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Contrasting effects of 5-HT₃ receptor stimulation of the nucleus accumbens or ventral tegmentum on food intake in the rat

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Abstract

Although serotonin (5-HT) signaling is known to regulate food intake and energy homeostasis, the roles of the 5-HT₃ receptor in feeding processes has been elusive. 5-HT₃ receptors are found throughout mesolimbic circuitry that promote feeding not only in response to hunger, but also to the palatable and rewarding properties of food. These experiments examined if stimulation or blockade of the 5-HT₃ receptor of the nucleus accumbens (NAcc) or ventral tegmentum affected food intake in the rat in response to hunger or the presence of a palatable diet. Rats (N = 6–9/group) received bilateral injections of the 5-HT₃ agonist m-chlorophenylbiguanide hydrochloride (mCPBG; at 0.0, 10.0, or 20.0 µg/0.5µl/side) or the 5-HT₃ antagonist ondansetron hydrochloride (at 0.0, 1.0, 2.0, or 5.0 µg/0.5µl/side) into either the NAcc or the ventral tegmentum. NAcc 5-HT₃ receptor stimulation significantly increased 2-hr food intake in food-deprived animals offered rat chow and in a separate group of unrestricted rats offered a sweetened fat diet. In contrast to the feeding increase seen with NAcc treatments, stimulation of 5-HT₃ receptors of the ventral tegmentum significantly reduced food and water intake in food-restricted animals; reductions of intake in non-restricted rats offered the palatable diet did not approach significance. Blockade of the 5-HT₃ receptor had no effect on feeding in either brain region. These data support a functional role for serotonergic signaling in the mesolimbic pathway on motivated behavior, and demonstrate that 5-HT₃ receptors differentially modulate food consumption in a region-dependent manner.

Keywords

Nucleus accumbens; ventral tegmental area; serotonin; 5-HT₃; food intake; motivation

1.0 Introduction

Although there are currently few approved pharmacological treatments to assist with long-term weight loss in overweight or obese patients, those that have targeted serotonergic signaling have a history of modest success. Two current FDA-approved drugs for weight

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loss, the phentermine/topiramate combination (Qsymia®) and lorcaserin (Belviq®), increase serotonin signaling. Phentermine increases serotonin (5-HT) tone in the synapse, whereas lorcaserin is a selective agonist at the 5-HT_{2C} receptor. Systemic stimulation of 5-HT_{1B} and 5-HT_{2C} receptors decreases food intake, causing reductions in meal size and duration that have been interpreted as advancing satiety processes [e.g., 1–5]. The recent success of lorcaserin in assisting weight loss provides hope that serotonin-based therapies that target individual serotonin subtypes may be of use in combatting obesity, without the cardiovascular side effects that resulted in the market removal of prior agents such as sibutramine and the phentermine/fenfluramine combination (phen-fen). The effects of serotonergic agents on satiety and meal size appear to be the result of their actions on the central nervous system, as injections of 5-HT_{1B} or 5-HT_{2C} agonists into the parabrachial and hypothalamic nuclei, respectively, also reduce feeding [e.g. 4,6].

Recent work has begun to examine what role, if any, the various 5-HT receptors of the meso-accumbens pathway may have on feeding and food-directed motivation. This pathway is of particular interest, as it has been argued to promote the overconsumption of palatable diets based upon their rewarding and reinforcing properties, and is differentially activated by food cues in individuals who are obese or are at risk of becoming obese [7–11]. Within the nucleus accumbens (NAcc) shell, stimulation of the 5-HT_{1A} (but not 5-HT_{1B} or 5-HT₇) receptors inhibits feeding, albeit with concomitant locomotor alterations [12,13]. Jean *et al.* reported that 5-HT₄ receptors of the NAcc inhibit food intake and are responsible for the anorexia observed following systemic 3,4-N-methylenedioxymethamphetamine (MDMA, ecstasy) treatment in mice [14]. In contrast to the reduced feeding caused by 5-HT_{1A} and 5-HT₄ receptor stimulation, 5-HT₆ receptor agonism of the NAcc increases food intake [13]. Additionally, stimulation of 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2B} receptors (but not 5-HT_{2A} or 5-HT_{2C} receptors) within the ventral tegmentum alters feeding in rats [15]. Clearly, serotonin afferents to the mesoaccumbens pathway are well-positioned to regulate motivated behavior, and serotonin's impact on food-directed motivation depends upon the particular receptor subtype engaged within these regions.

