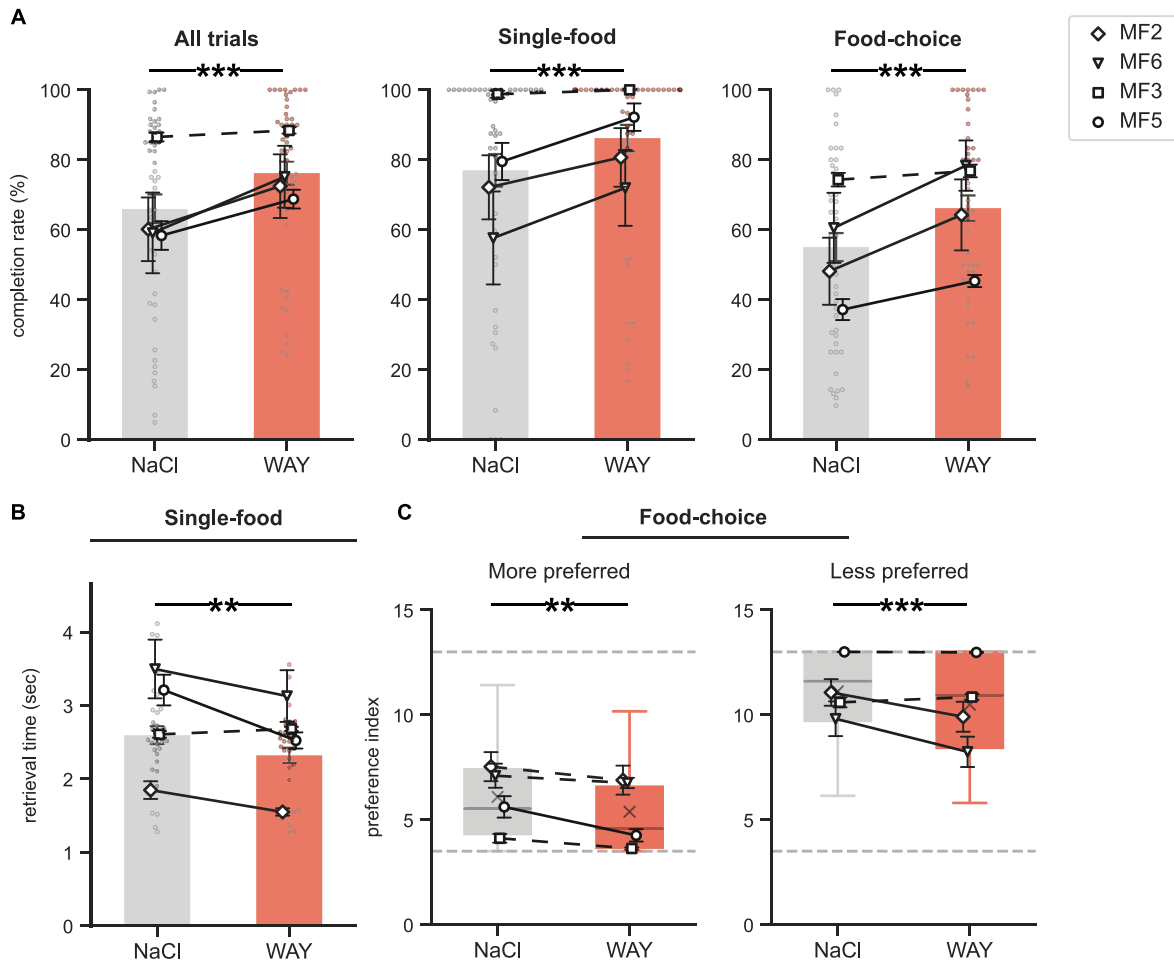


Fig. 1. Individual and group effects of acute WAY-208466 injections (0.1 mg/kg) in the food-intake task. Three parameters assessed food motivation in each condition (NaCl vs. WAY). **A.** Mean completion rate. Bar plots illustrate group completion rate (mean \pm SEM; T01-T12; $n = 48$ T periods per condition) for all trials and for each type of trial (single-food or food-choice). Stars highlight significant group differences between the two conditions (one-sided Wilcoxon signed rank test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Line plots with markers represent individual variations (mean \pm SEM; $n = 12$; solid lines: $P < 0.05$, dotted lines: non-significant (ns); one-sided Wilcoxon signed rank test). **B.** Mean retrieval time (RT) in single-food trials. Bar plots show group means (T01-T08; $n = 32$), and line plots show individual means ($n = 9$ to 12). **C.** Mean preference index based on food preference in choice trials. The preference index ranges from 3.5 to 13 and is inversely related to preference for an item. Boxplots show 1st and 3rd quartiles; gray crosses and lines, respectively, show group means (cross) and medians (solid line) in each condition ($n = 48$). Line plots show individual means ($n = 12$).

$= 0.002$, MF5: $T = 1.5$, $p = 0.002$). MF3 showed no treatment effect on this parameter.

In single-food trials, WAY-208466 significantly reduced average RT (Fig. 1B) in the group (from period T01 to period T08; $T = 417.0$, $p = 0.002$) and individually in three animals (MF2: T01-T12, $T = 72.0$, $p = 0.003$; MF6: T01-T09, $T = 39.0$, $p = 0.027$; and MF5: T01-T12, $T = 78.0$, $p < 0.001$). No significant differences in MF3 were found, but its average CR in single-food trials rapidly reached 100% in the NaCl condition.

The preference index score (Fig. 1C) is inversely related to relative preference for one item over the other in choice trials. WAY-208466 non-specifically decreased the preference index for both food items at group level (more preferred: $T = 291.5$, $p = 0.002$; less preferred: $T = 256.5$, $p < 0.001$). At individual level, the preference index of either the more preferred (MF5: $T = 1.5$, $p = 0.003$) or less preferred (MF2: $T = 1.5$, $p = 0.003$; MF6: $T = 0.0$, $p < 0.001$) item decreased. MF3's preference index did not show any significant difference following WAY-208466 acute injections but decreased for the more preferred food (MF3: $T = 14.5$, $p = 0.064$).

