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Serotonin 1A, 1B, and 7 receptors of the rat medial nucleus accumbens differentially regulate feeding, water intake, and locomotor activity

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Abstract

Serotonin (5-HT) signaling has been widely implicated in the regulation of feeding behaviors in both humans and animal models. Recently, we reported that co-stimulation of 5-HT_{1&7} receptors of the anterior medial nucleus accumbens with the drug 5-CT caused a dose-dependent decrease in food intake, water intake, and locomotion in rats (Pratt *et al.*, 2009). The current experiments sought to determine which of three serotonin receptor subtypes (5-HT_{1A}, 5-HT_{1B}, or 5-HT₇) might be responsible for these consummatory and locomotor effects. Food-deprived rats were given 2-hr access to rat chow after stimulation of nucleus accumbens 5-HT_{1A}, 5-HT_{1B}, or 5-HT₇ receptors, or blockade of the 5-HT_{1A} or 5-HT_{1B} receptors. Stimulation of 5-HT_{1A} receptors with 8-OH-DPAT (at 0.0, 2.0, 4.0, and 8.0 microg/0.5 microl/side) caused a dose-dependent decrease in food and water intake, and reduced rearing behavior but not ambulation. In contrast, rats that received the 5-HT_{1B} agonist CP 93129 (at 0.0, 1.0, 2.0 and 4.0 microg/0.5 microl/side) showed a significant dose-dependent decrease in water intake only; stimulation of 5-HT₇ receptors (AS 19; at 0.0, 1.0, and 5.0 microg/0.5 microl/side) decreased ambulatory activity but did not affect food or water consumption. Blockade of 5-HT_{1A} or 5-HT_{1B} receptors had no lasting effects on measures of food consumption. These data suggest that the food intake, water intake, and locomotor effects seen after medial nucleus accumbens injections of 5-CT are due to actions on separate serotonin receptor subtypes, and contribute to growing evidence for selective roles of individual serotonin receptors within the nucleus accumbens on motivated behavior.

Keywords

Feeding; food intake; motivation; serotonin; serotonin receptors; nucleus accumbens; reward

1. Introduction

With the prevalence of obesity continuing to rise in both adult and child populations, and the average associated medical costs for obesity estimated to top \$190 billion dollars a year within the United States alone (Cawley and Meyerhoefer, 2012, Finkelstein *et al.*, 2009)

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there continues to be a significant need for the development of pharmacological interventions that reduce food consumption and promote weight loss. Although there has been limited success in developing such drug therapies, many of those that have been or are being prescribed to promote weight loss increase serotonergic tone peripherally and centrally. For example, both sibutramine (a 5-HT and norepinephrine reuptake antagonist) and the combination treatment of phentermine and fenfluramine (fen-phen; which releases 5-HT, dopamine, and norepinephrine directly through actions on the transporter protein) enhance serotonin at the synapse, and were used as anti-obesity agents until they were withdrawn from the market due to cardiovascular side effects (Glazer, 2001, Halford *et al.*, 2007, Ioannides-Demos *et al.*, 2006, Rothman and Baumann, 2009). These side effects (hypertension and cardiac valve pathology, respectively) were likely due to generalized enhancement of serotonin (5-HT) output, impacting peripheral sympathetic tone. With the recent FDA approval of lorcaserin (Belviq®), which is a selective agonist at the 5-HT_{2C} receptor, there is reason to hope that targeted pharmacotherapy at individual 5-HT receptor subtypes will provide efficacy for weight loss treatment with fewer deleterious side effects.