The 5-HT₃ receptor is unique amongst the serotonin receptor classes, as it is the only serotonin receptor that directly opens a cation channel, rather than acting as a g-protein coupled receptor. 5-HT₃ receptors are found peripherally and in the central nervous system, and drugs that antagonize this receptor have been useful for their anti-emetic properties [16]. With regards to the central control of food intake, it has been shown that 5-HT₃ receptors of the nucleus of the solitary tract regulate meal size and suppress feeding in response to cholecystokinin [17,18]. 5-HT₃ receptors are also found within the ventral tegmental area (VTA) and the NAcc in rats and humans [19–21], and it is likely that they serve important regulatory functions. Specifically, 5-HT₃ receptor stimulation in either the VTA or NAcc increases dopamine output [22,23], and 5-HT₃ receptor antagonists reduce meso-accumbens dopamine signaling in response to several abuse-prone drugs, such as alcohol [for review, see, 24], cocaine [e.g., 25,26], and morphine [e.g., 25,27]. Given that drugs of abuse impact the same neural pathways implicated in the reinforcing and rewarding properties of food, these experiments were conducted to test whether stimulation or blockade of 5-HT₃ receptors of the NAcc and ventral tegmentum might regulate feeding, motivated either by energy need or by the presence of a palatable sweetened fat diet.

2.0 Materials and Methods

2.1 Animals and Housing

Sixty-six adult male Sprague-Dawley rats (Harlan, Madison, WI; 275–300 g at arrival) were acclimated to dual housing in a colony room maintained at ~21 °C with a 12-hr light–dark cycle (lights on at 7 a.m.). All experiments were conducted in accordance to NIH animal care guidelines and were approved by the Wake Forest University Animal Care and Use Committee.

2.2 Surgical Procedures

Following acclimation to the housing environment and daily handling, rats were anesthetized with a Ketamine-Xylazine cocktail (100 mg/kg–10mg/kg). Standard aseptic surgical procedures were used to implant indwelling stainless steel guide cannulas (23 gauge) bilaterally above the NAcc shell (with the nose bar set at 5 mm above interaural zero: 3.1 mm anterior and 1.0 mm lateral to bregma, 5.0 mm ventral to the skull surface) or the VTA (with the skull flat; –5.6 mm posterior and 0.6 mm lateral to bregma, 7.3 mm ventral to skull surface). Stylets were placed within the cannulas to maintain patency. Rats were given at least one full week of recovery before acclimation to the feeding chambers and subsequent behavioral testing.

2.3 Apparatus

Food intake was monitored during 2-hr feeding sessions in experimental chambers. The feeding chambers were constructed from clear acrylic, with internal dimensions 42 cm wide, 30.5 cm deep and 33 cm tall. A water bottle was hung at one end of the chamber, and a food hopper was available at the opposite end of the chamber. The hopper was filled with diet and mounted on a food intake monitor (head entry at 6.4 cm above the wire floor). Each food intake monitor consisted of a calibrated potentiometer which allowed for continuous monitoring of the mass/weight of the food hopper during the experimental sessions (Med Associates, St. Albans, VT). Infra-red eyebeams were located along the floor at three locations (5 cm above the wire floor) to measure ambulation; four additional IR beams were placed at a height of 16 cm above the floor to index rearing behavior. IR beam interruption (including at a sensor at the entry to the food intake monitor) was continually recorded by Med-PC software (Med Associates, St. Albans, VT). The weights of the food hoppers were monitored by the computer at 10-sec intervals. A speaker maintained an ambient level of white noise at 65 dB in the experimental room.

