Selective Serotonin Receptor Stimulation of the Medial Nucleus Accumbens Causes Differential Effects on Food Intake and Locomotion

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Abstract

Substantial evidence suggests that pharmacological manipulations of neural serotonin pathways impact ingestive behaviors. Despite the known role of the nucleus accumbens in directing appetitive and consummatory behavior, there has been little examination of the influences that serotonin receptors may play in modulating feeding within nucleus accumbens circuitry. In these experiments, we examined the effects of bilateral nucleus accumbens infusions of the 5-HT₁/₇ receptor agonist 5-CT (at 0.0, 0.5, 1.0, or 4.0 µg/0.5 µl/side), the 5-HT₆ receptor agonist EMD 386088 (at 0.0, 1.0, and 4.0 µg/0.5 µl/side), or the 5-HT₂C preferential agonist RO 60-0175 (at 0.0, 2.0, or 5.0 µg/0.5 µl /side) on food intake and locomotor activity in the rat. Intra-accumbens infusions of 5-CT caused a dose-dependent reduction of food intake and rearing behavior, both in food-restricted animals given 2-hr free access to chow, as well as in non-deprived rats offered 2-hr access to a highly palatable fat/sucrose diet. In contrast, stimulation of 5-HT₆ receptors with EMD 386088 caused a dose-dependent increase of intake under both feeding conditions, without affecting measures of locomotion. Infusions of the moderately selective 5-HT₂C receptor agonist RO 60-0175 had no effects on feeding or locomotor measures in food-restricted animals, but did reduce intake of the fat/sucrose in non-restricted animals at the 2.0 µg, but not the 5.0 µg dose. Intra-accumbens infusions of selective antagonists for the 5-HT₇ (SB 269970), 5-HT₆ (SB 252585), and 5-HT₂C (RS 102221) receptors did not affect locomotion, and demonstrated no lasting changes in feeding for any of the groups tested. These data are the first to suggest that the activation of different serotonin receptor subtypes within the feeding circuitry of the medial nucleus accumbens differentially impact consummatory behavior.
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Given the fundamental importance of feeding for survival of both individuals and species, the mammalian brain and periphery have evolved multiple interconnected networks that serve to direct behaviors toward foraging and feeding during times of negative energy balance, as well as to promote food intake in the presence of highly caloric and palatable foods (Berthoud, 2007; Woods, Seeley, Porte, & Schwartz, 1998). Due to this complex and diverse feeding circuitry, very few pharmacological interventions have demonstrated lasting benefits for the obese or morbidly obese. The only centrally-active drug that is FDA-approved for long-term use in human weight maintenance is sibutramine, which modestly reduces food intake and attenuates the decline in metabolic activity caused by caloric restriction (Hansen, Toubro, Stock, Macdonald, & Astrup, 1999; Lechin et al., 2006; Levin & Dunn-Meynell, 2000; Walsh, Leen, & Lean, 1999, although see Rotstein, Inbar, & Vaisman, 2008). Sibutramine acts by blocking serotonin and norepinephrine reuptake at the synapse, and likely has its anorectic effects by increasing central concentrations of serotonin. Other drugs that stimulate central serotonin output have also been historically effective in curbing food intake (Douglas & Munro, 1982; Silverstone, 1992).

Animal models suggest that serotonin signaling plays an important (although certainly not exclusive) role in the brain’s regulation of food intake and energy balance. In the rat, it has been shown that central depletion of serotonin results in hyperphagia and weight gain (Breisch, Zemlan, & Hoebel, 1976; Saller & Stricker, 1976). Likewise, pharmacological inhibition of the serotonin-producing neurons of the medial raphe nuclei causes robust increases of ingestive behaviors (for a review, see Wirtshafter, 2001). With regards to feeding, it has been argued that
much of this effect is mediated via serotonin influences on hypothalamic circuitry. Serotonin infusions directly into the medial nuclei of the hypothalamus alter dietary preferences and reduce food intake (Currie & Coscina, 1996; Leibowitz, Weiss, & Suh, 1990); selective serotonin agonists that activate 5-HT$_{1A}$ or 5-HT$_{2C}$ receptor subtypes also reduce feeding in rats when injected into the paraventricular nucleus of the hypothalamus (Lopez-Alonso, Mancilla-Diaz, Rito-Domingo, Gonzalez-Hernandez, & Escartin-Perez, 2007). However, serotonergic mechanisms also influence food intake by actions in brain regions beyond the hypothalamus. There are substantial projections from the serotonin-producing raphe nuclei to neural pathways that are important for the perception and evaluation of the hedonic value of food. For example, gustatory information enters the brain at the level of the nucleus of the solitary tract, and is subsequently relayed to the parabrachial nucleus of the pons. Direct serotonergic manipulation of both of these regions has been shown to impact feeding behavior (Hayes & Covasa, 2006; Lee, Aloyo, Fluharty, & Simansky, 1998; Simansky & Nicklous, 2002). Therefore, it appears that the pharmacological effects of serotonin receptor activation on feeding are distributed through many of the neural circuitries involved in modulating food intake.

It is possible that serotonin may also modulate feeding by actions within brain regions involved in processing the motivational significance of food rewards. The nucleus accumbens, for instance, receives converging inputs from brain structures involved in sensory evaluation, emotional valence, and mnemonic processing, all of which converge on the neurons within the ventral striatum to impact motivated behavior (Kelley, Baldo, Pratt, & Will, 2005; Mogenson, Jones, & Yim, 1980). Manipulation of multiple neurotransmitter systems within the nucleus accumbens have been shown to impact feeding, as well as the incentive properties of food and cues that are associated with food (Berridge, 2007; Kelley et al., 2002; Pecina & Berridge, 2000;
Salamone & Correa, 2002). However, despite a known role for the nucleus accumbens in modulating food intake, as well as a rising interest in the role of serotonin within mesostriatal circuitry on drug-reinforced behavior (e.g., Nic Dhonnchadha & Cunningham, 2008; Rothman, Blough, & Baumann, 2008) and cognitive function (e.g., Gonzalez-Burgos & Feria-Velasco, 2008; Mitchell & Neumaier, 2005; Olvera-Cortes, Anguiano-Rodriguez, Lopez-Vazquez, & Alfaro, 2008), very little research has examined the role of selective serotonergic agents within the nucleus accumbens on ingestive behaviors. What has been shown, however, is that nucleus accumbens injections of serotonin attenuate the hyperphagia observed in rats following median raphe inhibition (Bendotti, Garattini, & Samanin, 1986). Furthermore, a recent report by Jean et al., (2007) suggests that 5-HT$_4$ receptors within the nucleus accumbens mediate the hypophagic actions of systemic MDMA (Ecstasy) administration in mice. These two reports support a hypothesis that serotonin in the nucleus accumbens may modulate food consumption, and that these effects might be attributable to individual serotonin receptor subtypes.