Serotonergic agents affect appetite and food intake via actions upon brain motivational circuitry. For example, the injection of serotonin directly into medial hypothalamus (which regulates appetite in response to peripheral metabolic signals) reduces food intake and alters dietary preferences (Currie and Coscina, 1996, Leibowitz *et al.*, 1990). Similar reductions of 4 feeding have been observed following more selective serotonin receptor stimulation of the 5-HT_{1A} or 5-HT_{2C} receptor subtypes within the paraventricular nucleus of the hypothalamus (Lopez-Alonso *et al.*, 2007). In the hindbrain, injection of the 5-HT_{2C/2A} antagonist mesulergine into the fourth ventricle blocks the food intake inhibition normally observed following systemic mCPP treatment (a 5-HT_{2C/1B} agonist) in Sprague-Dawley rats (Kaplan *et al.*, 1998), and stimulation of 5-HT_{1B} receptors in the parabrachial nucleus of the pons inhibits food intake (Lee *et al.*, 1998, Simansky and Nicklous, 2002). Our laboratory and others have recently identified the nucleus accumbens as an additional region where serotonin signaling regulates food-directed motivation. Specifically, Jean *et al.* (2007) reported that the 5-HT₄ receptor in the mouse nucleus accumbens is responsible for the hypophagic actions of systemic MDMA (Ecstasy) treatment. We have reported that stimulation of rat medial nucleus accumbens 5-HT₆ receptors increases food intake and enhances appetitive motivation for sugar reinforcement (Pratt *et al.*, 2009, Pratt *et al.*, 2012). In contrast, nucleus accumbens 5-HT_{2C} receptor agonism or blockade has little effect on food motivation. However, stimulation of 5-HT_{1-type/7} receptors with 5-CT robustly affected locomotor and feeding behavior in the rat by reducing rearing behavior (but not ambulation or total approaches to the food hopper), inhibiting both food and water intake across a 2-hr feeding test, and affecting lever pressing for sugar reward on a progressive ratio schedule of reinforcement. Together, these data suggest that there are separable effects for individual serotonin receptors within the ventral striatum in promoting food-seeking and consumption.

That 5-CT treatment of the nucleus accumbens altered food-motivated behavior suggests that at least one of the 5-HT_{1-type/7} receptors regulate feeding and/or locomotor (rearing) behavior. Prior experiments have implicated both the 5-HT_{1A} and 5-HT_{1B} receptors in regulating food intake (Bovetto and Richard, 1995, Currie *et al.*, 1994, Dourish *et al.*, 1986, Lee *et al.*, 1998, Lee *et al.*, 2002, Lee and Simansky, 1997, Lopez-Alonso *et al.*, 2007, Schreiber *et al.*, 2000). 5-HT_{1A} and 5-HT_{1B} receptors tend to reduce neuronal activity through activation of G-protein gated K channels, and also inhibit intracellular adenylyl cyclase activity through actions on the G_i/G_o G protein family (Hannon and Hoyer, 2008, Matthys *et al.*, 2011, Sari, 2004). In contrast, 5-HT₇ receptors increase intracellular adenylyl cyclase via the recruitment of G_s proteins. Although the 5-HT₇ receptor has not been implicated in the regulation of food intake from systemic drug studies, it regulates the firing properties of striatal acetylcholine-containing neurons (Bonsi *et al.*, 2007), which have been

argued to impact food motivation and satiety processes within striatum (Hoebel *et al.*, 2007, Pratt and Kelley, 2005). The current experiments were designed to determine the specificity by which the individual serotonin 1A, 1B, or 7 receptor subtypes may be responsible for the feeding and locomotor effects observed following 5-CT treatment of the medial nucleus accumbens.

2. Materials and methods

2.1. Animals

Forty adult male Sprague-Dawley rats (Harlan, Madison, WI; 275-300 g at arrival) were dually housed in a colony room maintained at ~21 °C with a 12-hr light–dark cycle (lights on at 7 a.m.). Animals were allowed to acclimate to housing conditions for no fewer than 5 days prior to surgical procedures. 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 and Food Restriction Parameters

Standard aseptic surgical procedures were used to implant indwelling stainless steel guide cannulas (23 gauge) bilaterally above the anterior medial nucleus accumbens (with the nose bar set at +5.0 mm above the interaural plane; 3.1 mm anterior and 1.0 mm lateral to bregma, 5.0 mm ventral to skull surface). Stylets were placed within the cannulas to maintain patency. After one week of recovery, rats were food restricted and gradually reduced to approximately 90% of their *ad libitum* body. This was done by reducing the daily ration of the rats to 8 g of standard rat chow across 5-6 days. Once the animals reached 90% body weight, each rat was provided sufficient chow on a daily basis (12-16 g each, in the home cage or during their 2 hr free access to the feeding chamber) to maintain their target weight. Water was available at all times.

