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A systematic investigation of the differential roles for ventral tegmentum serotonin 1- and 2-type receptors on food intake in the rat

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Abstract

Central serotonin (5-HT) pathways are known to influence feeding and other ingestive behaviors. Although the ventral tegmentum is important for promoting the seeking and consumption of food and drugs of abuse, the roles of 5-HT receptor subtypes in this region on food intake have yet to be comprehensively examined. In these experiments, food restricted rats were given 2-hr access to rat chow; separate groups of non-restricted animals had similar access to a sweetened fat diet. Feeding and locomotor activity were monitored following ventral tegmentum stimulation or blockade of 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, or 5-HT_{2C} receptors. 5-HT_{1A} receptor stimulation transiently inhibited rearing behavior and chow intake in food-restricted rats, and had a biphasic effect on non-restricted rats offered the palatable diet. 5-HT_{1B} receptor agonism transiently inhibited feeding in restricted animals, but did not affect intake of non-restricted rats. In contrast, 5-HT_{1B} receptor antagonism decreased palatable feeding. Although stimulation of ventral tegmental 5-HT_{2B} receptors with BW723C86 did not affect hunger-driven food intake, it significantly affected palatable feeding, with a trend for an increasing intake at 2.0 µg/side but not at 5.0 µg/side. Antagonism of the same receptor modestly but significantly inhibited feeding of the palatable diet at 5.0 µg/side ketanserin. Neither stimulation nor blockade of 5-HT_{2A} or 5-HT_{2C} receptors caused prolonged effects on intake or locomotion. These data suggest that serotonin's effects on feeding within the ventral tegmentum depend upon the specific receptor targeted, as well as whether intake is motivated by food restriction or the palatable nature of the offered diet.

Keywords

serotonin; feeding; motivation; ventral tegmental area; reward

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1.0 Introduction

Of the many attempts to develop pharmacological tools for weight loss, those drugs that impact central serotonin (5-HT) signaling have had the most success to date. Indeed, two of the three current FDA-approved agents for assisting long-term efforts at dieting (the phentermine/topiramate combination, Qsymia®; and lorcaserin, Belviq®) both affect serotonergic mechanisms. Phentermine generally increases 5-HT tone in the synapse, whereas lorcaserin is a selective agonist that acts upon the 5-HT_{2C} receptor. There are over 14 individual subclasses of 5-HT receptors, and given the modest successes of lorcaserin, there is hope that selective agonists/antagonists at individual receptors may assist with weight loss without causing the cardiovascular side effects that have resulted in the market withdrawal of some previous serotonin-based pharmacotherapies, such as the phentermine/fenfluramine combination that was prescribed briefly in the late 1990s (fen-phen) and the more recently withdrawn sibutramine.

Although serotonin is found both peripherally and within the central nervous system, it is generally accepted that its actions in regulating meal size and satiety are accomplished within the brain. Particular attention has been paid to the roles of serotonin signaling in the brain stem, which receives input from the tongue and the gut, as well as the hypothalamus, which promotes food-seeking and intake based upon the current energy state of the organism (for reviews, see Lenard and Berthoud, 2008; Simansky, 1996; Woods and D'Alessio, 2008). Specific 5-HT receptor subtypes in both regions have been implicated in regulating food intake and satiety processes. For instance, 5-HT_{1B} receptors in the parabrachial nucleus of the pons inhibit feeding (Lee et al., 1998; Simansky and Nicklous, 2002), and 5-HT₃ receptors of the nucleus of the solitary tract mediate CCK-induced reductions of feeding (Hayes and Covasa, 2006a, 2006b). In the hypothalamus, 5-HT_{2C} receptor activation reduces food intake, likely by affecting melanocortin signaling (Heisler et al., 2003; Lam et al., 2008; López-Alonso et al., 2007; Zhou et al., 2005). These data suggest that serotonin signaling is an important modulator of food motivation across multiple levels of the nervous system.

Thus far, examination of the potential regulation of serotonin systems on feeding has largely neglected its impact on circuits of the brain that process the reinforcing and hedonic aspects of food. This is striking because it has been argued that the current rise in the obesity epidemic may largely be due to heightened availability and consumption of cheap, hyperpalatable, and energy-rich foods that promote intake beyond energy needs (i.e., Berridge et al., 2010; Berthoud et al., 2011; Kelley et al., 2005; Stice et al., 2013). Recent research suggests that serotonin can also regulate food intake and motivation through action in forebrain regions, including the prefrontal cortex (Mena et al., 2011; Stanquini et al., 2015) and the nucleus accumbens (Clissold et al., 2013; Francis et al., 2011; Jean et al., 2007; Pratt et al., 2009). Our laboratory and others have investigated the role of a subset of 5-HT receptors of the nucleus accumbens on food intake and motivation. The examination thus far suggests that stimulation of 5-HT_{1A} and 5-HT₄ receptors of the anterior nucleus accumbens shell inhibit food intake, whereas stimulation of the 5-HT₆ receptor increases feeding. Furthermore, nucleus accumbens 5-HT_{1A} and 5-HT₆ receptor stimulation decreases and increases motivation, respectively, in a progressive ratio task where rats work to earn

sugar pellets (Pratt et al., 2012). These findings suggest that serotonin, in addition to modulating brain stem and hypothalamic homeostatic circuitry, may regulate food motivation by directly impacting brain reward pathways.