There exist, at this time, 14 identified receptor subtypes for the 5-HT family, subdivided into seven separate classes (5-HT$_1$ through 5-HT$_7$; Barnes & Sharp, 1999). Three of these receptor subtypes, 5-HT$_7$, 5-HT$_6$, and 5-HT$_2C$, have recently been shown to modulate ion currents in the cholinergic interneurons of the striatum (Bonsi et al., 2007). We have previously reported that activation of acetylcholine muscarinic receptors within the nucleus accumbens is critical for normal consummatory behavior (Pratt & Blackstone, 2008; Pratt & Kelley, 2004, 2005), and other laboratories have also suggested an important role for cholinergic outflow in satiety processes (Avena, Rada, Moise, & Hoebel, 2006; Mark, Rada, Pothos, & Hoebel, 1992). Given that 5-HT$_7$, 5-HT$_6$, and 5-HT$_2C$ receptors modulate the electrophysiological output of striatal cholinergic interneurons (Blomeley & Bracci, 2005; Bonsi et al., 2007), these experiments were
designed to systematically test whether nucleus accumbens stimulation or antagonism of those serotonin receptors would affect food intake in rats. Additionally, prior work has suggested that the nucleus accumbens may be particularly important in modulating food intake on palatable diets. *Mu*-opioid receptor stimulation within the medial accumbens, for instance, preferentially increases feeding on palatable diets; both *mu*-opioid and cannabinoid receptor stimulation has been shown to increase positive hedonic responses to palatable solutions (Kelley et al., 2005; Mahler, Smith, & Berridge, 2007; Pecina, Smith, & Berridge, 2006). However, there has been no investigation to date of whether a similar distinction may be made with regard to nucleus accumbens serotonin on feeding. Therefore, these experiments assessed both need-driven and palatability-induced feeding to determine if there might be qualitatively different effects of drug treatment between the separate food intake conditions.

Method

*Subjects and Housing*

Adult male Sprague-Dawley rats (approximately 300 g at experiment onset; Harlan, Madison, WI) were acclimated to dual housing in a colony room maintained at ~21 °C with a 12-hr light–dark cycle. During acclimation to the laboratory and surgical recovery, standard rat chow and water were available *ad libitum*. All experiments were conducted in accordance to NIH animal care guidelines and approved by the Wake Forest University Animal Care and Use Committee.

*Surgery*

Following acclimation to the housing environment, rats were anesthetized with a Ketamine-Xylazine cocktail (100 mg/kg-10mg/kg). Standard aseptic procedures were used to implant indwelling stainless steel guide cannulas (23 gauge) bilaterally above the nucleus
accumbens 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). Guide cannulas were affixed to the skull with the use of screws and dental acrylic, and stylets were placed to prevent obstruction. Rats recovered for at least 7 days prior to food restriction and/or behavioral testing.

Apparatus

Food intake was monitored during 2-hr feeding sessions in experimental chambers. The 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 intake monitor (Med Associates, St. Albans, VT) was filled with either standard rat chow or a high fat/high sucrose diet (see below) at the opposite end (head entry at 6.4 cm above the wire floor). 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) during experimental sessions. The weights of the food monitors were recorded at 10-sec intervals. A speaker maintained an ambient level of white noise at 65 dB in the experimental room. All sessions were conducted during the last half of the lights-on period.

Drugs

These experiments assessed the effects of Acb injection of several serotonergic receptor agents upon feeding in food restricted and non-restricted rats (see Table 1). Separate groups of animals received intra-accumbens infusions of vehicle (0.0 µg drug/0.5 µl/side) and the 5-HT_{1/7} receptor agonist 5-CT (at 0.5, 1.0, or 4.0 µg/0.5 µl/side), the 5-HT_{7} receptor antagonist SB 269970 (at 1.0, 2.0, or 4.0 µg/0.5 µl/side), the 5-HT_{6} receptor agonist EMD 386088 (at 1.0, and
4.0 µg/0.5 µl/side), the 5-HT<sub>6</sub> receptor antagonist SB 252585 (at 1.0, 2.0, and 4.0 µg/0.5 µl/side), 5-HT<sub>2C</sub> receptor agonist RO 60-0175 fumarate (at 2.0, or 5.0 µg/0.5 µl/side), or the 5-HT<sub>2C</sub> receptor antagonist RS 102221 hydrochloride (at 1.0, and 2.0 µg/0.5 µl/side). RO 60-0175 fumarate, 5-CT, and SB 269970 were dissolved in sterile saline; all other drugs were dissolved in sterile saline containing 10% 2-Hydroxypropyl-β-cyclodextrin (Sigma). To maintain solubility of RO 60-0175 and RS 102221, pH levels of the solutions were raised to ~7.0 and ~8.0, respectively. 5-CT drug solutions were pH-balanced to saline vehicle; 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.

**Food-restriction feeding paradigm**

The effects of drug infusion on food intake were tested in rats given free access to rat chow during a food-restricted state. Following one week of surgical recovery, six groups of 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 two days of habituation, rats received mock injections to allow acclimation to microinfusion procedures. On the first day, mock injectors were lowered flush to the end of the guide cannula; the second mock injection utilized cannulas that were lowered to the infusion site, 2.5 mm below the end of the guides. No solutions were delivered on mock injection days. Experimental treatments began 48 hrs after the last mock injection. Rat within each
experimental group received all treatment doses of a single drug across 3-4 experimental days, the order of which was randomly determined for each rat. During vehicle and drug infusions, injection cannulas (30 gauge) were lowered bilaterally into the Acb shell and 0.5 μl of solution was delivered (at a rate of 0.32 μl per minute) by a Harvard Apparatus (Holliston, MA) microinfusion pump. Injectors remained in place for one minute to allow for diffusion, and rats were immediately placed in the feeding chambers. Drug infusions were separated by a minimum of 48 hours. On days that rats were not run in the food intake chambers, they were weighed and provided with sufficient rat chow to maintain their 90% weight.