2.3. Apparatus

For these experiments, food intake was monitored during 2-hr feeding sessions. Feeding chambers were constructed from clear acrylic, with internal dimensions of 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 filled with standard rat chow and mounted on a food intake monitor (Med Associates, St. Albans, VT) at the opposite end of the chamber (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. 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 recorded 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. Microinjections and behavioral testing

Rats received six days of habituation to the feeding chambers prior to pharmacological treatments. Each session consisted of 2 hrs of free access to rat chow and water. On the final two days of habituation, rats received mock injections to allow acclimation to microinfusion procedures, as previously described (Pratt *et al.*, 2009). Experimental treatments began 48 hrs after the last mock infusion. During vehicle and drug infusions, injection cannulas (30 gauge) were lowered into the nucleus accumbens and 0.5 µl of solution was delivered (at a

rate of 0.32 μ l per minute) by a Harvard Apparatus (Holliston, MA) microinfusion pump. Injectors remained in place for one minute following drug delivery to allow for diffusion. After injectors were removed and stylets replaced, rats were immediately put into the feeding chambers. Dependent measures included the amount of chow eaten across the 2-hr period, the number of approaches to the food chamber, ambulation within the chamber (assessed as the number of non-consecutive beam breaks of the three IR beams distributed across the length of the chamber), number of rears recorded, and total water intake across the feeding session.

Within each experimental group, rats received six days of habituation (including the two mock injection days). Two days after the second mock injection day, drug injections began. Each rat within each experimental group (see below) received all doses of a single serotonin receptor agonist or antagonist in a randomized order over 3-4 infusion day. Rats were placed into the feeding chamber for a 2 hr test immediately following the drug injection. Each drug treatment was separated by a minimum of 48 hrs, to allow for wash-out of the drug. On days that rats were not run in the food intake chambers (in between drug injection days), each was weighed and provided with sufficient rat chow to maintain their 90% weight.

2.5. Drugs and Experimental Groups

Five individual experiments were conducted to examine the roles of nucleus accumbens 5-HT_{1A}, 5-HT_{1B}, and 5-HT₇ receptors on feeding and locomotion. Separate groups of animals received intra-accumbens infusions of vehicle (0.0 μ g drug/0.5 μ l/side) and the 5-HT_{1A} receptor agonist 8-hydroxy-DPAT hydrobromide (8-OH-DPAT; at 0.0, 2.0, 4.0 or 8.0 μ g/0.5 μ l/side; Experiment 1), the 5-HT_{1A} receptor antagonist WAY 100135 (at 0.0, 2.5, 5.0 & 10.0 μ g/0.5 μ l/side; Experiment 2), the 5-HT_{1B} receptor agonist CP 93129 (at 0.0, 1.0, 2.0, or 4.0 μ g/0.5 μ l/side; Experiment 3), the 5-HT_{1B} receptor antagonist GR 55562 (at 0.0, 2.0, 5.0, or 10.0 μ g/0.5 μ l/side; Experiment 4), or the 5-HT₇ receptor agonist AS 19 (0, 1.0, or 5.0 μ g/0.5 μ l/side; Experiment 5). These experiments did not test the behavioral effects of 5-HT₇ receptor antagonism, as we previously reported that medial nucleus accumbens 5-HT₇ blockade with SB 269970 had no effect on food intake in an identical paradigm (Pratt *et al.*, 2009). All drugs were obtained from Tocris Biosciences. The agents 8-OH-DPAT, CP 93129, and GR 55562 were dissolved in sterile saline. WAY 100135 and AS 19 were dissolved in sterile saline containing 10% and 50% dimethyl sulfoxide, respectively. WAY 100135 and CP 93129 drug solutions were pH-balanced to the vehicle solution; the remaining drugs required no adjustment. Concentrations for each agent were chosen based upon solubility and consistency with behaviorally-effective doses in other paradigms, when available (Egashira *et al.*, 2006, Fletcher *et al.*, 2002, Przegalinski *et al.*, 2004).