The ventral tegmental area (VTA) is heavily interconnected with the hypothalamus and the nucleus accumbens (Oades and Halliday, 1987), and has been shown to be critically involved in promoting the intake of drugs of abuse as well as food. For instance, pharmacological manipulations of dopaminergic and opioid neurotransmission within the VTA impact food intake and motivation (e.g., MacDonald et al., 2004, 2003; Mucha and Iversen, 1986; Noel and Wise, 1995; Sharf et al., 2005). However, there has been no systematic investigation of the role of individual 5-HT receptor subtypes of the ventral tegmentum on feeding. As part of a broader effort to understand the potential modulatory roles of meso-accumbens 5-HT receptor signaling on food intake and motivation, here we examined the impact of pharmacological stimulation or blockade of ventral tegmentum 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors on rats' food intake in response to energy need or to the presence of a palatable sweetened fat diet.

2.0 Results

2.1 VTA 5-HT_{1A} stimulation and blockade on hunger- and palatability-driven feeding

In food restricted animals offered rat chow, stimulation of 5-HT_{1A} receptors of the ventral tegmentum with 8-OH-DPAT yielded a significant effect of drug ($F_{3,15} = 4.02$, $p = .028$) and a significant drug X time interaction effect ($F_{69,345} = 3.42$, $p < .001$). As can be seen in Figure 1, 5-HT_{1A} receptor stimulation caused a dose-dependent inhibition of feeding within the first hour of the feeding session. Follow-up ANOVAs at individual time points yielded significant differences between drug doses for all points examined within the first hour. Even when rats received the highest dose of 8-OH-DPAT, their feeding caught up to baseline levels during the second hour of testing. There was no significant effect of drug on ambulation as measured by cage crossings ($F_{3,15} = 2.33$, $p = .116$) nor water intake ($F_{3,15} = 0.94$, $p = .445$), although the 8.0 μg dose of the 8-OH-DPAT significantly reduced rearing behavior across the session (drug effect: $F_{3,15} = 11.44$, $p = .02$; Tukey's HSD pairwise comparisons $p < .05$).

Though consumption of the palatable diet was more variable than that of rat chow in food restricted animals, stimulation of the 5-HT_{1A} receptors also affected intake of the sweetened fat diet. There was a significant effect of drug ($F_{3,18} = 4.36$, $p = .018$), as well as a significant drug X time interaction effect ($F_{69,414} = 1.58$, $p = .004$). As can be seen in the bottom panels of Figure 1, rats that received the highest dose of drug ate the least amount of food across the session. Significant differences between drug treatments were apparent by 15 minutes into the session, although Tukey's HSD analyses at 5-min intervals suggest that the difference between the 2.0 μg and 8.0 μg drug treatments was primarily responsible for this effect, as neither dose was significantly different from vehicle treatment at the time points tested. This suggests that under these testing conditions, there was a biphasic impact of 5-HT_{1A} receptor stimulation on palatable feeding. Furthermore, there were significant effects of drug treatment on ambulation ($F_{3,18} = 3.21$, $p = .048$), although post-hoc analysis did not determine a significant difference of any of the drug conditions from the vehicle injection

day. There were no effects on rearing ($F_{3,18} = 1.14$, $p = .358$), nor was there a significant effect of drug on water intake ($F_{3,18} = 2.73$, $p = .075$).

Despite the effects of ventral tegmental 5-HT_{1A} receptor stimulation on food intake in both the food-restricted and palatable-feeding groups, blockade of the same receptor with WAY 100635 did not significantly change food intake or locomotor measures. There was no significant effect of drug dose ($F_{3,12} = 2.30$, $p = .129$), nor a drug X time interaction ($F_{69,276} = 1.32$, $p = .062$), on intake of rat chow in hungry rats. Neither did hungry animals exhibit significant changes in ambulation ($F_{3,15} = 0.19$, $p = .905$), rearing behavior ($F_{3,15} = 1.85$, $p = .182$) or water intake ($F_{3,15} = 2.02$, $p = .155$) across the drug doses tested. There were also no effects of ventral tegmental antagonism of 5-HT_{1A} receptors on palatable intake in non-restricted rats (drug effect: $F_{3,24} = 0.49$, $p = .689$; drug X time interaction: $F_{69,552} = 0.443$, $p = .999$). Ambulation, rearing, and water intake were also unaffected by drug treatment (all p 's > 0.1).

2.1 VTA 5-HT_{1B} stimulation and blockade on hunger- and palatability-driven feeding

In food-restricted animals offered rat chow, stimulation of 5-HT_{1B} receptors of the ventral tegmentum yielded a significant interaction of drug X time ($F_{69,345} = 1.65$, $p = .002$), though there was not a significant main effect of the drug ($F_{3,15} = 1.51$, $p = .253$). As can be seen in Figure 2, drug treatment slowed initial feeding. Both the 2.0 and 4.0 µg/side doses of the 5-HT_{1B} agonist CP 93129 caused significantly less feeding in the first five minutes compared to vehicle injections, and this difference was sustained for the first 25 minutes of the session for the 4.0 µg/side condition. Though there was a trend for the drug to also inhibit feeding during the middle of the second hour, there were no significant differences between the vehicle condition and any drug dose when examined at the individual time points (all ANOVA p 's > .05). Certainly, by the end of the second hour, rats had eaten equivalent amounts of food across all treatment conditions ($F_{3,15} = 0.73$, $p = .552$, as assessed at 2 hr). Although there was a tendency for the rats to rear less after receiving the high dose of drug, neither rearing ($F_{3,15} = 2.21$, $p = .129$), ambulation ($F_{3,15} = 0.93$, $p = .924$), nor water intake ($F_{3,15} = 0.04$, $p = .988$) significantly differed across drug treatments in food-deprived rats. Blockade of the receptor had no effect on food intake or locomotion; a separate set of rats, tested with ventral tegmentum injections of the 5-HT_{1B} antagonist GR 55562, showed no effects on food intake (drug effect: $F_{3,21} = 1.82$, $p = .174$; drug X time interaction: $F = 0.04$, $p = .988$), ambulation ($F_{3,21} = .012$, $p = .949$), rearing ($F_{3,21} = 0.606$, $p = .618$), or water intake ($F_{3,21} = 0.652$, $p = .590$).