On post-treatment days (+24h), two out of three parameters were still slightly modified compared to saline days at group level (Table S1): CR increased significantly in all trials ($T = 188.5$, $p < 0.001$) and the

preference index for the more preferred food decreased significantly ($T = 371.0$, $p = 0.026$). Individually, MF6's completion rate remained increased (all trials: $T = 1.5$, $p = 0.002$). Interestingly, MF3's daily completion rate increased significantly in all trials after 24 h ($T = 11.5$, $p = 0.015$).

3.1.2. Subchronic protocol

We tested whether these results could be reproduced at group level in a brief treatment-like paradigm over four weeks. Subchronic (daily consecutive) injections of the molecule at two doses resulted in similar effects on food-intake as shown in the acute protocol (Fig. 2).

At group level, CR means (Fig. 2A) differed significantly between conditions in all trials ($X2(3) = 18.341$, $p < 0.001$) and in both contexts (single-food: $X2(3) = 24.516$, $p < 0.001$; food-choice: $X2(3) = 13.664$, $p = 0.003$). The average values of both WAY treatment weeks increased significantly compared to the NaCl pre-treatment week in all trials (WAY-0.03: $\text{padj} = 0.024$; WAY-0.1: $\text{padj} < 0.001$) and in the single-food trials (WAY-0.03: $\text{padj} < 0.001$; WAY-0.1: $\text{padj} = 0.002$). Compared to the post-treatment week, the CR increase was still observed for the WAY-0.1 condition considering all trials ($\text{padj} = 0.024$) and for WAY-0.03 in single-food trials ($\text{padj} = 0.009$). CR also increased in food-choice trials

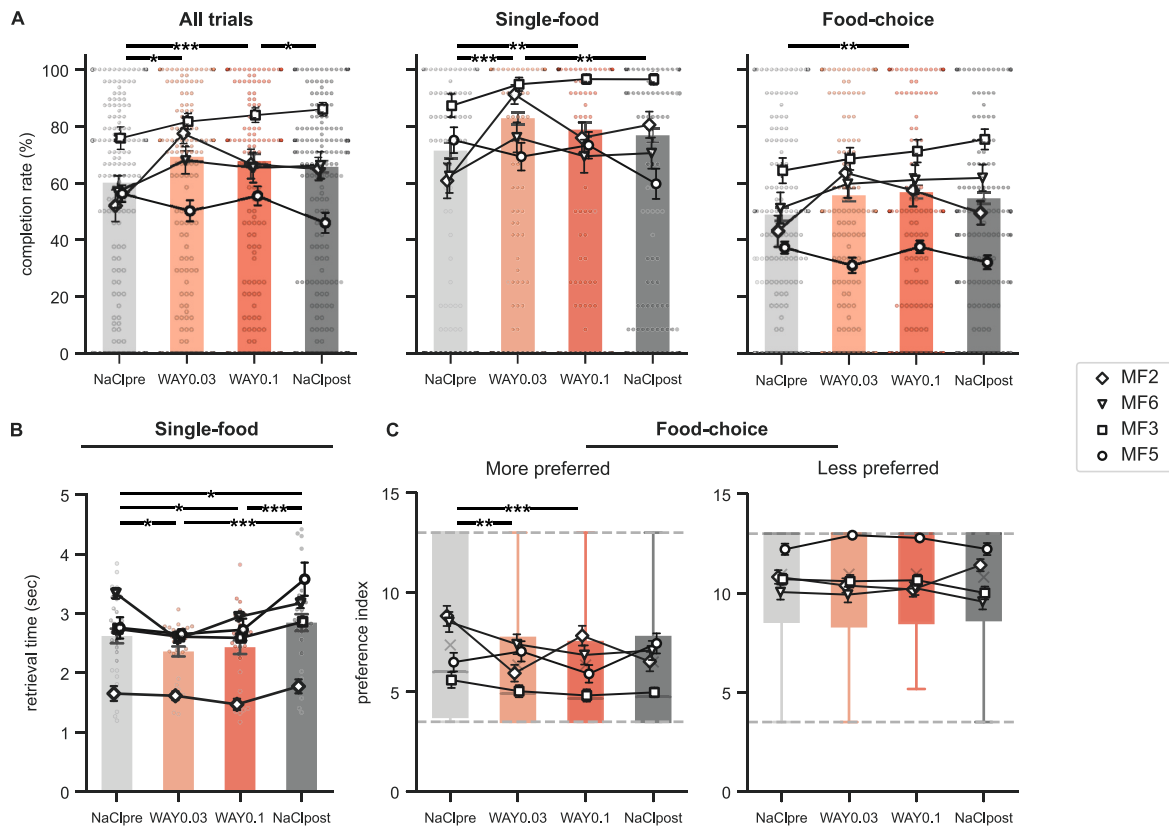


Fig. 2. Group effects of WAY-208466 subchronic injections (0.033–0.1 mg/kg) in the food-intake task. For panels A, B, and C, group differences between all conditions were assessed using paired tests (Friedman Conover post-hoc tests with Holm correction; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). **A.** Mean completion rate. Bar plots illustrate group completion rate (mean \pm SEM; $n = 240$ trials per condition). Line plots with markers illustrate individual variations (mean \pm SEM; $n = 60$ trials). **B.** Mean retrieval time (RT) in single-food trials. Bar plots show group means (T01-T08; $n = 32$ T periods), and line plots show individual means (T01-T08; $n = 8$ T periods). **C.** Mean preference index for each item in choice trials. In boxplots, gray crosses and lines, respectively, show group means and medians in each condition ($n = 240$ trials), which are inversely related to preference for an item. Line plots show individual means ($n = 60$ trials).

with the higher treatment dose compared to NaCl-pre (WAY-0.1: $\text{padj} = 0.001$). There was no significant difference in CR between pre- and post-treatment weeks, or between the two treatment doses.