2.6 Histology

Once the experiments were complete, rats were euthanized and the brains were collected for standard histological analysis. Brain sections were taken through the level of the nucleus accumbens and mounted for Nissl staining; placement of the injection sites was confirmed by light microscopy and charted with reference to (Paxinos and Watson, 1998). Six animals were excluded from further analysis due to either misplacement of the cannula or necrosis to the injection site. The results reported below represent data from animals whose injectors were bilaterally placed within the medial nucleus accumbens (Ns = 6-7 rats/group; see Figure 1).

2.7 Data analysis

Food intake was assessed at 5-min intervals across each 2-hr session. Ambulation, rearing behavior, and head entry measures were binned across 30-min intervals for analysis. Data were analyzed utilizing two-way repeated measures ANOVAs for each independent variable

as assessed across drug doses and time. Total water intake was determined across the feeding session, and analyzed with one-way repeated measures ANOVA across drug doses. For groups that had significant drug and/or drug $\times$ time interaction effects on food intake, ANOVAs were run comparing the main effects of drug dose at the time points of 30, 60, 90 and 120 min to further assess the consistency and time course of drug effects. Tukey's HSD post-hoc analyses were conducted to compare behavior across vehicle and drug treatment days, as appropriate.

3. Results

3.1 5-HT_{1A} receptor manipulations

Stimulation, but not blockade, of medial nucleus accumbens 5-HT_{1A} receptors reduced food and water intake in rats given 2-hr free access to food and water. As shown in Figure 2A, infusions of the 5-HT_{1A} receptor agonist 8-OH-DPAT delayed the onset of feeding and decreased total food intake across the entire session (drug effect: $F_{3,18} = 32.069$, $p < .001$; drug $\times$ time interaction: $F_{69,414} = 1.381$, $p = .031$). Delivery of 8 μ g 8-OH-DPAT/side significantly reduced food intake by 30 min into the test, and the inhibition of feeding lasted until the session's end. This reduction in food intake was matched by a significant decline in total water intake at the same dose ($F_{3,18} = 3.710$, $p = .031$).

Although there was not a significant main effect of drug dose on total ambulation ($F_{3,18} = 1.97$, $p = .16$), rearing ($F_{3,18} = 3.02$, $p = .057$), or food approaches ($F_{3,18} = 1.05$, $p = .39$), there were significant time $\times$ drug dose interactions for each measure (ambulation: $F_{9,54} = 3.21$, $p = .003$; rears: $F_{9,54} = 2.33$, $p = .027$; food approaches: $F_{9,54} = 8.32$, $p < .001$). Figure 2A shows that both rearing and approaches to food were inhibited in the first 30 min of the session by the highest level of 5-HT_{1A} receptor stimulation (8 μ g 8-OH-DPAT/side). The initial suppression of food approaches observed in the first 30 minutes of the session were mirrored by a significant increase (compared to control injections) in the second 30-min bin, paralleling the food intake that was on the increase by 1 hr into the session. The significant time $\times$ drug interaction on ambulation was not driven by this highest drug dose, but was due to a transient but significant increase that occurred 60-90 minutes after the injection on the days that the rats received 2 or 4 μ g 8-OH-DPAT/side.

As shown in Figure 1B, there were no effects of 5-HT_{1A} receptor blockade upon measures of food intake, water intake, or locomotor measures (all ps for drug and time $\times$ drug interactions $> .10$).

3.2. 5-HT_{1B} receptor manipulations

Activation of 5-HT_{1B} receptors in the nucleus accumbens decreased water intake ($F_{3,18} = 20.897$, $p < .001$) but did not affect food consumption across the 2 hr test (Figure 2A; drug effect: $F_{3,15} = .645$, $p = .598$; drug $\times$ time interaction: $F_{69,345} = .559$, $p = 1.0$). There were no effects of CP 93129 treatment on ambulation, rearing, or approaches to the feeding chamber (all ps for drug and drug $\times$ time interaction effects $> .10$).