2.4 Food-restriction feeding paradigm

The effects of drug infusion on hunger-driven food intake were tested in four groups of rats given free access to rat chow during a food-restricted state. Following one week of surgical recovery, rats were gradually reduced and maintained at approximately 90% of their *ad libitum* body weight. The animals then received six consecutive days of habituation to the feeding chambers prior to pharmacological treatments. Each session consisted of 2 hours of free access to rat chow (Prolab RMH 3000, Purina Lab Diets, 3.46 Kcal/gr) and water. When required, rats were given additional chow outside of the experimental chambers to maintain

their 90% weight; most rats consumed the daily ration required to maintain their weight within the 2-hr testing sessions.

On the final 2 days of habituation, rats received mock infusions to allow acclimation to microinfusion procedures, as previously described [13]. Experimental treatments began 48 hrs after the last mock infusion. During vehicle and drug infusions, injection cannulas (30 gauge) were lowered into the NAcc (2.5 mm beyond the end of the guide cannulas) or the ventral tegmentum (1.0 mm beyond the end of the guide cannulas), and 0.5 μ l of solution was delivered (at a rate of 0.32 μ l/min) by a Harvard Apparatus microinfusion pump (Holliston, MA). Injectors remained in place for one minute after drug delivery to allow for diffusion. Injectors were removed, stylets replaced, and rats were immediately placed into the feeding chambers. Dependent measures included the amount of chow eaten across the 2-hr period, ambulation within the chamber (assessed as the number of complete crossings of the chamber from end to end), number of rears recorded, and total water intake across the feeding session.

2.5 Feeding paradigm on a palatable fat/sucrose diet

Separate groups of rats were tested for the effects of NAcc or ventral tegmentum treatment with 5-HT₃ receptor agonists and antagonists on the intake of a highly palatable diet in non-deprived rats. Following one week of surgical recovery, *ad libitum* fed rats were given at least six days of habituation to the palatable diet for 2-hr sessions in the feeding chambers. The high-fat diet contained 278.3 g/kg vitamin free casein, 100.0 g/kg sucrose, 4.2 g/kg DL-methionine, 441.2 g/kg shortening, 77.7 g/kg safflower oil, 26.3 g/kg cellulose, 53.3 g/kg mineral mix, 15.2 g/kg vitamin mix and 3.8 g/kg choline chloride (Kilocaloric value of diet=6.2 kcal/g; Teklad Diets, Madison, WI, USA). Rats typically eat this diet when available, and we have previously shown that its intake is sensitive to intracranial injections of serotonergic, opiate or cholinergic drugs [e.g., 12,15,28–30]. On experimental days, mock injections and drug infusions were delivered as described for the food restriction paradigm above.

2.6 Drugs and Experimental Groups

These experiments assessed the behavioral effects of 5-HT₃ receptor stimulation or blockade of the NAcc or ventral tegmentum on food intake following injection of the selective agonist m-chlorophenylbiguanide (m-CPBG, at 0.0, 10.0, or 20.0 mg/0.5 μ g/side) or the selective antagonist ondansetron (at 0.0, 1.0, 2.0, or 5.0 μ g/side). Separate groups of animals were tested for each brain region (NAcc and ventral tegmentum), for each drug (agonist and antagonist), and for each dietary condition (food-restricted or palatable eating), for a total of eight experimental groups. Within each experiment, rats received all doses of a single drug in a randomized order over 3–4 infusion days. Drug infusions were separated by a minimum of 48 hrs to allow for washout and metabolism of drug between injections. Drug concentrations for each agent were chosen based upon solubility and consistency with behaviorally-effective doses in other paradigms, when available [17,31,32].

2.7 Histology

Once the experiments were complete, rats were euthanized and the brains were collected for standard histological analysis. Brain sections were taken through the extent of the NAcc or ventral tegmentum and stained with cresyl violet. Placement of the injection sites was confirmed by light microscopy and charted with reference to Paxinos and Watson [33]. Only the fifty-seven animals who completed all days of the experiment and whose injectors were bilaterally placed within the anterior medial NAcc or ventral tegmentum were included in the behavioral analysis.