**Feeding paradigm on a palatable fat/sucrose diet**

Six additional groups of rats were tested for the effects of Acb serotonin receptor agonists and antagonists on intake of a highly palatable diet in non-deprived rats. Following one week of surgical recovery, ad libitum fed rats were given 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 its intake has been shown to be sensitive to Acb injections of opiate or cholinergic drugs (e.g., Perry, Baldo, Andrzejewski, & Kelley, 2009; Will, Pratt, & Kelley, 2006). On experimental days, mock injections and drug infusions were delivered as described above.

**Data analysis**

The primary dependent measure was the amount of diet eaten over the 2-hr food intake session. In order to determine whether any observed effects could be attributed to locomotor
disturbances following drug treatment, the number of approaches to the food intake monitor, ambulation within the chamber (assessed as the number of complete crossings of the chamber from end to end), and the number of rears recorded were measured. Total water intake during the sessions was also recorded, to assist in determining whether feeding effects were due to general changes in consummatory behavior, or if it they were specific to food intake. Feeding data was analyzed utilizing two-way repeated measures ANOVAs, comparing food intake assessed across time (at 5-min intervals within each 2-hr session) and drug doses. For groups that had significant drug and/or drug x time interaction effects, 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 the drug effects. Locomotion, water intake, and head entry measures were 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.

Histology

Once the experiments were complete, rats were deeply anesthetized with sodium pentobarbital and perfused through the heart with a 0.9% buffered NaCl solution, followed by 10.0% formalin. Brains were removed and allowed to sink in 10.0% sucrose formalin. The brains were frozen and sliced into 60-μm sections with a cryostat. Sections were stained with cresyl violet. The tips of the cannulas were confirmed by light microscopy and charted with reference to Paxinos & Watson (1998). Only animals whose injectors were bilaterally placed within the medial nucleus accumbens were included in the behavioral analysis (see Figure 1).
Results

Stimulation of nucleus accumbens 5-HT_{1/7} receptors reduces feeding and locomotor behaviors.

In general, injection of the 5-HT_{1/7} receptor agonist 5-CT into the medial nucleus accumbens dose-dependently reduced food and water intake in rats (see Figure 2). Rearing behavior was also reduced following drug infusion; a small but significant decline of ambulatory behavior was observed in response to drug treatment for ad libitum fed rats offered the palatable diet. There were no significant effects of 5-HT_{7} receptor antagonism on any of the assessed behavioral measures. Statistical presentation of the data for both experiments is detailed in subsequent sections.

Drug effects in food-restricted rats. Repeated-measures ANOVA comparing the effects of 5-CT infusion on the intake of rat chow across time yielded significant effects of drug, F(3, 12) = 7.05, p < .01, and a significant drug x time interaction, F(69, 276) = 6.66, p < .01. As shown in Figure 2, stimulation of nucleus accumbens 5-HT_{1/7} receptors reduced chow intake; the highest dose of drug given (4.0 µg/side) delayed feeding onset and reduced the total amount of food consumed during the 2-hr period. Follow-up ANOVAs at 30 min intervals confirmed a consistent effect of 5-CT on food intake at 30 minutes F(3,12) = 12.22, p = .001; this effect continued until the end of the session, F (3, 12) = 3.95, p = .036.

Water intake [F(3, 12) = 21.46, p < .01] and rearing behaviors [F(3,12) = 6.10, p < .01] were also reduced in a dose-dependent manner following nucleus accumbens stimulation of 5-HT_{1/7} receptors. Post-hoc comparisons utilizing Tukey’s HSDs revealed that treatments with 1.0 and 4.0 µg/side of 5-CT significantly reduced water intake and rearing compared to vehicle infusion days. For food-deprived rats, there was no effect of drug treatment on ambulatory activity within the feeding chamber (as assessed by total number of complete crossings of the
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entire length of the feeding chamber). Although the number of approaches to the food intake monitor declined with increasing doses of the drug, this decrease did not meet significance, \( F(3, 12) = 0.493, p = .69 \).

There was no significant effect of nucleus accumbens infusions of the 5-HT\(_7\) receptor antagonist SB 269970 on food intake, \( F(3, 12) = 1.21, p = .35 \), nor was there a significant drug x time interaction, \( F(69, 276) = 1.48, p = .94 \) (see Figure 2). Furthermore, there were no systematic changes in water intake, locomotor measures, or approaches to the food chamber across increasing doses of SB 269970 (analysis not shown; descriptive statistics for each condition are reported in Table 2).

**Drug effects in non-deprived rats offered palatable food.** Repeated-measures ANOVA comparing the effects of 5-CT drug across time on the intake of the high fat diet resulted in significant effects of drug, \( F(3, 15) = 4.03, p = .028 \), and a significant drug x time interaction, \( F(69, 345) = 1.47, p = .015 \). As shown in the bottom panels of Figure 2, stimulation of 5-HT\(_7\) receptors dose-dependently reduced intake on the high fat diet, with reduced intake occurring primarily during the early phases of the feeding session. Significant differences in the amount of diet consumed across conditions were verified at 30 min and 60 min; \( F(3, 15) = 4.76, p = .016 \), and \( F(3, 15) = 3.37, p = .047 \), respectively. Although rats continued to show declines in fat diet intake following medium and high doses late into the session, the differences between the drug conditions were no longer significant at 90 or 120 min (\( p > .10 \) for ANOVAs at both time points).

Similar to the effects seen in hungry rats, both water intake \( [F(3, 15) = 4.67, p = .017] \) and rearing behaviors \( [F(3,15) = 13.629, p < .001] \) were reduced following nucleus accumbens infusions of 5-CT in rats receiving access to the palatable diet. Post-hoc comparisons revealed that treatments with all doses of the drug significantly reduced rearing compared to vehicle
infusion days; the water intake reduction was only apparent at the highest dose of the drug (4.0 µg/side). Furthermore, there was a significant effect of drug treatment on ambulation, F (3, 15) = 4.51, p = .019; Tukey’s HSD verified a significant decline of ambulation at the intermediate dose of the drug. There was no significant effect of drug on the number of approaches to the food intake monitor during the feeding session, F (3, 15) = 1.49, p = .26.