These effects contrast with the impact of the same agents in ad libitum-fed animals that were offered a sweetened fat diet. In those conditions, stimulation of ventral tegmental 5-HT_{1B} receptors had no effect on feeding (main effect of drug: $F_{3,24} = 0.24$, $p = .870$; drug X time interaction effect: $F_{69,552} = 1.13$, $p = .234$). There were also no effects of CP 93129 treatment on ambulation ($F_{3,24} = 2.36$, $p = .097$), rearing ($F_{3,24} = 1.46$, $p = .250$), or water intake ($F_{3,24} = 0.87$, $p = .469$). However, blockade of the 5-HT_{1B} receptor caused a significant drug effect on food intake ($F_{3,18} = 4.42$, $p = .022$), although there was no drug X time interaction ($F_{69,414} = 1.17$, $p = .177$). As can be seen in the bottom panels of Figure 2, rats ate on average only about half of their baseline intake of the sweetened fat diet when

given 10.0 $\mu\text{g}/\text{side}$ of GR 55562 into the ventral tegmentum. No significant effects of drug were observed for ambulation ($F_{3,18} = 2.16$, $p = .129$), rearing behaviors ($F_{3,18} = 1.60$, $p = .224$), or water intake ($F_{3,18} = 1.55$, $p = .236$) in the same animals.

2.2. VTA 5-HT_{2A} stimulation and blockade on hunger- and palatability-driven feeding

Stimulation of ventral tegmentum 5-HT_{2A} receptors did not cause a drug effect on feeding in food restricted animals ($F_{3,21} = 0.59$, $p = .628$). There was a significant drug X time interaction effect ($F_{69,483} = 1.37$, $p = .034$), which was likely due to inhibition of feeding following the highest dose (10.0 $\mu\text{g}/\text{side}$) of TCB-2 during the latter half of the first hour (see Figure 3), relative to the other groups. However, this effect was modest; follow-up ANOVAs comparing feeding across drug doses at each 5-minute interval did not yield a significant difference across drug conditions at any individual time point during the feeding session (all p 's $> .05$). There was no significant effect of drug on ambulation ($F_{3,21} = 2.63$, $p = .077$), rearing behavior ($F_{3,21} = 0.90$, $p = .456$), or water intake ($F_{3,21} = 0.14$, $p = .933$). Similarly, blockade of tegmental 5-HT_{2A} receptors did not affect feeding (drug effect: $F_{3,18} = 1.32$, $p = .298$; drug X time interaction: $F_{69,414} = 0.66$, $p = .982$), water intake, or locomotor measures (Table 1, all p 's $> .05$) under the same conditions.

Likewise, there was no impact of 5-HT_{2A} receptor manipulations on feeding for non-restricted animals that were offered a palatable diet (Figure 3, bottom panels). Sweetened fat diet intake was unaffected by ventral tegmental injections of the 5-HT_{2A} agonist TCB-2 (drug effect: $F_{3,18} = 1.55$, $p = .236$; drug X time interaction: $F_{69,414} = 1.01$, $p = .469$) or the 5-HT_{2A} antagonist (drug effect: $F_{3,12} = 0.44$, $p = .726$; drug X time interaction: $F_{69,276} = 1.02$, $p = .442$). There were no significant effects of either drug treatment on locomotor or water intake measures across the 2-hr session (all p 's $> .05$).

2.3. VTA 5-HT_{2B} stimulation and blockade on hunger- and palatability-driven feeding

Food-restricted animals showed no changes in feeding in response to 5-HT_{2B} receptor stimulation of the ventral tegmentum (Figure 4; drug effect: $F_{2,12} = 1.02$, $p = .389$; drug X time interaction: $F_{46,276} = 0.907$, $p = .999$). The apparent (but not significant) decrease in feeding and the high variability at the highest dose of the drug (5.0 $\mu\text{g}/\text{side}$ BW 723C86) was due to the data of a single animal who ate less on that day; all other animals ate consistently across drug and vehicle treatments. Neither were there differences in ambulation, rearing, nor water intake across drug doses (Table 1; all p 's $> .01$). No differences on hunger-driven feeding or locomotion were observed following blockade of the 5-HT_{2B} receptor, either (drug effect: $F_{2,12} = 0.28$, $p = .758$; drug X time interaction: $F_{46,276} = 0.386$, $p = 1.0$; locomotion, rearing, water intake p 's all $> .05$).