RTs differed between conditions (Fig. 2B; $X^2(3) = 36.375$, $p < 0.001$), decreasing significantly during both treatment weeks compared to NaCl pre- (WAY-0.03: $\text{padj} = 0.042$; WAY-0.1: $\text{padj} = 0.033$) and post-treatment (WAY-0.03: $\text{padj} < 0.001$; WAY-0.1: $\text{padj} < 0.001$). RT increased significantly after treatment and exceeded pre-treatment values (NaCl-post $>$ NaCl-pre, $\text{padj} = 0.041$). No significant difference in RT means was found between the two drug doses ($\text{padj} = 0.847$).

The preference index (Fig. 2C) differed significantly only for the more preferred food ($X^2(3) = 20.045$, $p < 0.001$). Each treatment dose significantly decreased the preference index compared to NaCl-pre (WAY-0.03: $\text{padj} = 0.008$; WAY-0.1: $\text{padj} < 0.001$). No other significant differences were found. The significance levels of the *post hoc* comparisons applied at the individual level are shown in Fig. S2.

3.2. WAY-208466 impacts behaviours differently depending on protocol, dose, and animal profile

In the acute protocol, measuring the duration proportion among different behavioural categories (Fig. 3) highlighted whether WAY-208466 induced a shift from one category to another. Group activity duration was unchanged between conditions (Fig. 3A), and increased only in MF3 ($T = 23.0$, $p = 0.035$). Active behaviour occurrences decreased very slightly at group level ($T = 620.0$, $p = 0.030$) and in MF5 ($T = 21.0$, $p = 0.026$). Consequently, duration ratio per active behaviour increased slightly in the group ($T = 577.0$, $p = 0.013$) and in MF3 ($T =$

4.0, $p < 0.001$).

Activity-related behaviours (Fig. 3B) were not affected, or only slightly, by the acute drug injections. Object-oriented duration increased significantly in the group ($T = 508.0$, $p = 0.003$) and in one animal (MF3: $T = 14.0$, $p = 0.007$), although occurrence remained unaffected. Movement occurrences increased only in MF2 ($T = 21.0$, $p = 0.026$), with no change observed at group level. Regarding activity and activity-related behaviours, few animals showed significant variation (Fig. 3A and B), while anxiety-related measures were significantly more affected by the drug (Fig. 3C).

Individual baseline profiles show two animals (MF6 and MF5) produced longer and/or more anxious-like behaviours compared to the rest of the group. In these most anxious animals, both duration (MF6: $T = 21.0$, $p = 0.026$, MF5: $T = 21.0$, $p = 0.026$) and occurrences (MF6: $T = 9.0$, $p = 0.002$, MF5: $T = 10.5.0$, $p = 0.003$) of anxiety-like behaviours decreased significantly. These parameters also decreased slightly in MF2 (duration: $T = 28.0$, $p = 0.073$, occurrences: $T = 16.0$, $p = 0.010$), while no effect was observed in MF3. The same measurements also decreased at group level (duration: $T = 425.0$, $p < 0.001$, occurrences: $T = 295.0$, $p < 0.001$). Regarding duration, the proportion of anxiety-like behaviours among overall activity decreased significantly in MF5 ($T = 22.0$, $p = 0.030$), as well as at group level ($T = 610.0$, $p = 0.025$), suggesting a transition from anxiety-like behaviours to other behaviours, rather than hypoactivity.

During post-injection days (+24h), anxiety-like behaviour measurements returned to baseline levels in MF6, MF5 and in the group (Table S2). However, duration of object-oriented behaviours remained significantly high in MF3 ($T = 21.0$, $p = 0.026$) and slightly high in the