As was the case in food-deprived rats, there was no effect of nucleus accumbens 5-HT_7 receptor blockade on feeding on the palatable diet in non-deprived animals: drug effect, F(3, 15) = 0.248, p = .86; drug x time interaction, F (69, 345) = 0.211, p = .85. 5-HT_7 receptor antagonism did not alter water intake, locomotor measures, or approaches to the food chamber in rats given access to the high-fat diet (analysis not shown; see values in Table 2).

*Stimulation of nucleus accumbens 5-HT_6 receptors increase food intake.*

For both food-restricted and non-restricted animal groups offered a high fat diet, stimulation of nucleus accumbens 5-HT_6 receptors with EMD 386088 increased food intake across the 2-hr feeding sessions, without affecting water intake or locomotor measures (see Figure 3). Antagonism of 5-HT_6 receptors with SB 252585 caused little effect on feeding or locomotion. Statistical presentation of the behavioral data for each experiment is detailed below.

*Drug effects in food-restricted rats.* As seen in Figure 3, stimulation of nucleus accumbens 5-HT_6 receptors with EMD 386088 dose-dependently increased the amount of rat chow that food-deprived rats ate across the two hour session. EMD 386088 treatment resulted in a significant drug effect, F(2, 10) = 9.56, p = .005, and a significant drug x time interaction, F(46, 230) =7.10, p < .001. A significant difference in feeding between the drug conditions was apparent by 1 hr, F(2, 10) = 7.79, p = .009, and continued until the end of the feeding session, F (2, 10) = 10.29, p = .004.
There were no significant effects of 5-HT$_6$ receptor stimulation on water intake, ambulation, or rearing activity within the chamber (statistics not shown, see figure 3). However, consistent with the effect of the drug on overall food intake, there was a trend for the 4.0 µg/side dose of EMD 386088 to increase the number of head entries into the food chamber, F(2, 10) = 2.95, p = .098.

Blockade of 5-HT$_6$ receptors of the medial nucleus accumbens with SB 252585 resulted in a significant drug x time interaction effect on food intake, F(69, 207) = 2.09, p < .001. This effect appears to be due to a slight, but significant divergence of the vehicle and high drug (4.0 µg/side) conditions during the last 30 minutes of the food intake session (see Figure 3). However, there was no significant effect of drug on food intake across the entire session, F (3, 9) = 0.44, p = .73, nor was there evidence of significant differences between drug doses at 30, 60, 90, or 120 minutes (all p values > .10), suggesting that the interaction effect was transient and not sustained until the end of the session. Drug treatment was also without effect on locomotor measures, water intake, or food approaches (see Table 2).

*Drug effects in non-deprived rats offered palatable food.* Intra-accumbens infusions of the 5-HT$_6$ agonist EMD 386088 increased food intake in non-restricted rats offered a palatable diet. A repeated measures ANOVA comparing fat diet intake across time and drug dose resulted in a significant drug x time interaction effect, F(46, 230) = 2.53, p < .001, as well as a trend for an overall drug effect, F(2, 10) = 3.61, p = .066. As shown in the bottom panels of Figure 3, rats treated with the high dose of the drug ate more of the palatable diet across the 2-hr feeding session. Analyses of the drug effects at 30 minute intervals yielded trends toward a difference between drug doses at 30, 60, and 90 minutes into the session (with p-values of .08, .06, and .06, respectively), and a significant drug effect at 120 min, F (2, 10) = 4.72, p = .036.
Although increasing drug doses increased ambulation and approaches to the food chamber, these differences were not significant [ambulation: F(2, 10) = 2.03, p = .18; approaches: F(2, 10) = 0.964, p = .41]. Neither did 5-HT₆ receptor stimulation alter water intake or rearing behavior (see Figure 3).

Antagonizing nucleus accumbens 5-HT₆ receptors had no effect on feeding on the palatable diet [drug effect: F (3, 15) = 0.248, p = .85; drug x time interaction: F(69, 345) = 0.848, p = .80]. Neither were there any effects on water intake, food approaches, or locomotor measures (all p values > .10; see Table 2).

Effects of 5-HT₂c receptor agents on food intake.

Drug effects in food-restricted rats. As can be seen in Figure 4, stimulation of 5-HT₂c receptors with RO 60-0175 had no effect on food intake in restricted rats [drug effect: F(2, 10) = 0.44, p = .66; drug x time interaction: F(46, 230) = 0.88, p =.69]. Neither were there any significant effects of the drug on water intake, food approaches, or locomotor activity in food-restricted animals offered standard rat chow.

Blockade of the 5-HT₂c receptor resulted in a significant drug x time interaction, F(46, 230) = 2.10, p < .001, but not a significant effect of drug, F (2, 10) = 0.99, p =.41. The interaction effect appears to be the result of a transient, but significant, decrease in food intake during the last 30 minutes of the feeding session on days that the rats received the low drug dose (1.0 µg/side). By the end of the session, all groups had eaten similar amounts of food; analysis of the drug effect at 30 min time points revealed no significant effects of drug on food intake at the 30, 60, 90 or 120 minute time points (all p-values > .10). There were no effects of intra-accumbens RS 102221 on water intake, locomotor measures, or food approaches (see Table 2).
**Drug effects in non-deprived rats offered palatable food.** Stimulation of nucleus accumbens 5-HT$_{2c}$ receptors did not cause a significant drug effect, F(2, 12) = 2.28, p = .145, but did result in a significant drug x time interaction effect, F(46, 276) = 1.87, p = .001. As can be seen in the bottom panels of Figure 4, this interaction appears to be due to a decrease in fat diet consumption on days that animals received the low dose of RO 60-0175 (2.0 μg(side)). This effect was not dose-dependent, however, as rats appear to have eaten normally on days that they received the high drug dose. ANOVAs comparing food intake across drug doses at the 30, 60, 90, and 120 min time points demonstrated trends toward differences between drug conditions at 90 and 120 min (p = .08 for both time points), but not at 30 and 60 min (p > .10).

Furthermore, there was a significant effect of drug on water intake in the non-deprived rats, F(2, 12) = 5.79, p = .017, although it should be noted that water intake was low in animals offered the high fat/sucrose diet. Tukey’s HSD comparing drug days against vehicle infusions suggest that the high (5.0 μg(side)) dose of RO 60-0175 significantly increased water intake (from 1.14 grams in the vehicle condition to 2.12 grams for the high drug dose). No effects of drug were observed on approaches to the food chamber or locomotor measures.