In contrast to its lack of effect on feeding in hungry animals, ventral tegmental 5-HT_{2B} receptor stimulation had significant drug ($F_{2,14} = 7.32$, $p = .007$) and drug X time interaction effects on food intake ($F_{46,322} = 14.789$, $p < .001$) in animals offered a palatable sweetened fat diet. As can be seen in Figure 4, this was due to an apparent biphasic effect of the drug as the dose increased. The 2.0 $\mu\text{g}/\text{side}$ dose of BW723C86 tended to increase feeding, particularly late in the session, whereas the 5.0 $\mu\text{g}/\text{side}$ dose modestly inhibited feeding. At 15 minutes into the session, the highest dose had significantly inhibited feeding. Though

there was a significant overall effect of drug over the course of the 2 hrs, follow-up analysis at individual time points late in the session with Tukey's HSD just missed significance when comparing the vehicle injection with the 2.0 μg drug dose. That the higher dose reversed the apparent increase in feeding seen at the 2.0 $\mu\text{g}/\text{side}$ injection may have been due to non-specific locomotor impacts of the drug; 5-HT_{2B} receptor stimulation significantly reduced rearing behavior at 5.0 $\mu\text{g}/\text{side}$ of BW723C86 ($F_{2,14} = 4.28$, $p = .036$). Water intake and ambulation were not significantly different after treatment (p 's > .05). Interestingly, blockade of the 5-HT_{2B} receptor with RS127445 caused a modest but significant reduction of sweetened fat diet intake (drug effect: $F_{2,10} = 7.10$, $p = .012$; drug X time interaction: $F_{46,230} = 1.19$, $p < .001$). Follow-up analysis at individual time points across the session revealed a significant difference across drug condition by 40 minutes into the session; ventral tegmentum 5-HT_{2B} antagonism with 5.0 $\mu\text{g}/\text{side}$ of RS127445 inhibited sweetened fat diet intake, and that effect persisted throughout the remainder of the feeding session. There were no significant changes in ambulation, rearing, or water intake across drug treatments (p 's > .05).

2.3. VTA 5-HT_{2C} stimulation and blockade on hunger- and palatability-driven feeding

Food intake in food-restricted animals did not differ across treatments that stimulated ventral tegmentum 5-HT_{2C} receptors (Figure 5; drug effect: $F_{2,12} = 1.07$, $p = .373$; drug X time interaction: $F_{46,276} = 0.646$, $p = .963$), nor those that blocked the receptors (drug effect: $F_{2,8} = 0.19$, $p = .830$; drug X time interaction: $F_{46,184} = 0.448$, $p = .999$). Neither locomotor nor water intake were affected by microinjections of RO 60-0175 or RS 102221 (Table 1; all p 's > .05).

In a separate group of non-restricted rats offered a palatable diet, neither stimulation nor blockade of VTA 5-HT_{2C} receptors affected food intake. Stimulation with RO 60-0175 had no effect on food consumption (drug effect: $F_{2,14} = 0.92$, $p = .421$; drug X time interaction: $F_{46,322} = 0.43$, $p = 1.0$). Antagonism with RS 102221 had no effects, either (drug effect: $F_{2,14} = 0.78$, $p = .479$; drug X time interaction: $F_{46,322} = 1.05$, $p = .39$). Neither group experienced significant differences in ambulation, rearing, or water intake across doses of the 5-HT_{2C} agonist or antagonist (Table 2; all p 's > .05).

3.0 Discussion

The goal of these experiments was to examine the impact of selective 5-HT 1- or 2-type receptor stimulation or blockade of the ventral tegmentum on food intake in the rat. To our knowledge, this is the first systematic investigation of intra-VTA injections of 5-HT receptor-specific ligands on food intake, motivated either by food restriction or by palatability. Consistent with prior research that examined the role of individual 5-HT receptors following systemic treatment or injections into other brain regions, feeding and locomotor effects varied across manipulations of the different 5-HT receptor subtypes. Furthermore, the effects of these treatments (notably that of the 5-HT_{1A}, 5-HT_{1B}, and 5-HT_{2B} receptors) also depended on whether rats were motivated to eat due to food-restriction or due to the presence of a palatable food. The remainder of this discussion will place the

behavioral effects observed here within the context of prior investigations examining the roles of each 5-HT receptor on feeding.

3.1 5-HT_{1A} receptor

Stimulation of ventral tegmental 5-HT_{1A} receptors transiently inhibited chow feeding in food-restricted rats. This is consistent with prior research that has linked the 5-HT_{1A} receptor to the regulation of food intake. For instance, systemic 5-HT_{1A} receptor stimulation reduces food intake in rats and other animals that have been food-restricted (e.g., Ebenezer, 1992; Ebenezer et al., 1999; Saadoun and Cabrera, 2008). Furthermore, feeding is inhibited following intracranial injection of 5-HT_{1A} agonists directly into the hypothalamus (López-Alonso et al., 2007; Steffens et al., 2010, 2008) and the nucleus accumbens (Clissold et al., 2013). In this experiment, chow intake in food-restricted rats was transiently reduced by ventral tegmental 8-OH-DPAT injections. This contrasts with effects of 8-OH-DPAT observed by Lopez-Alonso et al. (2007) after injections into the paraventricular nucleus of the thalamus, which did not initially decrease feeding but instead suppressed overall carbohydrate intake consistent with an advancement of satiety processes. Similar to what we have reported following 8-OH-DPAT injections into the nucleus accumbens shell, the feeding effect seen here was paralleled by a significant decrease in rearing behavior. High doses of 8-OH-DPAT can and do elicit the low body posture characteristic of serotonin syndrome (e.g., Pranzatelli and Pluchino, 1991; Tricklebank et al., 1984). Anecdotally, this was evident in some of these animals during the first few minutes post-injection at the 8.0 µg/side dose. However, this locomotor effect did not impact water intake across the session; thus, it is possible that the transient food intake reduction observed here in food-restricted animals was due to a reduced motivation to consume the food, locomotor impairment, or both.