2.8 Data analysis

Feeding data were analyzed utilizing two-way repeated measures ANOVAs, comparing food intake assessed across time (at 5 minute intervals within each 2-hr session) and drug doses. For groups that had significant drug and/or drug $\times$ time interaction effects, ANOVAs were run to compare the main effects of drug dose at 5 minute time points across the session, in order to further assess consistency and time course of the drug effects. Total ambulation, rearing behavior, and water intake was analyzed with one-way repeated measures ANOVAs with drug dose as the independent variable; Tukey HSD post-hoc analyses were conducted to compare behaviors between vehicle and drug treatment days, as appropriate.

3.0 Results

3.1 Effects of 5-HT₃ receptor manipulations of the NAcc on intake and locomotion

In food-restricted animals offered rat chow, 5-HT₃ receptor stimulation of the NAcc yielded a significant effect of drug ($N = 7$; $F_{2,12} = 17.27$, $p < .001$, $\eta_p^2 = 0.74$) and a significant drug $\times$ time interaction effect ($F_{46,276} = 7.09$, $p < .001$, $\eta_p^2 = 0.54$). As can be seen in the top panels of Figure 1, 5-HT₃ receptor stimulation resulted in a dose-dependent increase in rat chow eaten over the 2-hr feeding test. Follow-up ANOVAs at individual time points determined that food intake began to diverge by 20 min into the session. After 1 hour of feeding, both doses of mCPBG had increased feeding as compared to the vehicle injection condition, and this effect continued throughout the remainder of the session for the 20.0 $\mu\text{g}/\text{side}$ condition. Despite this increase of food intake, 5-HT₃ receptor stimulation had no effect on ambulation ($F_{2,12} = 2.20$, $p = .153$, $\eta_p^2 = 0.27$), rearing ($F_{2,12} = 2.60$, $p = .115$, $\eta_p^2 = 0.30$), or water intake ($F_{2,12} = 0.61$, $p = .559$, $\eta_p^2 = 0.09$) at these doses.

A similar feeding increase was observed following NAcc 5-HT₃ receptor stimulation in non-restricted rats offered a palatable sweetened fat diet ($N = 7$; drug effect: $F_{2,12} = 20.20$, $p < .001$, $\eta_p^2 = 0.77$; drug $\times$ time interaction: $F_{46,276} = 2.14$, $p < .001$, $\eta_p^2 = 0.26$). Compared to days on which the rats received a vehicle infusion, both 10.0 and 20.0 $\mu\text{g}/\text{side}$ of mCPBG increased food intake by 10 minutes into the session, and this effect continued through the remainder of the session for the highest dose tested (bottom panels, Fig 1). Neither ambulation ($F_{2,12} = 1.05$, $p = .380$, $\eta_p^2 = 0.15$) nor rearing ($F_{2,12} = 0.42$, $p = .419$, $\eta_p^2 = 0.07$) were affected by drug treatment, although water intake was significantly reduced ($F_{2,12} = 6.53$, $p = .012$, $\eta_p^2 = 0.52$; both doses significantly differed from vehicle according to Tukey's HSD).

There were no effects of NAcc 5-HT₃ receptor antagonism on the food intake of food-restricted rats offered rat chow (N = 8; drug effect: $F_{3,21} = 1.03$, $p = .398$, $\eta_p^2 = 0.13$; drug $\times$ time interaction: $F_{69,483} = 1.18$, $p = .171$, $\eta_p^2 = 0.14$) or in sated rats given access to a sweetened high fat diet (N = 7; drug effect: $F_{3,18} = 0.10$, $p = .957$, $\eta_p^2 = 0.02$; drug $\times$ time interaction: $F_{69,414} = 0.56$, $p = .998$, $\eta_p^2 = 0.09$). Ondansetron did not affect ambulation, rearing, or water intake for food-restricted rats (all p 's $> .10$); for rats offered the palatable diet, ondansetron increased rearing at 2.0 $\mu\text{g}/\text{side}$ ($F_{3,18} = 3.67$, $p = .041$, $\eta_p^2 = 0.36$), but did not impact ambulation or water intake (both p 's $> .10$).