Antagonizing nucleus accumbens 5-HT$_{2c}$ receptors had no effect on feeding on the palatable diet [drug effect: F (2, 10) = 0.398, p = .40; drug x time interaction: F(46, 230) = 1.28, p = .13]. Neither were there any effects on water intake, food approaches, or locomotor measures (all p values > .10; see table 2).

**Discussion**

To our knowledge, this report is the first to examine the effects of stimulation of 5-HT$_{1/7}$, 5-HT$_{6}$, or 5-HT$_{2c}$ receptors within the medial nucleus accumbens on food intake elicited by food restriction or the offering of a palatable diet to non-deprived rats. Overall, the behavioral effects
following drug treatments in both feeding paradigms were consistent, suggesting that the behavioral role of these serotonin receptors were similar for rats that were food-restricted or motivated to feed by the presence of a palatable diet. Specifically, stimulation of 5-HT$_{17}$ receptors with 5-CT within the medial accumbens (primarily the shell, see Figure 1) caused reduced intake of food and water, as well as a concurrent reduction in rearing behavior (and ambulation in the non-deprived animal group). In contrast, stimulation of nucleus accumbens 5-HT$_6$ receptors with EMD 386088 increased feeding in both of the paradigms tested here. Activation of 5-HT$_{2C}$ receptors with physiologically effective doses of RO 60-0175 (Filip & Cunningham, 2002; Fletcher, Chintoh, Sinyard, & Higgins, 2004) had no effect on chow consumption in hungry rats, and modestly decreased fat diet intake in sated animals at the 2.0 µg/side (low), but not the 5.0 µg/side (high) dose. These data, combined with a recent report that stimulation of nucleus accumbens 5-HT$_4$ receptors reduces food intake in the mouse (Jean et al., 2007), suggest that distinct nucleus accumbens serotonin receptor subtypes serve different roles in the modulation of food intake and locomotor activity within nucleus accumbens feeding circuitry.

These experiments were also designed to determine whether antagonism of each receptor subtype would impact ingestive behavior. In contrast to the effects of 5-HT$_7$, 5-HT$_6$, or 5-HT$_{2C}$ receptor stimulation, blockade of these receptors had no impact on food consumption in either food access condition. Neither did antagonism of these receptors affect water intake or locomotor measures. Therefore, it appears that a tonic level of endogenous activation of these receptors in the medial nucleus accumbens is not required for normal feeding or locomotor behaviors to occur.
All of the agonists utilized here have been shown to excite or depolarize striatal cholinergic neurons (Blomeley & Bracci, 2005; Bonsi et al., 2007), and cholinergic signaling within the nucleus accumbens has itself been argued to modulate food intake and satiety processes (Avena et al., 2006; Mark et al., 1992; Pratt & Blackstone, 2008; Pratt & Kelley, 2004, 2005). However, given that the behavioral effects of each agonist were qualitatively different, and that striatal serotonin receptors are not exclusively expressed within cholinergic interneurons (Ward & Dorsa, 1996), it seems likely that the differential behavioral effects seen here are not the result of a unitary action on local acetylcholine outflow. The remainder of the discussion, therefore, places the behavioral effects of each drug manipulation within the broader context of known effects of serotonin receptors on food intake and motivation.

**Stimulation of nucleus accumbens serotonin receptors**

5-HT₁/7 receptors. Few previous reports have examined the role of central 5-HT₇ receptors on food intake or food-directed motivation. Knockdown of hypothalamic 5-HT₇ receptors by ICV injections of antisense oligonucleotides does not selectively reduce feeding or locomotor activity (Clemett, Cockett, Marsden, & Fone, 1998), and systemic blockade of these receptors (along with those of D₂, 5-HT₁A, and sigma receptors) by tiospirone only impact lever-pressing for food reward at levels that also induce catatonia (Arolfo & McMillen, 1999). However, in these experiments, stimulation of nucleus accumbens 5-HT₁/7 receptors with the drug 5-CT reduced feeding in rats in a dose-dependent manner. Increasing doses of 5-CT also attenuated water intake, rearing behavior, and ambulatory activity (although the latter was significantly decreased only in the rats fed the palatable diet). These are important observations, as they suggest that food intake alterations may be due to a reduced motivation to consume the food, locomotor impairment, or both. 5-CT was chosen for use in these experiments due to its known affinity and
activation of 5-HT7 receptors, and because prior reports had successfully utilized this agent intracranially to affect motivated behavior (Fletcher & Korth, 1999).

It is important to note, however, that 5-CT also has strong affinity for other serotonin receptor subtypes, most notably 5-HT1-type receptors. There is a relatively large literature suggesting that serotonin receptors of the 5-HT1 family impact ingestive behaviors. It has been shown that 5-HT1A and 5-HT1B agonists reduce food intake and shift feeding patterns when given systemically (De Vry & Schreiber, 2000; Simansky, 1996). Within the brain, activation of 5-HT1B receptors in the parabrachial nucleus of the pons inhibit food intake (Lee et al., 1998; Simansky & Nicklous, 2002), and stimulation of 5-HT1A receptors within the hypothalamus have been argued to advance satiety processes (Lopez-Alonso et al., 2007). There is a moderate distribution of 5-HT1B receptors in striatum (Palacios, Raurich, Mengod, Hurt, & Cortes, 1996; Verge et al., 1986); 5-HT1A receptor expression is low relative to other brain regions (Kia et al., 1996; Lanfumey & Hamon, 2000). Therefore, despite the notable behavioral effects of 5-CT in the current paradigm, it is unclear at this time whether the locomotor and ingestive effects are mediated by selective stimulation of 5-HT7 or 5-HT1-type receptors, or as the result of a constellation of activation of these subtypes. Future experiments, utilizing more selective ligands, will be required in order to determine if separate receptor subtypes may be responsible for the feeding and locomotor effects seen here.