In contrast to its impact on feeding in hungry animals, systemic treatment with 5-HT_{1A} agonists has been shown to increase feeding across multiple species in animals that are not food-deprived upon testing (i.e., Baldwin and de la Riva, 1995; Dourish et al., 1988, 1986; Ebenezer and Surujbally, 2007; Shepherd and Rodgers, 1990). It is therefore notable that these experiments observed a biphasic effect of VTA 5-HT_{1A} receptor stimulation on palatable food intake (See Figure 1). This suggests that the impact of 5-HT_{1A} receptor stimulation in rats varies depending upon the energy state of the animal, with regards to both systemic treatments and those receiving 8-OH-DPAT into the ventral tegmentum. It has been argued that the increase in feeding observed after systemic treatment may be due to autoreceptor activation on serotonin-containing neurons within the raphe nuclei, which would subsequently decrease satiety-promoting serotonergic signaling to hypothalamic regions (Dourish et al., 1986, although see Fletcher and Coscina, 1993). Consistent with this hypothesis, 8-OH-DPAT injections directly into the raphe nuclei also increase feeding behavior (e.g., Bendotti and Samanin, 1986; Currie et al., 1994). Interestingly, at least part of this effect involves dopaminergic mechanisms, as systemic or striatal injections of dopaminergic antagonists block the increased feeding seen following raphe 8-OH-DPAT injections (Fletcher, 1991; Fletcher and Davies, 1990). A modest number of 5-HT_{1A} receptors are present in the VTA (Doherty and Pickel, 2001; Pazos and Palacios, 1985), therefore it is tempting to suggest that intra-VTA injections of 8-OH-DPAT may impact

feeding by altering dopamine output. However, although systemic injections of 8-OH-DPAT and other 5-HT_{1A} agonists increase midbrain dopamine firing and dopamine outflow in both the VTA and the nucleus accumbens, this effect does not appear to be mediated by 5-HT_{1A} receptors found in the VTA. Specifically, the increase in dopamine cell firing observed following systemic 8-OH-DPAT infusions is not replicated by iontophoretic application directly onto dopamine neurons (Prisco et al., 1994). Neither does 5-HT_{1A} receptor stimulation in the VTA increase nucleus accumbens dopamine (Guan and McBride, 1989). Other evidence suggests that the increase in dopaminergic output is caused by actions of the 5-HT_{1A} agonists in other brain regions that interact with dopamine signaling, such as the prefrontal cortex (Díaz-Mataix et al., 2005) and the raphe nuclei (Yoshimoto and McBride, 1992). Therefore, it appears unlikely that the biphasic effects of 8-OH-DPAT observed on feeding here are due to direct regulation of dopamine outflow from the VTA due to 5-HT_{1A} receptor stimulation in sated rats; the precise mechanism by which these receptors impact feeding within ventral tegmental circuits will require further study.

3.2 5-HT_{1B} receptor

The 5-HT_{1B} receptor has also received significant attention with regards to feeding processes. Systemic 5-HT_{1B} receptor stimulation dose-dependently decreases food intake, resulting in reductions in meal size and duration (Bovetto and Richard, 1995; Lee et al., 1998; Lee and Simansky, 1997; Schreiber et al., 2000). Consistent with this effect, blockade of the 5-HT_{1B} receptor reduces the anorectic effect of relatively non-selective serotonin agonists (Curzon, 1991; Simansky and Nicklous, 2002). Some of these effects are likely mediated by actions within the parabrachial nucleus of the pons. 5-HT_{1B} receptor stimulation of the parabrachial nucleus reduces food consumption, and pretreatment with a 5-HT_{1B} receptor antagonist blocks the hypophagic effects of fenfluramine or CP 93129 injections into the same region (Lee et al., 1998; Simansky and Nicklous, 2002). It is less clear if these receptors impact satiety processes within the hypothalamus. Although there is evidence suggesting a role for the 5-HT_{1B} receptor in regulating melanocortin signaling in the arcuate nucleus (Halford et al., 2007; Heisler et al., 2006), injections of 5-HT_{1B} agonists into the medial hypothalamus or the paraventricular nucleus have not yielded consistent results across laboratories (Fletcher et al., 1992; Hutson et al., 1988). Additionally, we have previously shown that 5-HT_{1B} receptor stimulation of the nucleus accumbens inhibits water intake but not chow consumption in food-restricted rats (Clissold et al., 2013), which is consistent with other reports suggesting a role of this receptor on water intake that is separable from that of its effect on food intake (Lee et al., 2002; Schreiber et al., 2000).

In these experiments, 5-HT_{1B} receptor stimulation of the ventral tegmentum caused a transient early inhibition of feeding behavior in rats that were food-restricted. The decrease is consistent with the feeding inhibition seen after systemic or parabrachial stimulation reviewed above, though in this case the inhibition was early in the feeding session and does not appear to be as a result of advanced satiety processes. Rats ultimately caught up to the control condition by the end of the two hour period. Although stimulation of the 5-HT_{1B} receptor in the nucleus accumbens has been shown to increase feeding without affecting water intake, the opposite was observed with stimulation in the ventral tegmentum in this study (specifically, feeding behavior decreased with no significant effect on water intake).

This suggests a complex role for this receptor in regulating food and water consumption across brain regions.