3.1 Effects of 5-HT₃ receptor manipulations of the ventral tegmentum on intake and locomotion

In contrast to the increases in food intake seen following NAcc treatment, stimulation of 5-HT₃ receptors of the ventral tegmentum dose-dependently decreased food intake in food-restricted rats offered rat chow (N = 9; drug effect: $F_{2,16} = 17.66$, $p < .001$, $\eta_p^2 = 0.69$; drug $\times$ time interaction: $F_{46,368} = 5.60$, $p < .001$, $\eta_p^2 = 0.41$). As can be seen in Figure 2, inhibition of feeding occurred within the first 5 minutes of the session and continued through the remainder of the 2 hour test. Likewise, water intake was also reduced at both drug doses ($F_{2,16} = 10.74$, $p = .001$, $\eta_p^2 = 0.57$). Neither ambulation ($F_{2,16} = 1.32$, $p = .296$, $\eta_p^2 = 0.141$) nor rearing ($F_{2,16} = 0.27$, $p = .765$, $\eta_p^2 = 0.03$) were impacted. Although a modest inhibition of feeding was observed following 20.0 $\mu\text{g}/\text{side}$ mCPBG, this effect did not approach significance for non-restricted rats offered the sweetened fat diet (N = 7; drug effect: $F_{2,12} = 0.51$, $p = .615$, $\eta_p^2 = 0.08$; drug $\times$ time interaction: $F_{46,276} = 0.74$, $p = .896$, $\eta_p^2 = .011$). It is possible that this statistic was limited due to the low overall feeding on the palatable diet in this group (creating a possible floor effect). Nonetheless, there was a qualitative difference in the pattern of feeding inhibition between the group tested under restriction conditions as compared to the palatable feeding condition group. The restricted group's feeding was most heavily inhibited early in the session, whereas the palatable diet group's feeding was modestly inhibited in the second hour of the session. Other behavioral measures were unaffected; drug treatment did not alter water consumption ($F_{2,12} = 0.34$, $p = .716$, $\eta_p^2 = .05$), ambulation ($F_{2,12} = 0.16$, $p = .851$, $\eta_p^2 = .03$) or rearing ($F_{2,12} = 0.14$, $p = .873$, $\eta_p^2 = .02$) in rats offered the palatable diet.

Blockade of the ventral tegmental 5-HT₃ receptor had no significant effects on behavior. Food intake was not altered by VTA treatment in food-restricted rats (N = 6; drug effect: $F_{3,15} = 0.22$, $p = .882$, $\eta_p^2 = 0.04$; drug $\times$ time interaction: $F_{69,345} = 0.54$, $p = .99$, $\eta_p^2 = 0.10$) or in non-restricted rats offered a palatable diet (N = 6; drug effect: $F_{3,15} = 0.89$, $p = .467$, $\eta_p^2 = 0.15$; drug $\times$ time interaction: $F_{69,345} = 0.403$, $p = 1.0$, $\eta_p^2 = 0.08$). Ondansetron treatments did not affect ambulation, rearing, or water intake measures (all p 's $> .05$).

4.0 Discussion

The goal of these experiments was to determine if 5-HT₃ receptors of the meso-accumbens pathway influence feeding in response to energy need, or as motivated by the presence of a palatable diet. To our knowledge, this is the first report to examine the role of meso-accumbens 5-HT₃ receptors on food-directed motivation. Our findings demonstrate that 5-

HT₃ receptor activation of the NAcc and ventral tegmentum affect feeding behavior in opposing manners. Specifically, stimulation of 5-HT₃ receptors in the anterior medial NAcc dose-dependently increased both hunger-driven and palatable feeding. In contrast, agonist treatments of 5-HT₃ receptors within the ventral tegmentum inhibited feeding in food-restricted rats offered rat chow, though intake was not significantly impacted in non-restricted animals presented with the palatable diet. Water intake was decreased by 5-HT₃ receptor stimulation in two groups: the VTA-treated group offered rat chow, and the NAcc-treated group given access to the palatable diet. In the former case, it is likely that the water intake decrease was linked to the reduced consumption of the dry rat chow. The decreased water consumption observed here after NAcc treatment has not been observed with other treatments that enhance feeding on the palatable diet, such as 5-HT₆ or mu-opioid receptor stimulation of the NAcc [13,29]. Despite the behavioral changes observed with 5-HT₃ receptor stimulation, there were no effects of regional 5-HT₃ receptor blockade on feeding following ondansetron injection into either the ventral tegmentum or the NAcc.