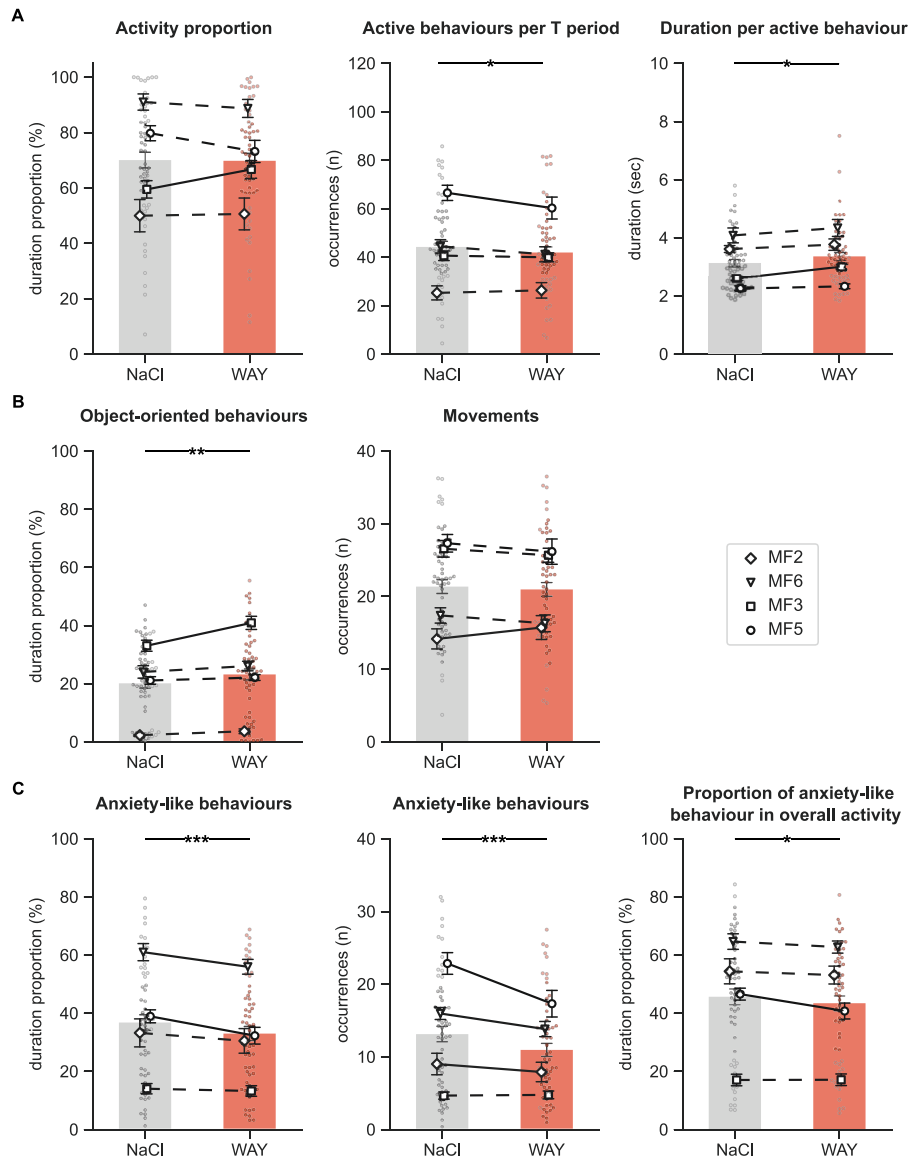


Fig. 3. Individual and group effects of acute WAY-208466 injections (0.1 mg/kg) on spontaneous behaviour.

Each behavioural category occurrence and duration was measured in each condition across T01 to T15 periods. Bar plots illustrate group means \pm SEM ($n = 60$ T periods per condition). Stars highlight significant group differences between the two conditions (two-sided Wilcoxon signed rank test; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Line plots with markers represent individual variations (mean \pm SEM; $n = 15$; solid lines: $P < 0.05$, dotted lines: ns; two-sided Wilcoxon signed rank test). **A.** Activity-related ratios. Panels respectively display: percentage of activity duration in a mean 180-s T period; total number of active behaviours occurring in a mean T period; and mean duration of a given active behaviour (i.e., behavioural maintenance). **B.** Behavioural categories related to activity. Panels respectively display: average proportion of time spent performing object-oriented behaviours per T period; and average number of non-goal movements produced per T period. **C.** Anxiety-related measurements and ratios. Panels respectively display: average proportion of time spent performing anxiety-like behaviours per T period; average number of anxiety-like behaviours produced per T period; and their proportion relative to overall activity duration.

group ($T = 589.0$, $p = 0.016$), as did mean duration of a given active behaviour (MF3: $T = 12.0$, $p = 0.004$; group: $T = 562.0$, $p = 0.009$).

Fig. 4 shows the behavioural effects of the subchronic injection protocol at group level. Interestingly, in this protocol, the proportion of activity duration increased with treatment, reaching statistical significance only at the highest dose (Fig. 4A; NaCl-pre vs. WAY-0.1: $\text{padj} = 0.004$). This effect was reversible (NaCl-pre vs. NaCl-post: ns). A similar effect was observed in active behaviour occurrences (NaCl-pre vs. WAY-0.1: $\text{padj} < 0.001$). No effect was observed in the mean duration per active behaviour.

As in the acute protocol, the duration proportion of object-oriented behaviours also increased significantly only at the highest dose (Fig. 4B; NaCl-pre vs. WAY-0.1: $\text{padj} = 0.006$). Movement occurrences increased significantly with both doses (NaCl-pre vs. WAY-0.03: $\text{padj} =$

0.006 ; NaCl-pre vs. WAY-0.1: $\text{padj} < 0.001$). These two behavioural measurements returned to baseline levels during the post-treatment week (NaCl-pre vs. NaCl-post: ns).

Contrary to the acute protocol, no treatment effect was detected on anxiety-like behaviour duration, occurrence, or proportion of overall activity (Fig. 4C; NaCl-pre vs. WAY-0.03 or WAY-0.1: ns). However, an increase in the last two measurements was observed during post-treatment week, compared to pre-treatment week (NaCl-pre vs. NaCl-post: occurrence: $\text{padj} < 0.001$; proportion of anxiety-like behaviour in overall activity: $\text{padj} = 0.025$). Inter-individual variability affected anxiety-like behavioural measurements, as two animals (MF2, MF6) had opposite anxiety-like profiles and responded inversely to treatment. Fig. S3 shows the significance of each *post hoc* test by subject and behavioural category.