Complicating the issue further, non-restricted rats did not change their intake of a palatable diet following VTA injection of the same 5-HT_{1B} agonist. In fact, under cases of palatable feeding, blocking the 5-HT_{1B} receptor caused a modest but significant decrease of the intake of the sweetened fat diet by the end of the session, without impacting locomotor measures. This suggests a differential role of VTA 5-HT_{1B} receptors on feeding that depends upon the energy state of the animal and/or whether feeding is motivated by the palatability of the diet. Again, the precise mechanism for this effect will require further investigation, but unlike the 1A receptor, 5-HT_{1B} receptor agonists, when directly injected into the ventral tegmentum, increase dopamine output within both the VTA and the interconnected ventral striatum (Guan and McBride, 1989; O'Dell and Parsons, 2004; Yan et al., 2004). Palatable meals can affect dopamine outflow and signaling with the nucleus accumbens (Bassareo et al., 2002; Bassareo and Di Chiara, 1997; Danielli et al., 2010; Hajnal and Norgren, 2001), a brain region thought to be important in mediating food intake in response to palatable diets (e.g., Berridge et al., 2010; Berthoud et al., 2011; Kelley et al., 2005). It is possible that under conditions where the rat is not food deprived and food intake is being promoted by the palatable/rewarding aspects of food, 5-HT_{1B} receptor blockade may alter this signaling between the VTA and nucleus accumbens and reduce the reinforcing aspects of the palatable diet.

3.3 5-HT_{2A} receptor

The serotonin 2A receptor has been linked to feeding behavior in several ways. First, the testing of early serotonergic drugs that stimulated 5-HT_{2-type} receptors often caused hypophagia (Aulakh et al., 1992; Kennett and Curzon, 1991; Schechter and Simansky, 1988). Many of those drugs possess affinities at both the 2A and 2C receptors. As will be described below, the 5-HT_{2C} receptor has been a successful drug target for weight loss. Although there has been some indication that 5-HT_{2A} receptors impact feeding through interactions with hypothalamic NPY signaling in the paraventricular nucleus (Currie et al., 1999; Currie and Coscina, 1998, 1997) and also at targets within the ventromedial prefrontal cortex (Mena et al., 2011), research investigating the role of the 5-HT_{2A} receptor has been limited due to issues with drug specificity and concerns that agents which stimulate this receptor are associated with undesirable psychotic side effects. Furthermore, it has been argued that systemic stimulation of the 5-HT_{2A} receptor disrupts feeding, rather than specifically advancing satiety processes (Simansky, 1996). Nonetheless, the role of the 5-HT_{2A} receptor in feeding processes is likely complex, as neuroimaging experiments in humans suggest that differences in this receptor's profile are present in the brains of bulimic and anorexic patients (for reviews, see Bailer and Kaye, 2011; Kaye et al., 2005).

5-HT_{2A} receptors are present in the VTA (Doherty and Pickel, 2000; Rodríguez et al., 2000), and ventral tegmental injections targeting these receptors have been shown to impact the locomotor effects of cocaine injections (McMahon et al., 2001). However, despite the evidence indicating that the 5-HT_{2A} receptor may serve a role in regulating food intake and motivation, the current experiments do not suggest that the ventral tegmentum serves as a

neural locus of those effects. Neither stimulation nor antagonism affected food intake, water intake, or locomotor measures in rats motivated by hunger or food palatability.

3.4 5-HT_{2B} receptor

Compared to the other 5-HT_{1&2} receptor subtypes, less research has examined the potential effects of central 5-HT_{2B} receptor agents on feeding. One potential reason for this is that peripheral stimulation of this receptor subtype in the heart is believed to have been the cause of heart valve pathologies following fenfluramine-phentermine co-treatment, which limits the practical application of the 5-HT_{2B} receptor as a pharmacological target (for reviews, see Glazer, 2001; Rothman and Baumann, 2009). Regardless, much of our understanding of this receptor's effect on food intake is derived from experiments that examined the efficacy of systemic 5-HT receptor subtype antagonism on reversing the hypophagia caused by the anorectic agents sibutramine and dexfenfluramine (both of which increase serotonin tone). There has been scant evidence suggesting a role for the 5-HT_{2B} receptor in these types of experiments. For instance, Jackson et al. (1997) argued that 5-HT_{2A/2C} receptors (in addition to alpha 1-adrenoreceptors) are responsible for the anorectic effects of sibutramine, and a modest recovery of food intake following 5-HT_{2B/2C} antagonist pretreatment was also reported by Grignaschi et al. (1999). Vickers et al. (2001) reported that it is primarily the 5-HT_{2C} receptor that mediates the hypophagic effects of dexfenfluramine, and argued that other 1- and 2-type serotonin receptors are not involved. Despite these findings, mice that lack the 5-HT_{2B} receptor fail to reduce food intake in response to dexfenfluramine treatment (Banas et al., 2011; Lee et al., 2004; Lucas et al., 1998). Furthermore, 5-HT_{2B} receptor agonists may be linked to mechanisms of stress and food regulation, as 5-HT_{2B} receptor antagonists modestly but significantly reverse the hypophagia observed in mice following novelty stress (Yamada et al., 2013). These data suggest that, to date, we have an incomplete account of 5-HT_{2B} receptor function in the regulation of feeding pathways and behavior.