Blockade of the 5-HT_{1B} receptor caused a significant drug $\times$ time interaction ($F_{69,345} = 1.710$, $p = .001$), but not a main effect of drug treatment ($F_{3,15} = 2.233$, $p = .127$) on food intake. As can be seen in Figure 3A, this effect was due to a decline in the rate of food consumption within the third 30-min segment of behavioral testing when the rats were treated with the 10 μ g dose of the GR 55562. However, this effect was transient, as the rats ate comparable amounts of rat chow by the end of the session regardless of drug treatment; there were no effects of drug treatment on total food intake as assessed at 30, 60, 90, or 120 minutes into the 2-hr session. 5-HT_{1B} antagonism at the highest dose also significantly reduced ambulatory behavior across the two hour session (main effect of drug: $F_{3,15} = 3.70$,

$p = .036$; drug $\times$ time interaction: $F_{9,45} = 0.92$, $p = .518$). There were no significant effects of 5-HT_{1B} receptor antagonism on water intake, rearing behavior, or the number of approaches to the food units (all p s for drug and drug $\times$ time interaction effects $> .05$).

3.3 5-HT₇ receptor stimulation

Stimulation of 5-HT₇ receptors caused a modest but significant reduction of ambulatory activity (main effect of drug: $F_{2,12} = 3.894$, $p = .050$; time $\times$ drug interaction effect: $F_{6,36} = 1.971$, $p = .096$), with the highest dose significantly reducing ambulation as compared to vehicle injections. Despite this locomotor decline, and in contrast to the effects of 5-HT_{1A} and 5-HT_{1B} receptor stimulation, injection of AS 19 within the nucleus accumbens did not affect food or water consumption (2-hr food intake: drug effect: $F_{2,12} = .444$, $p = .651$; drug $\times$ time interaction: $F_{46,276} = .548$, $p = .992$; water intake: $F_{2,12} = .022$, $p = .887$). There were also no significant differences in rearing behavior or approaches to the food chamber following 5-HT₇ receptor stimulation (all p s $> .10$).

4. Discussion

The current experiments were conducted to investigate the potentially independent roles of nucleus accumbens serotonin 1A, 1B, and 7 receptor subtypes in regulating food intake and locomotor activity in food-restricted rats. Pharmacological specificity of function was indeed observed across the three 5-HT receptor agonists utilized here. Specifically, activation of nucleus accumbens 5-HT_{1A} receptors decreased food and water intake in food-deprived animals. Locomotor and ambulatory measures were also transiently impacted by 5-HT_{1A} stimulation; the highest treatment reduced rearing and approaches to the food cup during the first 30 min, though food approaches increased in the second half of the first hour. Ambulation was significantly increased (in the 60-90 min time frame), but only for the intermediate doses that did not show significant changes in feeding behavior over time. Thus, it is unlikely that the food intake decline was secondary to increases in competing locomotor behavior, though an early suppression of rearing and food approaches at the high dose suggests that either a motivational or locomotor inhibition may have caused the initial delay in feeding behavior (see Tricklebank et al., 1984). This suggests a functional contrast with the effects of other neurotransmitter manipulations in the nucleus accumbens that block food intake; for instance, muscarinic acetylcholine receptor blockade decreases overall food intake in rats but increases their locomotor activity and total number of approaches to the food chamber (Pratt and Blackstone, 2009, Pratt and Kelley, 2004). In contrast to the effects of 5-HT_{1A} receptor stimulation, 5-HT_{1B} receptor agonism reduced water consumption without affecting feeding or locomotion. Stimulation of 5-HT₇ receptors reduced ambulation within the food chambers (suggesting that the chosen doses were physiologically relevant) but had no effects on food or water intake. Previously, we reported that intra-accumbens injections of the 5-HT_{1/7} receptor agonist 5-CT dose-dependently decreased chow intake, water intake, and rearing behavior (Pratt *et al.*, 2009). The current results suggest that the reported 5-CT-induced decrease in food intake and rearing behavior were most likely due to the effects of activation of the 5-HT_{1A} receptor, while the dose-dependent decrease in water intake may have caused through actions of both 5-HT_{1A} and 5-HT_{1B} receptors.