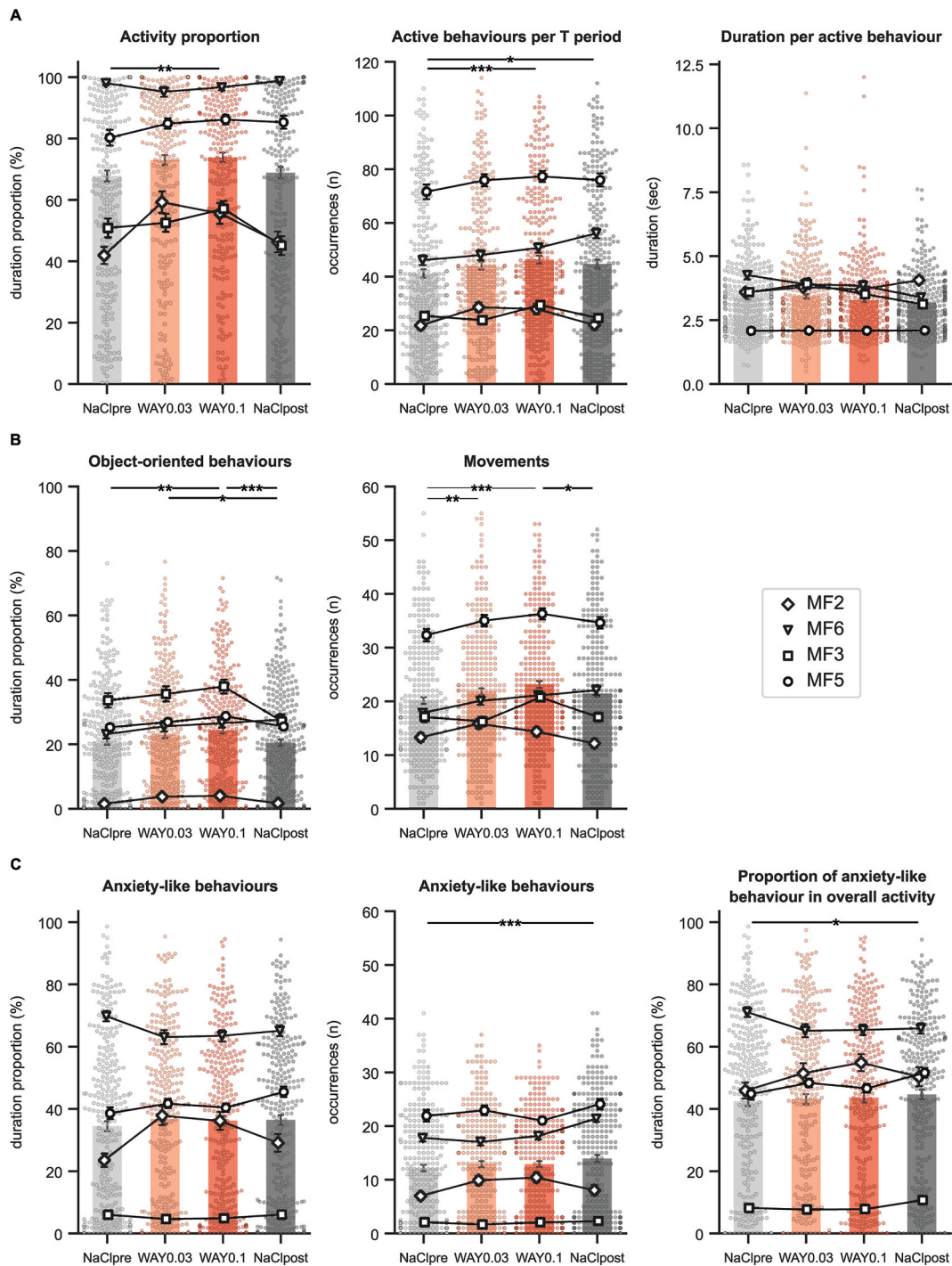


Fig. 4. Group effects of subchronic WAY-208466 injections (0.03–0.1 mg/kg) on spontaneous behaviour. Each behavioural category occurrence and duration was measured in each condition across T01 to T15 periods. For panels A, B, and C, bar plots illustrate group means \pm SEM ($n = 300$ T periods per condition). Stars highlight significant group differences between all conditions (Friedman Conover post-hoc tests with Holm correction; * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$). Line plots with markers show individual variations (mean \pm SEM; $n = 75$). **A.** Activity-related ratios. **B.** Behavioural categories related to activity. **C.** Anxiety-related measurements and ratios. (Panel description as in Fig. 3).

3.3. WAY-208466 reduced the 5-HT₆R specific radiotracer binding potential in striatal and cortical regions

In the four animals, WAY-208466 was pre-injected at 0.1 mg/kg prior to the [¹⁸F]2FNQ1P radiotracer, to compare its BP_{ND} between treatment and baseline scans (Fig. 5). The group average map of control scans (Fig. 5A) shows that regions with the highest radioligand binding (BP_{ND} > 0.3) include the basal ganglia (caudate nucleus (CdN), putamen

(Put) and external globus pallidus (GPe)), as well as the thalamus, amygdala (Amyg), insula, and posterior cingulate cortex (PCC). These latter regions are the only cortical regions with high [¹⁸F]2FNQ1P BP_{ND}.

WAY-208466 administration induced displacement of [¹⁸F]2FNQ1P from 5-HT₆R, resulting in a decreased signal in cortical and subcortical regions. The resulting delta map (WAY-208466 – control) shows the primary regions where decreased [¹⁸F]2FNQ1P BP_{ND} occurred, such as antero-posterior dorsal striatum, and VS (Fig. 5A). This decrease was