Although these experiments suggest that 5-HT₃ receptors of the NAcc and VTA are involved in food intake, there is a possibility that the observed effects resulted from impacts of the drugs on nearby regions. It should be noted, however, that the volume and injection rate of the drug solutions used here have been successfully utilized by a number of laboratories to identify functional differences between the closely-positioned NAcc core and shell on learning and motivation [e.g., 34–36]. Additionally, Mena and colleagues [37] recently reported on the potential function of serotonergic agents of the medial prefrontal cortex in feeding. In their report, stimulation of serotonin receptors of the ventromedial prefrontal cortex (with serotonin hydrochloride) had no effect on feeding during a 60-minute session. Assuming that those injections stimulated 5-HT₃ receptors (along with other 5-HT receptor subtypes), it seems unlikely that the effects we observed in the NAcc groups were due to diffusion of mCPBG to the nearby medial prefrontal cortex.

4.1 5-HT₃ receptor modulation of the meso-accumbens circuit

The 5-HT₃ receptor is an ionotropic cation channel, and its activation rapidly depolarizes the membrane [24,38]. Further experimental scrutiny will be required to determine which of the several cell populations within the tegmentum and ventral striatum possess the receptor, and how their activation may alter the functional output of the circuitry. Though 5-HT₃ receptors are known to modulate multiple neurotransmitter systems in cortical and hindbrain systems, including both glutamate and GABA signaling [e.g., 39–42], the examination of their influence on signaling within the ventral tegmentum and NAcc has been primarily concentrated on dopamine output. 5-HT₃ receptor stimulation of either region increases dopamine outflow [22,23], and mCPBG injections into the posterior VTA support self-administration behavior [32]. Despite the similar effects of 5-HT₃ receptor stimulation on dopamine output in both regions, here we report that 5-HT₃ receptor stimulation increased food intake when applied to the NAcc and inhibited feeding in restricted rats offered rat chow when injected into the ventral tegmentum. One possible reason for this difference across brain regions is that the temporal character of the dopamine release likely differs following tegmental or NAcc 5-HT₃ agonism. For instance, NAcc dopamine release may be enhanced by 5-HT₃ receptor stimulation in a manner that is consistent with afferent

signaling of natural reinforcement by midbrain dopamine neurons, whereas VTA injections may directly impact dopamine output by altering the firing properties of dopaminergic neurons in a reinforcement-independent manner. Thus, the latter may be more disruptive of natural reward/reinforcement mechanisms than the former.

Additional insight into the functional role of 5-HT₃-dopamine interactions has been provided by researchers interested in the possible effects of 5-HT₃ receptor treatments on drug abuse. For instance, a robust literature has demonstrated that systemic alcohol increases dopamine output within the NAcc, and this increase can be reversed by blockade of 5-HT₃ receptors [e.g., 24,25,43,44]. In a set of elegant studies, McBride and colleagues demonstrated that this may be mediated by 5-HT₃ receptors within the ventral tegmentum, as VTA injections of a 5-HT₃ antagonist reduces dopamine output within the NAcc in response to i.p. injections of ethanol [22]. Furthermore, 5-HT₃ antagonist treatments also block lever-pressing for posterior VTA infusions of alcohol or cocaine [45]. Indeed, preclinical studies have shown that under certain access conditions, 5-HT₃ receptor antagonism can reduce ethanol intake in rats [see, 24 for review]. Similar reduced dopamine outflow in the NAcc following 5-HT₃ receptor antagonism has also been shown following injections of cocaine [25,26] or morphine [25,27], although the preclinical efficacy of 5-HT₃ receptor antagonism is less compelling than it is for alcohol [24]. Regardless, such data suggest an important role for 5-HT₃ receptors for modulating dopamine release in the meso-accumbens pathway.