We did not observe food intake changes in food-restricted rats following either stimulation or blockade of the ventral tegmental 5-HT_{2B} receptor. However, in sated animals offered a palatable diet, the 2.0 µg/side dose of BW723C86 increased feeding on the palatable diet, whereas the 5.0 µg/side reversed this effect and inhibited food intake. In the only other study that we are aware of that has examined food intake following systemic and intracerebroventricular treatments of a 5-HT_{2B} agonist, a similar U-shaped function was observed in rats with increasing doses of drug. Specifically, Kennett et al. (1997) reported an increase in frequency and duration of feeding following 1–10 µg of BW 723C86 I.C.V., which reversed at 30 µg. As was the case for the animals in the present experiment, their rats were not food deprived at test. We also observed a modest but significant inhibition of palatable feeding following 5-HT_{2B} receptor blockade in the ventral tegmentum with RS 127445. Altogether, these data suggest that 5-HT_{2B} receptors in the ventral tegmentum modulate food intake, but only for animals who are choosing to feed during a non-restricted state.

3.5 5-HT_{2C} receptor

Of all the serotonin receptor subclasses, the 5-HT_{2C} receptor has received the most attention with regards to feeding and food motivation. Systemic infusions of 5-HT_{2C} agonists reduce

feeding behaviors, and lorcaserin (Belviq®) was approved in 2012 for assisting with weight loss in obese patients (Halford et al., 2007; Nilsson, 2006; “Press Announcements - FDA approves Belviq to treat some overweight or obese adults,” n.d.; Simansky, 1996). Research from several laboratories suggested that 5-HT_{2C} receptors within the hypothalamus are an important site of action for this effect (Heisler et al., 2003; Lam et al., 2008; Zhou et al., 2005). Nonetheless, 5-HT_{2C} receptors are also found within the ventral tegmentum (Bubar et al., 2011; Bubar and Cunningham, 2007), and there is ample evidence to suggest they serve important functional roles. For instance, Di Matteo and colleagues have shown that VTA dopamine cell firing and striatal dopamine output is inhibited by systemic stimulation of the 5-HT_{2C} receptor (see Di Matteo et al., 2002 for a review). Furthermore, direct stimulation of VTA 5-HT_{2C} receptors attenuates dopamine output in the nucleus accumbens following cocaine treatment (Navailles et al., 2008). Ventral tegmental treatment with doses of RO-0175 consistent with those used here also reduce cocaine self-administration and locomotor activation following acute cocaine injections in rats (Fletcher et al., 2004).

Despite their known effects on dopamine neuronal firing and output, we found no effects of 5-HT_{2C} receptor stimulation or blockade on food intake in rats feeding due to hunger or in response to palatability. Recently, we reported that similar injections into the nucleus accumbens also did not impact the feeding of food-restricted rats (Clissold et al., 2013). It should be noted that, in these experiments, rats are not required to work for food access; in all cases the food is freely available. This is likely a relevant methodological detail, as 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 and Kelley, 2007; Berridge, 2007; Salamone and Correa, 2002). Regardless of the impact of VTA 5-HT_{2C} receptors on dopamine output, the current data suggest that these receptors within the ventral tegmentum or the nucleus accumbens are not directly involved in the hypophagia observed following systemic treatment with serotonin receptor agonists.

3.6 Interpretational Caveats

Although these are the first experiments to examine the efficacy of selective serotonin receptor manipulation of the ventral tegmentum on two separate feeding paradigms, there are three caveats that need to be made in terms of the interpretation of the results. First, although the current experiments were specifically designed to examine feeding induced by food restriction (need) or the palatable nature of the diet (in the absence of energy deficit), the rate of food intake across the food-restriction and palatable feeding conditions differed. Thus, it is possible that different drug effects across the two feeding paradigms resulted not from the different motivational conditions *per se*, but from an interaction of the drug action on the varying rates of food intake in the two feeding conditions. Future experiments will be needed to address this directly. Second, the ventral tegmental area is a relatively diffuse brain region, intermingling with other midbrain nuclei and not distant from hindbrain centers that also regulate food intake. Our intracranial injections are consistent with past studies that examined the role of ventral tegmental pharmacological manipulations on behavior; however, we cannot rule out the possibility that the drug may have diffused to nearby brain regions over the course of the 2-hr feeding session.

Finally, as noted in section 4.6, the selective serotonin receptor agents used in this study were chosen based upon past successes in their use for investigating the roles of individual serotonin receptor subtypes in pharmacological assessments. Despite relative selectivity, off-target activation or inhibition of other receptor types is possible. For instance, WAY 100635 possesses D₄ receptor agonist properties in addition to its antagonism at the 5-HT_{1A} receptor. This may not be of relevance, given the lack of effect of WAY 100635 on feeding shown here. However, 8-OH-DPAT is a partial agonist at 5-HT₇ receptors, albeit with lower potency than its stimulation of the 5-HT_{1A} receptor (Wood et al., 2000). In the nucleus accumbens, we have found that the doses of 8-OH-DPAT used here inhibit feeding and locomotion, whereas injections of the selective 5-HT₇ agonist AS 19 do not (Clissold et al., 2013). This may suggest that the actions of 8-OH-DPAT are predominantly due to its impact on the 5-HT_{1A} receptor and/or that 5-HT₇ receptors of the nucleus accumbens are not involved in the regulation of feeding. Additionally, GR 55562 has been shown to antagonize human 5-HT_{1D} receptors in addition to its impact at the 5-HT_{1B} receptor (Walsh et al., 1995). And, although BW 723C86 has been repeatedly used as a selective 5-HT_{2B} agonist, high concentrations may activate other serotonin receptor subtypes (Kennett et al., 1996). It is possible that the U-shaped response pattern of BW 723C86 on palatable feeding observed here may be due to differential co-activation of 5-HT receptor subtypes as the dose increased.