5-HT6 receptors The 5-HT6 receptor has received recent attention as a potential target for pharmacotherapies with regard to both cognition and weight maintenance (Mitchell & Neumaier, 2005; Upton, Chuang, Hunter, & Virley, 2008). Several recent reports have suggested that manipulations targeting the 5-HT6 receptor result in reduced food intake and body weight (see Heal, Smith, Fisas, Codony, & Buschmann, 2008; Woolley, Marsden, & Fone, 2004). Mutant
mice lacking a functional 5-HT$_6$ receptor show attenuated weight gain on a high-fat diet as compared to wild-type controls offered the same diet (Frassetto et al., 2008). Pharmacological studies confirm a role for this receptor in food intake and body weight regulation, but the mechanisms underlying the effect are unclear, as there is evidence that both 5-HT$_6$ receptor agonists and antagonists, when applied systemically, reduce feeding and body weight (Fisas et al., 2006; Heal et al., 2008; Woolley, Bentley, Sleight, Marsden, & Fone, 2001).

5-HT$_6$ receptors are distributed throughout the striatum (Gerard et al., 1997; Ruat et al., 1993; Ward & Dorsa, 1996). In the current experiments, pharmacological activation of nucleus accumbens 5-HT$_6$ receptors increased feeding in both food-deprived rats offered rat chow, and in ad libitum fed rats given access to a palatable diet. This increased intake appeared to be specific to food consumption, as there was no increase in water intake following drug treatment, nor were there any significant alterations in locomotor measures. The increased feeding is not predicted from studies using systemic treatments of 5-HT$_6$ agonists, and suggests that hypophagia following such treatments may have another neural locus. The current data does, however, suggest that there is an important modulatory role for nucleus accumbens 5-HT$_6$ receptor activation on feeding. Although no formal measures were taken of the behavioral satiety sequence (see Halford, Wanninayake, & Blundell, 1998), the feeding curves across time suggest that food intake in both conditions began as normal, but that nucleus accumbens 5-HT$_6$ receptor stimulation caused a delay in the cessation of feeding (as shown by the later plateaus in feeding seen following drug treatment; see Figure 3). This pattern of intake is suggestive of a delay in the onset of satiety following drug treatment.

5-HT$_{2c}$ receptors
Systemic infusions of 5-HT\textsubscript{2C} agonists reduce feeding behaviors, and targeted stimulation of these receptors may be effective as a means to treat obesity (Halford, Harrold, Boyland, Lawton, & Blundell, 2007; Nilsson, 2006; Simansky, 1996). Research from several laboratories has suggested that 5-HT\textsubscript{2C} receptors within the hypothalamus are the primary site of action for this effect (e.g., Heisler et al., 2003; Lam et al., 2008; Zhou et al., 2005). Despite a dense distribution of 5-HT\textsubscript{2C} receptors in the nucleus accumbens shell, the current data suggest that the nucleus accumbens 5-HT\textsubscript{2C} receptors play a limited role (at best) in modulating feeding behavior. Infusions of the moderately selective agonist RO 60-0175 (see Higgins & Fletcher, 2003) into the medial accumbens had no effect on intake in food-deprived rats. The pattern of feeding in non-restricted rats offered palatable food was less clear; low doses (2.0 µg/side) of RO 60-0175 attenuated feeding, but the effect was not evident following infusions of the high drug dose (5.0 µg/side). This apparent lack of a consistent effect of nucleus accumbens 5-HT\textsubscript{2C} manipulation on food intake is interesting, given that intra-accumbens infusions of RO-0175 (at doses from 0.5 to 5.0 µg/side) are known to increase cocaine-induced locomotion, and also enhance rats’ discrimination of low doses of cocaine (Filip & Cunningham, 2002). Furthermore, blockade of 5-HT\textsubscript{2C} receptors in the nucleus accumbens shell with RS 102221 (at doses lower than those used in the current experiments) attenuates cocaine-induced locomotor activity. All of these effects are consistent with a possible role for 5-HT\textsubscript{2C} receptors in regulating meso-accumbens dopamine outflow (Navailles, De Deurwaerdere, Porras, & Spampinato, 2004; Willins & Meltzer, 1998; Yan, 2000). Dopamine within the meso-accumbens circuit has been argued to play a fundamental role in promoting preparatory/appetitive processes, but may be less involved in regulating consummatory behavior if food is freely available (Baldo & Kelley, 2007; Berridge, 2007; Salamone & Correa, 2002). Thus, if a predominant role of the 5-HT\textsubscript{2C} receptor in the
nucleus accumbens is to modulate dopaminergic activity, then these manipulations might not be expected to impact intake of the freely-available food. At the very least, the current data suggest that intra-accumbens 5-HT$_{2C}$ receptor treatments that may be effective in modulating the behavioral effects of drugs of abuse may not themselves have a significant impact on food-directed consummatory behaviors, an important consideration in terms of deriving effective and specific pharmacotherapies targeted toward addiction.

**Concluding remarks**

These experiments suggest that individual serotonin receptors within the medial nucleus accumbens serve important and diverse roles in modulating food-directed behavior. This may not be a surprise, as manipulations of several neurotransmitter systems within the nucleus accumbens have been shown to impact consummatory behavior, as well as the incentive properties of food and cues that are associated with food. Dopaminergic manipulations, for instance, affect the amount of effort that animals will elicit to earn food, and dopamine receptor stimulation increases the incentive salience of stimuli that are predictive of food delivery (Berridge, 2007; Salamone & Correa, 2002). Opioid and cannabinoid receptor stimulation of the nucleus accumbens preferentially increases feeding upon palatable foods, possibly by positively affecting hedonic responses (Kelley et al., 2002; Mahler, Smith, & Berridge, 2007; Pecina & Berridge, 2000). Blocking glutamate receptors within the rat medial nucleus accumbens shell, or inhibiting the region by stimulating GABA receptors, initiates voracious feeding (Basso & Kelley, 1999; Stratford, Swanson, & Kelley, 1996). Certainly, the effects of stimulation of 5-HT$_{1A}$ or 5-HT$_{6}$ receptors on food intake seen in the current experiments may result from interactions with one or many of the other neurotransmitter systems within the nucleus accumbens shell. In situ hybridization, immunohistochemical, and electrophysiological
techniques have shown that several classes of serotonin receptors are expressed and functional throughout the nucleus accumbens and striatum. Little is yet known about the specific cellular distribution of these receptors or how they interface with the other striatal neurotransmitters known to be involved in motivational processes (although see Ward & Dorsa, 1996). As it becomes apparent that serotonergic mechanisms within reward circuits modulate both natural and drug-reinforced behaviors, it will be of interest to determine how serotonergic signaling impacts the motivational effects of other neurochemical influences within the striatum.
References


Author Note

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Table 1: Summary of drugs and drug doses infused into the medial nucleus accumbens during these experiments. The final column shows the number of animals that contributed data to the analyses for each experimental group. Each group of rats received all doses of a single drug, the order of which was randomly determined for each rat.