Dopamine has a long history of being associated with motivated behavior. For instance, it regulates some aspects of incentive processes, the learning about natural reinforcers, and the allocation of effort expended to earn reinforcement [e.g., 10,46–48]. Furthermore, its signaling is a critical element for the addictive nature of drugs of abuse [e.g., 49]. Thus, it is interesting that the potential effects of 5-HT₃ antagonists on the seeking and consumption of alcohol, which may be due in part to reductions of dopamine signaling, are not mirrored in this set of experiments or in other studies that have looked at the effects of 5-HT₃ receptor antagonism on feeding. Several laboratories have failed to report changes in normal food intake following systemic 5-HT₃ receptor antagonism in food-restricted rats [50–53]. Similarly, mice treated with the 5-HT₃ antagonist tropistron showed no effects on food intake, even in a glucose-induced obesity model [54]. In a paradigm designed to test the effects of drug treatment on reinforcement, 5-HT₃ receptor blockade of the ventral tegmentum was without effect on lever pressing for saccharin or water reinforcement, though the same treatments reduced alcohol seeking in an operant task [55]. Furthermore, systemic ondansetron treatment alone does not appear to impact lever pressing for a food-associated secondary reinforcer, though dopaminergic receptor blockade does [56]. That 5-HT₃ receptor blockade does not affect normal feeding is striking given that 5-HT₃ receptor antagonists are routinely used to ameliorate nausea, vomiting, and lack of appetite after chemotherapy treatment in human patients [16], and they also reverse anorexia in rats presented with diets with amino acid imbalances [57]. Together with the present data, this suggests that 5-HT₃ receptor antagonism, which appears to reduce the rewarding properties of drugs of abuse under some conditions, may not impact reinforcement for natural rewards in non-addictive settings [but see, 50,53]. Although more work needs to be done to better understand when and how 5-HT₃ receptor antagonism may interact with peripheral and

central mechanisms of nausea and food intake, reports that demonstrate that 5-HT₃ receptor antagonists reduce alcohol intake without affecting feeding is promising in terms of their potential for treating drug abuse without reducing motivation for natural reinforcers.

4.2 5-HT₃ receptor stimulation, food intake, and motivation

The current data suggest that stimulation of 5-HT₃ receptors may serve differential motivational functions across disparate brain regions. These findings may help clarify why prior studies that examined the impact of systemic 5-HT₃ receptor stimulation on feeding or diet choice have yielded inconsistent outcomes. For instance, treatment with the peripheral agonist 2-methyl-5-HT does not affect food intake in deprived rats [58], suggesting a limited role for peripheral receptors in normal feeding. With drugs that penetrate brain, male and female Wistar rats maintained ad-libitum on a choice of diets (high-fat, high carbohydrate, and high-protein sources) reduced their carbohydrate ingestion following systemic 5-HT₃ stimulation, though only females reduced their total diet consumption across a 2 or 12 hour test [59]. Mazzola-Pomietto and colleagues [52] reported a transient inhibition of feeding (within the first hour), followed by transient hyperphagia (between 1 and 4 hr), after systemic mCPBG treatment (10 mg/kg) in food-restricted male Wistar rats, though chin-rubbing behavior may have interfered with food intake early in the session. Recently, a similar transient inhibition of acute feeding was observed following SR 57227 (a 5-HT₃ agonist) treatment in food restricted, but not fed, mice [60]. That 5-HT₃ receptor stimulation does not appear to consistently reduce feeding when given systemically may suggest differential involvement of 5-HT₃ receptors in food intake under different dietary and deprivation conditions across the hindbrain (see introduction), the ventral tegmentum, and the NAcc.