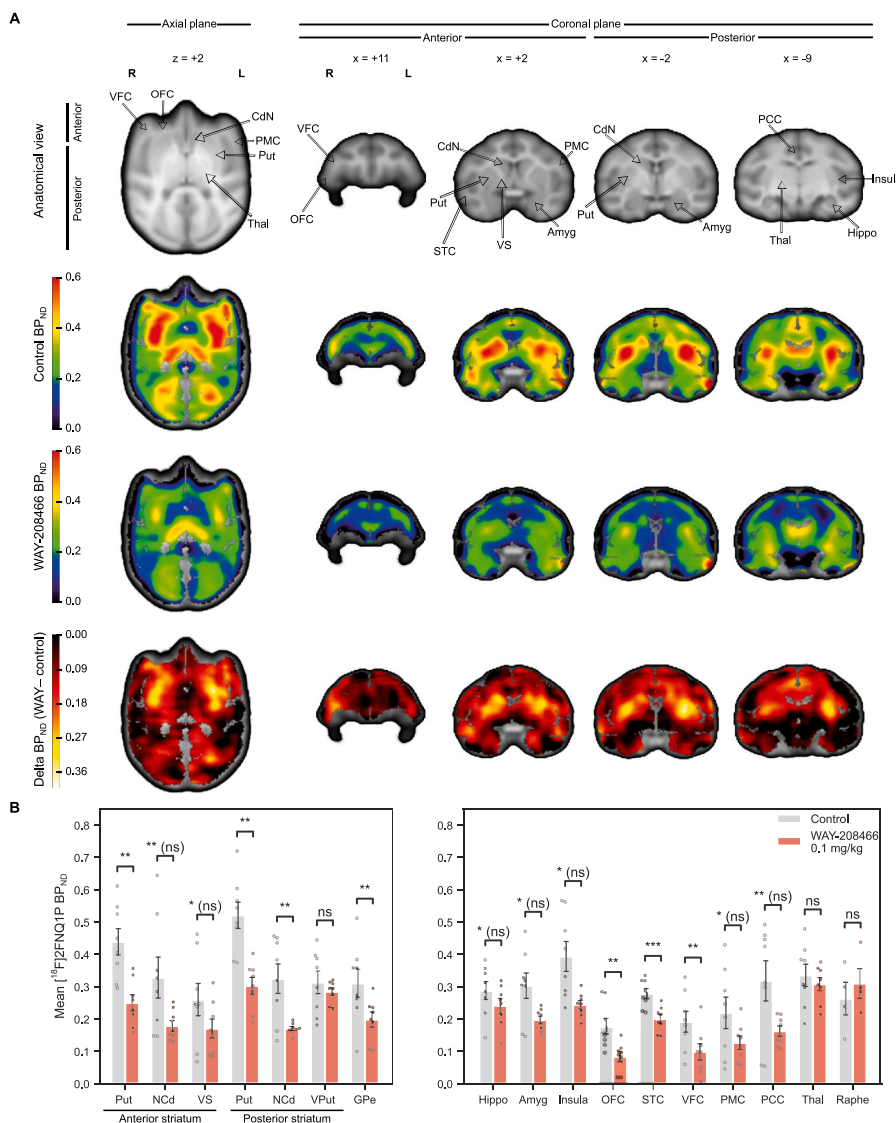


Fig. 5. [¹⁸F]2FNQ1P BP_{ND} reduction induced by WAY-208466 pre-injection (0.1 mg/kg) across cortico-striatal ROIs. **A.** The 5-HT₆R agonist [¹⁸F]2FNQ1P binding on 5-HT₆R. The antero-posterior indications refer to the anterior commissure (AC). One axial (z = +2) and four coronal (x = +11, +2, -2, -9) brain slices are displayed. The first panel displays an anatomical view of the different ROIs. The second and third panels respectively show [¹⁸F]2FNQ1P BP_{ND} parametric maps of the four macaques averaged in baseline (upper panel) and WAY-208466 conditions. In the last panel, delta values illustrate decrease in [¹⁸F]2FNQ1P BP_{ND} (WAY-208466 minus control) throughout brain slices. **B.** ROI statistical analysis. Striatal subregions are shown in the left panel, with limbic and cortical ROIs shown in the right panel. Each bar shows average [¹⁸F]2FNQ1P BP_{ND} in the group in baseline versus WAY-208466 scans. Data points from the four monkeys are lateralized (i.e., for each brain region and condition, n = 8; except for the raphe n = 4). Stars indicate significant p-values (one-tailed paired t-test; *P < 0.05, **P < 0.01, ***P < 0.001). “(ns)” identifies the regions that did not survive Benjamini-Yekutieli correction for multiple comparisons (applied for 17 ROIs). Abbreviations: BP_{ND}: non-displaceable binding potential; CdN: caudate nucleus; Put: putamen; VS: ventral striatum; VPut: ventral putamen; GPe: globus pallidus external segment; Hippo: hippocampus; Amyg: amygdala; OFC: orbitofrontal cortex; STC: superior temporal cortex; VFC: ventral frontal cortex; PMC: pre-motor cortex; PCC: posterior cingulate cortex; Thal: thalamus.

also evidenced in other brain regions, particularly in the amygdala, insula, orbitofrontal cortex (OFC), PCC and, to a lesser extent, the hippocampus. Other cortical regions, the ventral frontal (VFC), superior temporal (STC), and primary motor (PMC) cortices, had a detectable BP_{ND} decrease following WAY-208466 injection. The thalamus and raphe, which have a high concentration of 5-HT₆R, were unaffected. After adjusting for multiple testing, ROI statistical analysis highlighted regions where the 5-HT₆R agonist was more likely to bind, i.e., the antero-posterior putamen, posterior CdN, GPe, OFC, STC, and VFC (Fig. 5B).

4. Discussion

This study in four macaques sought to demonstrate the impact of a

selective, full 5-HT₆R agonist on food motivation and spontaneous behaviours. Both acute and subchronic injections of the agent successfully increased food motivation parameters in the group, but only acute injections reduced anxiety-like behaviours. Additionally, PET imaging provided evidence that the striatum and other brain regions may be involved in WAY-2084466’s behavioural and motivational effects.

4.1. WAY-208466, a 5-HT₆R agonist, increases food motivation in non-human primates

In this study, the food-intake task used two contexts to assess potential changes in both appetitive motivation in any type of trial and decision-making related to food choice. In the choice context, acute administration of the agent decreased the preference index of both the

more and the less preferred items. This suggests increased valuation of both foods, but no shift in their relative preference. This phenomenon was observed only for the more preferred item during subchronic administration. In both protocols, the overall increase in CRs in both trial types, in addition to the decrease in RT, reflects improved motivation towards food. Thus, acute and subchronic administration of WAY-208466 had similar effects on food motivation. These effects on food behaviour persisted 24 h post-treatment when administered acutely at a 0.1 mg/kg dose, but were reversible during the washout week following subchronic treatment. This demonstrates that the observed changes were treatment-induced rather than time- or training-induced. Post-treatment RT increased above pre-treatment levels in the group. This rebound effect, suggesting a reduction in food motivation beyond the pre-treatment period, could be attributed to subsequent sub-stimulation or desensitization of 5-HT₆Rs, although this does not appear to be the case *in vivo* in rats (Schechter et al., 2008). Interestingly, no dose effect was observed in the subchronic treatment. The 0.03 mg/kg dose was sufficient to increase food motivation, while the 0.1 mg/kg dose had no additional benefit or side effect on food intake. A limitation is that no significant weight changes were observed during the subchronic protocol (results not shown), probably because food intake was controlled outside the task. Another limitation is that, although *anorexia nervosa* is more common in women than in men, we used male monkeys. On the other hand, this avoids any confounding cyclic hormonal influence on the behavioural effects of WAY-208466. It would be interesting to evaluate off-task calorie intake along with body weight follow-up in a larger *ad libitum* female animal sample. Nevertheless, these results validate the potential of low-dose WAY-208466 to increase food intake by enhancing appetitive motivation. This preclinical NHP study is the first to show the orexigenic effect of a 5-HT₆R agonist.