<table>
<thead>
<tr>
<th>Drug Treatment</th>
<th>Pharmacological action</th>
<th>Doses used (in 0.5 µl/side)</th>
<th>N of rats/group: (Palatable diet)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-Carboxamidotryptamine maleate (5-CT)</td>
<td>5-HT$_{1/7}$ agonist</td>
<td>0.0, 0.5, 1.0, or 4.0 µg</td>
<td>5 (6)</td>
</tr>
<tr>
<td>SB 269970 hydrochloride</td>
<td>5-HT$_{7}$ antagonist</td>
<td>0.0, 1.0, 2.0, or 4.0 µg</td>
<td>5 (5)</td>
</tr>
<tr>
<td>EMD 386088 hydrochloride</td>
<td>5-HT$_{6}$ agonist</td>
<td>0.0, 1.0, or 4.0 µg</td>
<td>6 (6)</td>
</tr>
<tr>
<td>SB 258585 hydrochloride</td>
<td>5-HT$_{6}$ antagonist</td>
<td>0.0, 1.0, 2.0, or 4.0 µg</td>
<td>5 (6)</td>
</tr>
<tr>
<td>Ro 60-0175 fumarate</td>
<td>5-HT$_{2C}$ agonist</td>
<td>0.0, 2.0, or 5.0 µg</td>
<td>6 (7)</td>
</tr>
<tr>
<td>RS 102221 hydrochloride</td>
<td>5-HT$_{2C}$ antagonist</td>
<td>0.0, 1.0, and 2.0 µg</td>
<td>6 (6)</td>
</tr>
</tbody>
</table>
Table 2. Water intake, locomotor measures, and food approaches following injections of selective serotonergic antagonists into the medial nucleus accumbens. No significant effects were observed on these behavioral measures for any of the groups that received antagonists for 5-HT$_7$, 5-HT$_6$, or 5-HT$_{2C}$ receptors.

<table>
<thead>
<tr>
<th>Food-deprived rats given standard rat chow.</th>
<th>Water Intake (grams ± SEM)</th>
<th>Ambulation (counts ± SEM)</th>
<th>Rears (counts± SEM)</th>
<th>Food Approaches (counts± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SB 269970 (5-HT$_7$ Ant)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (0 µg/side)</td>
<td>12.9 ± 1.2</td>
<td>48.4 ± 9.3</td>
<td>51.0 ± 12.5</td>
<td>458.2 ± 101.9</td>
</tr>
<tr>
<td>1 µg/side</td>
<td>14.1 ± 0.5</td>
<td>64.4 ± 14.6</td>
<td>68.0 ± 17.0</td>
<td>358.4 ± 104.3</td>
</tr>
<tr>
<td>2 µg/side</td>
<td>14.9 ± 1.2</td>
<td>73.2 ± 20.9</td>
<td>67.6 ± 17.2</td>
<td>410.0 ± 31.1</td>
</tr>
<tr>
<td>4 µg/side</td>
<td>15.6 ± 0.9</td>
<td>67.0 ± 28.0</td>
<td>48.0 ± 15.2</td>
<td>555.4 ± 160.6</td>
</tr>
<tr>
<td><strong>SB 252585 (5-HT$_6$ Ant)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>17.6 ± 0.6</td>
<td>76.2 ± 16.9</td>
<td>71.17 ± 18.0</td>
<td>345.2 ± 54.9</td>
</tr>
<tr>
<td>1 µg/side</td>
<td>17.2 ± 1.5</td>
<td>57.2 ± 9.1</td>
<td>74.17 ± 19.6</td>
<td>445.0 ± 109.8</td>
</tr>
<tr>
<td>2 µg/side</td>
<td>15.9 ± 0.7</td>
<td>72.0 ± 10.9</td>
<td>78.50 ± 13.1</td>
<td>370.5 ± 83.8</td>
</tr>
<tr>
<td>4 µg/side</td>
<td>17.9 ± 0.6</td>
<td>75.0 ± 17.6</td>
<td>65.75 ± 17.5</td>
<td>356.3 ± 36.1</td>
</tr>
<tr>
<td><strong>RS 102221 (5-HT$_{2C}$ Ant)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>16.6 ± 1.6</td>
<td>63.5 ± 6.0</td>
<td>66.5 ± 8.0</td>
<td>326.17 ± 35.4</td>
</tr>
<tr>
<td>1 µg/side</td>
<td>16.7 ± 1.4</td>
<td>52.83 ± 4.1</td>
<td>71.0 ± 11.5</td>
<td>332.00 ± 37.6</td>
</tr>
<tr>
<td>2 µg/side</td>
<td>15.9 ± 1.0</td>
<td>56.83 ± 7.3</td>
<td>53.83 ± 7.7</td>
<td>336.67 ± 18.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Ad libitum fed rats given high fat/sucrose diet.</th>
<th>Water Intake (grams ± SEM)</th>
<th>Ambulation (counts ± SEM)</th>
<th>Rears (counts± SEM)</th>
<th>Food Approaches (counts± SEM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>SB 269970 (5-HT$_7$ Ant)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle (0 µg/side)</td>
<td>1.7 ± 0.3</td>
<td>34.2 ± 5.7</td>
<td>51.6 ± 11.9</td>
<td>66.2 ± 29.5</td>
</tr>
<tr>
<td>1 µg/side</td>
<td>1.2 ± 0.2</td>
<td>52.4 ± 16.8</td>
<td>96.4 ± 31.3</td>
<td>69.4 ± 16.5</td>
</tr>
<tr>
<td>2 µg/side</td>
<td>1.1 ± 0.1</td>
<td>37.8 ± 9.1</td>
<td>61.0 ± 18.8</td>
<td>44.8 ± 13.7</td>
</tr>
<tr>
<td>4 µg/side</td>
<td>1.2 ± 0.1</td>
<td>52.4 ± 8.9</td>
<td>82.4 ± 19.3</td>
<td>56.0 ± 18.4</td>
</tr>
<tr>
<td><strong>SB 252585 (5-HT$_6$ Ant)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.2 ± 0.2</td>
<td>46.2 ± 5.4</td>
<td>75.8 ± 9.2</td>
<td>50.2 ± 8.3</td>
</tr>
<tr>
<td>1 µg/side</td>
<td>1.7 ± 0.2</td>
<td>54.2 ± 11.4</td>
<td>93.0 ± 20.8</td>
<td>55.2 ± 12.4</td>
</tr>
<tr>
<td>2 µg/side</td>
<td>2.0 ± 0.3</td>
<td>60.5 ± 16.8</td>
<td>90.2 ± 31.5</td>
<td>41.3 ± 9.0</td>
</tr>
<tr>
<td>4 µg/side</td>
<td>1.4 ± 0.3</td>
<td>44.5 ± 10.9</td>
<td>74.8 ± 28.4</td>
<td>46.3 ± 14.6</td>
</tr>
<tr>
<td><strong>RS 102221 (5-HT$_{2C}$ Ant)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vehicle</td>
<td>1.1 ± 0.3</td>
<td>70.0 ± 16.8</td>
<td>118.3 ± 25.2</td>
<td>54.8 ± 6.6</td>
</tr>
<tr>
<td>1 µg/side</td>
<td>1.4 ± 0.6</td>
<td>71.5 ± 18.0</td>
<td>118.83 ± 35.2</td>
<td>66.3 ± 9.9</td>
</tr>
<tr>
<td>2 µg/side</td>
<td>1.1 ± 0.5</td>
<td>74.8 ± 19.4</td>
<td>106.5 ± 20.9</td>
<td>49.8 ± 11.4</td>
</tr>
</tbody>
</table>
Figure Captions