Both the ventral tegmentum and the striatum are important for regulating motivational and learning processes. In these experiments, we specifically targeted the anterior medial nucleus accumbens (as opposed to other striatal regions) for two prominent reasons: 1) this region of the NAcc connects directly and indirectly to hypothalamic regions responsible for regulating food intake, and 2) the anterior NAcc shell has been heavily implicated in both feeding and hedonic evaluation. Stimulation of mu-opioid receptors within this “hedonic hotspot” is known not only to increase food intake (“wanting”), but also enhance the hedonic responses that rats show to sapid sucrose solutions (“liking”), unlike other regions of striatum [61]. Little is known as to whether 5-HT receptors impact hedonic evaluation. This study suggests that mCPBG stimulation of 5-HT₃ receptors in the anterior medial NAcc enhances “wanting”, but it cannot determine if “liking” is also increased by such treatment. Ventral tegmental drug treatments, although capable of altering food intake, have not yet been reported to increase hedonic evaluation [62]. Future studies, looking at affective facial reaction or licking microstructure to sapid solutions in response to local drug infusions, will be needed to determine if and how serotonin modulation within these circuits affects hedonic evaluation.

4.3 Concluding remarks

These experiments demonstrate for the first time that 5-HT₃ receptors of the NAcc and ventral tegmentum are important modulators of feeding behavior. Stimulation, but not

blockade, of these receptors impacted feeding in a differential manner dependent upon the region targeted and the motivational state of the animal. These data add to a growing understanding of the roles of serotonin receptors of the meso-accumbens pathway on food intake and motivation, and provide important basic insight to the functional impact of 5-HT₃ receptor agents. Given current interest in the development of 5-HT₃-targeting compounds (particularly antagonists) for potential use in the treatment of addiction and mental disorders [24,63–66], it remains of interest to understand how 5-HT₃ (and other serotonin receptors) modulate the motivational functions of the meso-accumbens pathway in both normal and addictive-like conditions.

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Highlights

- 5-HT₃ receptors are found in brain regions that regulate food-directed motivation
- Nucleus accumbens 5-HT₃ receptor stimulation increased feeding in rats
- In contrast, 5-HT₃ receptor stimulation of the ventral tegmentum reduced feeding
- 5-HT₃ receptor blockade of either region did not alter food intake
- The impact of 5-HT₃ receptors on motivation depends upon the brain region affected

Figure 1.

Effects of NAcc stimulation or blockade of the 5-HT₃ receptor on feeding, water intake, and locomotion. In food-restricted rats offered rat chow, 5-HT₃ receptor stimulation dose-dependently increased food intake; water intake and locomotor measures were not affected. A similar dose-dependent increase in food intake was also observed following mCPBG treatment in non-deprived rats offered a palatable diet. In this case, water intake was significantly reduced, though locomotor measures were unaffected. Antagonism of NAcc 5-HT₃ receptors with ondansetron did not impact feeding or water intake in either paradigm,

though 2.0 µg/side increased rearing behavior in rats offered the palatable diet. Statistical symbols: * $p < .05$, ** $p < .01$ for drug effects; single and double crosses demark $p < .05$ and $p < .01$ for drug X time interaction effect, respectively. Plus signs along the bottom of feeding graphs indicate times when there were significant simple effects of drug on food intake across drug doses. White stars within the graphs denote significant differences from the vehicle control injection, as assessed by Tukey's HSD. The histology figures to the right represent the injection sites for each experiment, as charted within Paxinos and Watson [33]. Black-filled circles represent agonist injection sites; gray-filled circles denote antagonist injection sites.

Figure 2.

Effects of ventral tegmentum stimulation or blockade of the 5-HT₃ receptor on feeding, water intake, and locomotion. Stimulation of 5-HT₃ receptors significantly inhibited food and water intake. This effect on feeding was seen within the first five minutes of food exposure for both doses of mCPBG, and continued through the remainder of the 2-hr session. No significant effects of the drug was observed on ambulation or rearing. Though a trend for a decrease in feeding was observed in sated animals offered a palatable diet for the 20.0 µg/side treatment of mCPBG, there was no significant effect of drug treatment on

consumption measures or locomotion in the non-deprived rats. Antagonism of ventral tegmentum 5-HT₃ receptors had no effect upon feeding or locomotion in either paradigm. Statistical symbols and histology as in Figure 1, with the exception that white stars were not placed in the figure to denote significant differences in feeding from the vehicle condition (as all time points were significantly different in the feeding condition).