To our knowledge, only preclinical rodent studies have attempted to modulate feeding behaviour using 5-HT₆R agonists. Most used EMD-386088, described as either a full or partial agonist. Consistent with the present study, in non-fasting rats, it increased palatable food intake by at least 80% when administered subcutaneously (da Silva et al., 2018) or locally into the NAc (Pratt et al., 2009). When injected into this ventral striatal region, it increased both appetitive motivation in a progressive ratio paradigm *ad libitum* and consummatory free food intake in fasted rats, suggesting it could be involved in various aspects of food motivation (Pratt et al., 2012; Anderson et al., 2023). However, when EMD-386088 was chronically administered via intraperitoneal injections, it reduced standard and palatable food intake and body weight in various rat feeding models (Kotanska et al., 2017). Several confounders may have led to these opposite results. Uncertainty remains regarding the *in vivo* properties of agonists (Hoyer and Boddeke, 1993; Heal et al., 2008). Most have been shown to have intrinsic activities ranging from partial to full agonism *in vitro*, depending on receptor density, constitutive activity, origin (rat or human) and other parameters. *In vitro*, several drugs behaving as full agonists (including WAY-208466, WAY-181187, E-6837, and EMD-386088) at a human 5-HT₆R (Holenz et al., 2005; Mattsson et al., 2005; Fisas et al., 2006) show only a submaximal response at the rat 5-HT₆R (Fisas et al., 2006; Dupuis et al., 2008; Jastrzębska-Więsek et al., 2013). Therefore, in rats, partial agonists may have produced either an agonistic or an antagonistic effect, (*i.e.*, increase or decrease in food motivation), depending on its concentration relative to endogenous serotonin levels (Jastrzębska-Więsek et al., 2013), and receptor density after potential treatment-induced desensitization. Thus, using a more selective drug, such as WAY-208466, through to behave as a full agonist (Schechter et al., 2008) at the human (and by extension macaque) 5-HT₆R, offers better translational prospects for AN.

4.2. WAY-208466 displays better anxiolytic-like activity in the most anxious individuals

The 5-HT₆R agonist's effects on spontaneous behaviours and the

potential benefits of this treatment were assessed in the second part of our study. At group level, acute administration showed anxiolytic-like activity (Fig. 3C). This was particularly significant in the two animals that exhibited the most anxiety-like behaviours, suggesting that the drug may have an impact in a population with anxiety disorders. In the group, the proportion of anxiety-like behaviour in overall activity decreased, while object-oriented behaviours increased, suggesting that the animals generally shifted from self-directed behaviours to outward-directed behaviours rather than to hypoactivity or drowsiness. Additionally, the consistent level of activity in the group, together with the increased ability to maintain a given behaviour, showed this 0.1 mg/kg acute dose to be suitable in avoiding undesirable hyperactivity effects. These results confirm the beneficial anxiolytic-like effects of acute systemic 5-HT₆R agonist injections, which have been reproduced without affecting locomotor activity in various rat paradigms with WAY-208466, WAY-181187, and EMD-386088 (Cole et al., 2007; Schechter et al., 2008; Carr et al., 2011; Jastrzębska-Więsek et al., 2014).

Nevertheless, to date, no study has assessed the chronic anxiolytic potential of a 5-HT₆R agonist. Researchers administered WAY-181187 to rats intraperitoneally at 3 mg/kg for 21 days, producing a non-significant anxiolytic-like effect in the elevated plus maze test, but no effect in the Vogel Conflict Drinking test or locomotor activity. It produced the same results acutely, suggesting this dose was probably too low to assess its anxiolytic potential. However, its specific effect when administered alone was not the purpose of the study (Wesolowska et al., 2021). Similarly, in our subchronic protocol, WAY-208466 did not achieve the anxiolytic goal at group level (Fig. 4C). This lack of effect may be due to inter-individual variability, as it produced an anxiogenic-like effect in the oldest animal and a hyperactive-like response in two of the least anxious and active animals (Fig. S3). This may account for its particular response profile, as in humans, 5-HT₆R availability may decrease with age (Radhakrishnan et al., 2018). For other individuals, we must also consider the potential stress of the subchronic injection protocol over time, which may have mitigated any anxiolytic effect, as the frequency and proportion of anxiety-like behaviour among overall activity were significantly higher than baseline during the post-treatment saline week. In addition, the 0.1 mg/kg dose resulted in an overall increase in activity, with more object-oriented behaviours and movements. However, no pathological hyperactivity features were observed as the animals displayed good behavioural maintenance. The 0.03 mg/kg dose appeared to be more appropriate, as it did not significantly affect activity-related behaviours.

The discrepancies observed between acute and chronic effects raise the question of the compound's intrinsic activity, as well as of whether the reported effects are direct or indirect. This may reflect accumulation, especially at the higher dose. Indeed, when WAY-208466 was administered acutely, its anxiolytic-like effects were not sustained 24 h later, while increased object-oriented behaviours persisted longer. These two types of behaviours may be driven by different neuronal populations in different brain regions that could dynamically adapt to changes induced in the chronic protocol. 5-HT₆Rs are known to modulate excitatory (glutamate) and inhibitory (GABA) signaling (Gérard et al., 1996; Woolley et al., 2004; Schechter et al., 2008; Marazziti et al., 2013; Helboe et al., 2015; de Jong and Mørk, 2017), potentially impacting the activity of several cortico-subcortical networks.

4.3. WAY-208466 targets cortico-basal ganglia circuit regions involved in anorexia nervosa and anxiety disorders

This study is the first to investigate a 5-HT₆R-specific agonist's occupancy regions. The [¹⁸F]2FNQ1P radiotracer allowed us to map baseline distribution of 5-HT₆R in the NHP brain. Consistent with other primate studies using various techniques (Kohen et al., 1996; Roberts et al., 2002; Hirst et al., 2003; Marazziti et al., 2012; Sgambato-Faure et al., 2017; Emery et al., 2020), the dorsal striatum was the most densely enriched region. PET imaging of the 5-HT₆R in the four animals

revealed that, as in our previous study using a 5-HT₆R antagonist (Pitoy et al., 2024), WAY-208466 acted mainly in the antero-posterior dorsal striatum (*i.e.*, CdN and putamen), targeting the VS to a lesser extent. Interestingly, no changes were observed in the thalamus or raphe nuclei. Additionally, WAY-208466 significantly targeted the GPe, OFC, VFC, and STC. Thus, all of these regions, which are organized in cortico-basal ganglia circuits, could directly contribute to WAY-208466's effects on behavioural and food motivation. Notably, the drug was also detected in other limbic regions that did not survive correction, in the insula, amygdala and hippocampus, which have received particular attention in 5-HT₆R-related rodent studies (Nikiforuk et al., 2011; Wang et al., 2016; Sun et al., 2018). The small number of animals and variability in BP_{ND} levels may account for the lack of significant effects in these structures.