In addition to assessing the effects of 5-HT receptor stimulation on food intake, two animal groups assessed the effects of blocking the 5-HT_{1A} or 5-HT_{1B} receptor on feeding and locomotion. Antagonism of the 5-HT_{1A} receptor was without effect on either consummatory or locomotor measures. Ambulation was modestly reduced across the 2 hr period following inhibition of 5-HT_{1B} receptors at the highest dose. This 5-HT_{1B} receptor antagonism also had a transient effect on food intake 60-90 min after the start of the feeding session, although total food consumption was equivalent to the vehicle condition by the end of the 2 hr session. This delayed effect of food intake may have been caused by alterations in satiety

process, or could possibly have been due to diffusion of the drug away from the injection site over time. Future mapping experiments, assessing the role of these receptors across other regions of the striatum, will be necessary to determine if the behavioral impact of the current treatments are limited to the medial nucleus accumbens shell (similar to the hyperphagia caused by GABA_A receptor stimulation that occurs selectively following medial shell treatment [Basso and Kelley, 1999]) or may also be effective within other regions of dorsal and ventral striatum (similar to mu-opioid receptor mediated hyperphagia within the striatum [Zhang and Kelley, 2000], or the hyperphagic effects of muscarinic receptor blockade, [Pratt and Kelley, 2004, 2005]). The medial aspects of the nucleus accumbens shell were targeted for these experiments because of its strong connections with hypothalamic feeding circuits (Kampe *et al.*, 2009, Stratford and Kelley, 1999) and its known impact in promoting regulating both food intake and hedonic reactions to palatable diets (Pecina and Berridge, 2000, Pecina *et al.*, 2006). The remainder of this discussion places the observed behavioral effects following manipulations of each receptor within the broader context of their known effects on food intake and motivation

4.1 5-HT_{1A} receptors

Serotonin 1A receptors have long been known to regulate feeding behavior, both systemically and within raphe and hypothalamic nuclei. The effects of systemic 5-HT_{1A} receptor stimulation depend upon the energy state of the animal. In the non-restricted animal, 5-HT_{1A} activation increases food intake (Dourish *et al.*, 1985, Ebenezer and Tite, 2003). Dourish and colleagues initially reported an increase of food intake in rats treated systemically with 8-OH-DPAT at doses below the threshold for eliciting serotonin-related stereotypical behavior (Dourish *et al.*, 1985). These effects were argued to be due to activation of 5-HT_{1A} autoreceptors within the raphe nuclei, reducing the influence that serotonin serves on satiety-promoting brain circuitry (Dourish *et al.*, 1986). Consistent with this hypothesis, 8-OH-DPAT injections directly into the raphe nuclei also increase feeding behavior (Currie *et al.*, 1994), though the precise mechanism of action remains unclear (Fletcher and Davies, 1990).

In food restricted rats, systemic treatment with 5-HT_{1A} agonists inhibit feeding (Ebenezer, 1992, Ebenezer *et al.*, 2007, Ebenezer and Tite, 2003). Furthermore, activation of 5-HT_{1A} receptors in the hypothalamus advances satiety processes (Lopez-Alonso *et al.*, 2007), suggesting that this receptor may post-synaptically inhibit feeding in neural circuits outside of the raphe nuclei. In the current experiments, we have shown for the first time that 5-HT_{1A} receptor activation of the medial nucleus accumbens also inhibits feeding and drinking behavior in hungry rats offered free access to rat chow. This is consistent with the effect of systemic 5-HT_{1A} receptor activation in deprived animals, as noted, and suggests that the nucleus accumbens and ventral striatum may be one neural locus of this 5-HT_{1A}-receptor mediated hypophagia. Future studies will be necessary to determine if this food intake suppression is attenuated when nucleus accumbens-treated rats are offered food in a non-deprived state.