Figure 1. Location of injector tips for animals included in behavioral analyses. Column A shows placement of tips for rats receiving the 5-HT$_{1/7}$ agonist 5-CT (filled symbols) or the 5-HT$_7$ antagonist SB 269970 (open symbols). Rats in B received the 5-HT$_6$ agonist EMD 386088 (filled symbols) or the 5-HT$_6$ antagonist SB 252585 (open symbols). Column C represents the placements for animals receiving the 5-HT$_{2c}$ receptor agonist RO 60-0175 (filled symbols) or the 5-HT$_{2c}$ receptor antagonist RS 102221 (open symbols). Rats tested in the food-restricted feeding paradigm are denoted with circles; those that fed on the palatable high fat/sugar diet are represented with squares. Photomicrographs show representative placement of the cannula tips for rats that received the agonist within each column. 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 5-HT$_{1/7}$ receptor stimulation on feeding and locomotion. Increasing doses of the 5-HT$_{1/7}$ agonist 5-CT dose-dependently reduced food and water intake across the 2-hr feeding session, within both food-restricted rats feeding on standard chow (top panels) and ad libitum fed animals offered access to a highly palatable diet (bottom panels). This feeding reduction was accompanied by reduced rearing behavior in both groups of rats, as well as inhibited ambulation for the ad libitum feeding group. The number of head entries within the feeding chamber was not significantly affected by drug treatment within either group of animals. Antagonism of the 5-HT$_7$ receptor with SB 269970 had no effect on food intake in either feeding condition (bottom right panel for each behavioral condition; increasing doses of drug 0, 1.0, 2.0, and 4.0 µg SB 269970/side, higher doses are demarked by darker fill). *p < 0.05, **p < 0.01 for drug effects; single and double crosses demark p < .05 and p < .01 for
drug x time interaction effect, respectively. D indicates a significant difference from vehicle infusion according to post-hoc tests (see text).

Figure 3. Effects of medial nucleus accumbens 5-HT$_6$ receptor stimulation on feeding and locomotion. Increasing doses of the 5-HT$_6$ agonist EMD 386088 dose-dependently increased food intake across the 2-hr feeding session, most notably within food-restricted rats feeding on standard chow (top panels), but also in *ad libitum* fed animals offered access to a highly palatable diet (bottom panels). There was no significant effect of EMD 386088 on water intake, ambulatory activity, or rearing behavior. Increases of head entries within the food chamber also did not reach statistical significance. Antagonism of 5-HT$_6$ receptors with SB 252585 had little effect on food intake in either feeding condition (bottom right panel for each behavioral condition; increasing doses of drug 0, 1.0, 2.0, and 4.0 µg/side, higher doses are demarked by darker fill), although a slight but transient divergence between high and vehicle conditions led to a significant drug x time interaction (see text). Statistical symbols as in Fig. 2.

Figure 4. Effects of medial nucleus accumbens 5-HT$_{2c}$ receptor stimulation on feeding and locomotion. Stimulation of 5-HT$_{2c}$ receptors with the agonist RO 60-0175 had no effect on food intake, water intake, or locomotor measures in food-restricted animals (top panels). A significant drug x time effect for the animal group that received the high fat/sucrose diet appears to be the result of a mild, but significant, feeding reduction following bilateral infusion of the 2.0 µg dose of RO 60-0175. This effect is not evident at the higher (5.0 µg/side) dose, although water intake showed a mild but significant increase on days that rats received the highest drug dose. There were no effects of RO 60-0175 on locomotor measures at any drug dose. Antagonism of 5-HT$_{2c}$ receptors with RS 102221 had little effect on food intake in either feeding condition (bottom right panel for each behavioral condition; increasing doses of drug 0, 1.0, and 2.0 µg/side,
higher doses are demarked by darker fill). A significant time x drug interaction effect of RS 102221 in the food-deprived animals seems to be due to a transient mild reduction in feeding late in the session for animals receiving the 1.0 µg dose, which recovers by the end of the entire 2-hr session. Statistical symbols as in Fig. 2.
Figure 1.
Figure 2.

Effects of 5-HT(1/7) receptor stimulation on feeding and locomotion.

Chow intake in food-restricted rats (N = 5).

Fat/sucrose diet intake in non-restricted rats (N = 6).
Figure 3.

Effects of 5-HT(6) receptor stimulation on feeding and locomotion.

Chow intake in food-restricted rats (N = 6).

Fat/sucrose diet intake in non-restricted rats (N = 6).
Effects of 5-HT(2c) receptor stimulation on feeding and locomotion.

Chow intake in food-restricted rats (N = 6).

Fat/sucrose diet intake in non-restricted rats (N = 7).