The GPe is a basal ganglia structure of the indirect pathway that receives projections from all striatal sub-territories, with a functional organization similarly divided into sensorimotor, associative and limbic territories (Tremblay et al., 2015). In the GPe and striatum, GABAergic disturbances (using bicuculline) in NHPs performing similar behavioural tasks induced: 1) abnormal movements in sensorimotor territories from the antero-dorsal putamen, posterior striatum and GPe; 2) hyperactivity with attentional impairment associated with touch or attraction to objects in the associative territories from the anterior CdN and dorsal GPe; and 3) anxiety-like behaviours from the limbic territories in the medial part of the VS and ventral GPe (Grabli et al., 2004; Worbe et al., 2009, 2013; Galineau et al., 2017). In subsequent NHP studies, anatomical tracer injections within these different functional territories of the anterior striatum identified distinct cortico-basal ganglia circuits: 1) a circuit passing through the dorsal striatum, the CdN, involved in decision-making processes, hyperactivity, and object-oriented behaviours; 2) a circuit connecting the OFC and VFC to the medial part of the VS and involved in anxiety-like behaviours and; 3) a circuit connecting the insula and STC to the ventral putamen and involved in food motivation (Worbe et al., 2013; Sgambato-Faure et al., 2016). Thus, WAY-208466 could act on these different circuits at both cortical and subcortical levels.

Brain imaging studies in AN using monetary (Wagner et al., 2007; Wierenga et al., 2015) or food-choice tasks (Foerde et al., 2015) have consistently shown functional circuit alterations. Regardless of their state of satiety, AN patients failed to activate the reward valuation circuitry (including the VS), but over-engaged the cognitive control circuitry between the lateral prefrontal cortex and CdN that play a role in maladaptive decision-making guiding food choices in AN. Thus, acting on the first described circuit via CdN, 5-HT₆R antagonists may facilitate more adaptive choices such as approaching food. AN is frequently comorbid with anxiety disorders (Bulik et al., 1997; Kaye et al., 2004), and some authors have suggested that these two pathologies are closely related (Steinglass et al., 2010; Compan et al., 2015). The medial VS, lateral OFC, VFC (or mPFC; area 25), and insula are regions known for their involvement in aversive anticipation and active avoidance due to uncertainty (Saga et al., 2019), two important mechanisms behind anxiety (Grube and Nitschke, 2013). Dysfunction in such regions is found in both AN and anxiety-related disorders, and may lead to excessive fear of negative outcomes (Etkin and Wager, 2007; Grube and Nitschke, 2013). The present PET imaging results showed that WAY-208466 may have produced acute anxiolytic-like effects in NHPs mainly through the OFC, which is associated with the second described circuit. Interestingly, the OFC has been shown to encode the value (*i.e.*, preference) of rewards in NHPs during choice tasks (Tremblay and Schultz, 1999). In humans, it has been implicated in emotional decision-making and the hedonic experience of food (Kringelbach, 2005). Thus, WAY-208466 may have increased the value attributed to both more and less preferred rewards and, more generally, increased willingness to retrieve food through 5-HT₆R modulation in the OFC-striatal circuit involved in incentive motivation. Finally, it has also been suggested that there may be impaired interoceptive information processing via an impaired VS-insular circuit in AN (Wagner et al., 2008; Oberndorfer et al., 2013).

Our results show that WAY-208466 may increase food motivation while targeting the anterior ventral putamen, the STC and, to a lesser extent, the insula, which are interconnected in the cortico-striatal (more lateral) circuit regulating food motivation (Sgambato-Faure et al., 2016).

5. Conclusion

Excessive dietary restriction in AN patients is a major public health concern, as there is no pharmaceutical treatment providing benefits for refeeding. Therefore, we propose WAY-208466 may be beneficial for food motivation AN-R patients not responding to SSRI treatment and potentially for treating anxiety-related comorbidities, after pharmacological safety evaluation. The 5-HT₆R target has recently been addressed in obesity, with some 5-HT₆R antagonists completing phase I clinical trials. This study could reopen avenues for the 5-HT₆R target in AN-R to modulate eating behaviour and deserves further investigation in specific models. In NHPs, injecting WAY-208466 directly into these regions should determine the precise involvement of the CdN, putamen, and VS in the processes underlying these symptoms. fMRI studies in NHPs using 5-HT₆R-specific modulation should determine the involvement of the aforementioned circuits in food motivation and anxiety-related disorders.

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Contributors

MP: Data curation, Formal Analysis, Investigation, Methodology, Software, Visualization, Writing – original draft, Writing - Review & Editing; JM: Investigation, Writing - Review & Editing; LG: Investigation, Writing - Review & Editing; JD: Data curation, Formal Analysis, Software, Visualization, Writing – original draft, Writing - Review & Editing; NC: Data curation, Software, Writing - Review & Editing; IM: Data curation, Software, Writing - Review & Editing; TB: Investigation, Resources, Writing - Review & Editing; KP: Investigation, Writing - Review & Editing; SL: Investigation, Resources, Writing - Review & Editing; BG: Funding acquisition, Writing - Review & Editing; LZ: Funding acquisition, Supervision, Writing - Review & Editing; LT: Conceptualization, Funding acquisition, Investigation, Methodology, Project administration, Resources, Supervision, Writing – original draft, Writing - Review & Editing.

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

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.nsa.2024.104086>.

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