4.2 5-HT_{1B} receptors

A number of previous reports have demonstrated an effect of systemic 5-HT_{1B} receptor stimulation on feeding behavior. In general, systemic activation of 5-HT_{1B} receptors causes a dose-dependent decrease in food intake through reductions in both meal size and meal duration (Bovetto and Richard, 1995, Lee *et al.*, 2002, Lee and Simansky, 1997, Schreiber *et al.*, 2000), and blockade of the 5-HT_{1B} receptor reduces the anorectic effect of relatively non-selective serotonin agonists (Curzon, 1991, Simansky and Nicklous, 2002). Furthermore, 5-HT_{1B} receptor activation potentiates the suppression of feeding that follows concurrent 5-HT_{2C} receptor agonist treatment (Doslikova *et al.*, 2013, Schreiber and De

Vry, 2002). Consistent with a potential effect on food-directed motivation, 5-HT_{1B} receptor stimulation also reduces the reinstatement of lever pressing for contingent reinforcement (Przegalinski *et al.*, 2008) or in the presence of food-associated cues (Acosta *et al.*, 2005).

Two prior reports that examined the impact of systemic 5-HT_{1B} receptor agonism on food intake also noted a hypodipsic action following treatment which did not mirror the changes in feeding (Lee *et al.*, 2002, Schreiber *et al.*, 2000). Such findings suggest that the neuronal mechanisms underlying 5-HT_{1B}-mediated hypophagia and hypodipsia may differ. Here, we report that stimulation of nucleus accumbens 5-HT_{1B} receptors caused a significant dose-dependent hypodipsia, but did not impact food intake in food-restricted rats. Lee *et al.* (1998) previously demonstrated that 5-HT_{1B} receptor stimulation of the parabrachial nucleus of the pons reduced food consumption without affecting water intake (Lee *et al.*, 1998). Taken together, these data suggest that separate neuronal substrates regulate the impact of systemic 5-HT_{1B} receptor activation on food and water ingestion, and suggest a unique role for nucleus accumbens 5-HT_{1B} receptors in moderating water consumption separate from food intake.

4.3 5-HT₇ receptors

Although 5-HT₇ receptors exist in relatively low concentrations within the striatum, activation of those receptors has been shown to affect the firing characteristics of cholinergic interneurons of the striatum (Bonsi *et al.*, 2007). Striatal acetylcholine has been implicated in the regulation of satiety and motivational processes relating to food-seeking and consumption (Baldo *et al.*, 2013, Hoebel *et al.*, 2007). However, neither 5-HT₇ receptor stimulation (in these experiments) nor blockade (Pratt *et al.*, 2009) of the medial nucleus accumbens significantly impacted food or water consumption, despite a significant reduction in ambulation following 5-HT₇ receptor stimulation. These data are consistent with other examinations targeting the role of 5-HT₇ receptor with regards to food motivation. For instance, Clemett and colleagues (1998) injected antisense oligonucleotides into the cerebral ventricles to knock down 5-HT₇ receptors. Rats did not show differential feeding or body weight between groups receiving the antisense or mismatched oligonucleotides. Additionally, systemic blockade of 5-HT₇ receptors (along with D₂, 5-HT_{1A}, and sigma receptors) with tiospirone influences lever-pressing for food reward only at levels that also induce catatonia (Arolfo and McMillen, 1999). At this point, there is no convincing evidence to link 5-HT₇ receptors with the regulation of feeding or food motivation, either systemically or during the targeting of the medial nucleus accumbens.

4.4 Concluding remarks

As the differential expression and function of serotonin receptors within central and peripheral tissues has become better understood, selective 5-HT receptor agents are being explored not only to assist with weight loss, but also to treat mood disorders (such as depression), drug abuse, and cognitive decline (Mitchell and Neumaier, 2005, Nic Dhonnchadha and Cunningham, 2008, Rothman *et al.*, 2008, Upton *et al.*, 2008). Given these clinical implications, as well as the known roles of other neurotransmitters within the nucleus accumbens upon the regulation of food intake, incentive processes, and hedonic responses to palatable foods (Berridge *et al.*, 2010, Kelley *et al.*, 2005), it is of interest to determine how selective serotonin receptor signaling within mesolimbic pathways impacts motivated behavior. The current experiments present, for the first time, a characterization of the individual roles for the 5-HT_{1A}, 5-HT_{1B}, and 5-HT₇ receptors of the medial nucleus accumbens on food and water intake and locomotion in the rat. The results suggest that the behavioral effects previously reported by our laboratory following similar treatments of the 5-HT_{1/7} receptor agonist 5-CT were caused by activation of both 5-HT_{1A} & 1B, but not 5-HT₇ receptors. These data add a novel contribution to the study of striatal neurotransmitters

and motivated behavior, and suggest that activation of individual serotonin receptors within the medial nucleus accumbens have differential roles in directing motivated behavior.

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Highlights

- Individual serotonin receptors in the nucleus accumbens serve unique behavioral roles
- Nucleus accumbens 5-HT_{1A} receptor activation reduces food and water intake in the rat
- 5-HT_{1B} receptor stimulation inhibits water intake without affecting feeding
- Nucleus accumbens 5-HT₇ receptor stimulation does not affect consummatory behavior

Figure 1.

Location of injector tips for animals included in the behavioral analyses. Column A shows placement of tips for rats receiving the 5-HT_{1A} agonist 8-OH-DPAT (filled circles) or the 5-HT_{1A} antagonist WAY 100135 (open circles). Rats in B received the 5-HT_{1B} agonist CP 93129 (filled circles) or the 5-HT_{1B} antagonist GR 55562 (open circles). Column C represents the placements for animals receiving the 5-HT₇ receptor agonist AS 19. The photomicrographs show a representative placement of the injection cannulas from each set of experiments. Drawings were adapted from *The Rat Brain in Stereotaxic Coordinates*, 4th ed., G. Paxinos and C. Watson, Figures 10, 11, and 13, copyright 1998.

Figure 2.

Effects of medial nucleus accumbens stimulation or blockade of the 5-HT_{1A} receptor on feeding, water intake, and locomotion. The 8 µg dose of the 5-HT_{1A} agonist 8-OH-DPAT significantly reduced food and water intake across the 2-hr feeding session in food-restricted rats feeding on standard chow (A). Additionally, rearing behavior was reduced during the first 30 min for the 4.0 and 8.0 µg doses. Fewer food approaches were recorded during the first 30 min session following the highest 8-OH-DPAT treatment, though this was compensated for by an increase in head entries (compared to vehicle injections) during the second 30 min period. Ambulation was increased during the 60-90 min time frame for the 2.0 and 4.0 µg treatments of the agonist, though this did not interfere with feeding behavior. In contrast, antagonism of the 5-HT_{1A} receptor with WAY 100135 had no effect on any of the behaviors measured here (B). * $p < .05$, ** $p < .01$ for main drug effects; single and double crosses demark $p < .05$ and $p < .01$ for drug $\times$ time interaction effect, respectively. Stars above or within the bar graphs denote significant differences from the respective vehicle control injection, as assessed by Tukey's HSD.

Figure 3.

Effects of medial nucleus accumbens stimulation or blockade of the 5-HT_{1B} receptor on feeding, water intake, and locomotion. Although stimulation of the 5-HT_{1B} receptor of the nucleus accumbens with CP 93129 had no effect on locomotor measures or the amount of rat chow eaten across a 2-hr feeding session, increasing amounts of the drug significantly and dose-dependently reduced water intake during the session (A). Blockade of the 5-HT_{1B} receptor yielded a significant drug × time interaction (B); when treated with the highest dose of GR 55562, rats transiently paused feeding 1 hr into the feeding session, but ultimately caught up to the other treatment days by the end of the 2 hrs. A modest but significant decrease in ambulation was also evident following 5-HT_{1B} receptor antagonism; GR 55562 caused no further effects on rearing, food approaches, or water intake across the session. Statistical symbols are as represented in Figure 2.

Figure 4.

Effects of medial nucleus accumbens stimulation of the 5-HT₇ receptor on feeding, water intake, and locomotion. Although treatment with AS 19 resulted in a significant inhibition of ambulatory behavior at the highest dose, there were no significant effects of drug treatment on food or water intake, the number of approaches to the food hopper, or on rearing behavior. Statistical symbols are as represented for Figure 2.