3.7 Conclusions

A significant amount of prior research has suggested that the ventral tegmentum is important for directing motivation towards natural or drug rewards. However, previous reports examining the neural loci of the effects of serotonergic drugs on appetite have largely neglected meso-accumbens circuitry. The experiments presented here extend our understanding of serotonergic modulation of food motivation by demonstrating that serotonin receptors within the ventral tegmentum are involved in the regulation of food intake. Consistent with the diversity of serotonin receptor subtypes found throughout the periphery and central nervous system, these effects on feeding behavior depend upon the specific serotonin receptor targeted, as well as whether the animal is motivated by food restriction or the palatable nature of the offered diet. A better understanding of how serotonin interacts with meso-accumbens circuitry to increase or decrease the motivational aspects of ingestive behaviors may lead to more efficacious treatments for obesity and drug addiction.

4.0. Materials and methods

4.1. Animals

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.

4.2. Surgical Procedures

Following acclimation to the housing environment, 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 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.

4.3. 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 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) and filled with either standard rat chow or the palatable diet, depending on the paradigm (described below). 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. The weights of the food hoppers were monitored by the computer at 10-sec intervals. Water intake was measured by weighing the water bottles before and after each feeding session. 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). A speaker maintained an ambient level of white noise at 65 dB in the experimental room.

4.4 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, ten 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 infusions 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 ventral tegmentum (1.0 mm beyond the end of the guide cannula) and 0.5 μ l 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, 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.

4.5 Feeding paradigm on a palatable fat/sucrose diet

Ten additional groups of rats were tested for the effects of ventral tegmental 5-HT 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 nucleus accumbens injections of opiate or cholinergic drugs (Perry et al., 2014, 2009; Pratt and Blackstone, 2009; Skelly et al., 2010; Will et al., 2006). On experimental days, mock injections and drug infusions were delivered as described for the food restriction paradigm, above.

4.6. Drugs and Experimental Groups

These experiments assessed the effects of ventral tegmentum injection of selective agonists and antagonists at the 5-HT_{1A}, 5-HT_{1B}, 5-HT_{2A}, 5-HT_{2B}, and 5-HT_{2C} receptors upon feeding in food restricted and non-restricted rats. Within each experimental group, 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 in between injections. The drugs used, tested doses, and number of animals included in the analyses for each experiment are shown in Table 1. All drugs were obtained from Tocris Biosciences. 8-OH-DPAT, CP 93129, GR 55562, TCB-2, ketanserin, and RO 60-0175 were dissolved in physiological saline; WAY 100635, BW 723C86, RS 127445, and RS 102221 were dissolved in DMSO concentrations of 10%, 10%, 50%, and 50%, respectively, in physiological saline (drug was dissolved first in DMSO, and then saline was added). WAY 100635, CP 93129, and RO 60-0175 solutions were Ph-balanced to vehicle with NaOH. Concentrations for each agent were chosen based upon solubility and consistency with behaviorally-effective doses in other paradigms, when available (Currie et al., 2002; Egashira et al., 2006; Filip et al., 2003; Filip and Cunningham, 2002; Fletcher et al., 2004, 2004, 2002; Fletcher and Korth, 1999; McMahon et al., 2001; Pobbe and Zangrossi, 2005; Przegalinski et al., 2004). Potency for each agent at its receptor target is listed in the final column of Table 1.

4.7 Histology

Once the experiments were complete, rats were euthanized and the brains were collected for standard histological analysis. 60 µm sections were taken through the level of the midbrain and mounted for Nissl staining with Cresyl Violet. Placement of the injection sites was confirmed by light microscopy and charted with reference to Paxinos and Watson (1998).

Only animals whose injectors were bilaterally placed within the ventral tegmentum were included in the behavioral analysis (Ns = 5–9 rats/group; see Table 1).

4.8 Data analysis

Feeding data were 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 to compare the main effects of drug dose at the individual time points to further assess the consistency and time course of drug effects. Total ambulation, rearing behavior, 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.

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References

- Arvidsson LE, Hacksell U, Nilsson JL, Hjorth S, Carlsson A, Lindberg P, Sanchez D, Wikstrom H. 8-Hydroxy-2-(di-n-propylamino)tetrinalin, a new centrally acting 5-hydroxytryptamine receptor agonist. *J Med Chem.* 1981; 24:921–923. [PubMed: 6460101]
- Aulakh CS, Hill JL, Yoney HT, Murphy DL. Evidence for involvement of 5-HT_{1C} and 5-HT₂ receptors in the food intake suppressant effects of 1-(2,5-dimethoxy-4-iodophenyl)-2-aminopropane (DOI). *Psychopharmacology (Berl).* 1992; 109:444–448. [PubMed: 1365860]
- Bailer UF, Kaye WH. Serotonin: imaging findings in eating disorders. *Curr Top Behav Neurosci.* 2011; 6:59–79. DOI: 10.1007/7854_2010_78 [PubMed: 21243470]
- Baldo BA, Kelley AE. Discrete neurochemical coding of distinguishable motivational processes: insights from nucleus accumbens control of feeding. *Psychopharmacology (Berl).* 2007; 191:439–459. DOI: 10.1007/s00213-007-0741-z [PubMed: 17318502]
- Baldwin BA, de la Riva C. Effects of the 5-HT_{1A} agonist 8-OH-DPAT on operant feeding in pigs. *Physiol Behav.* 1995; 58:611–613. [PubMed: 8587972]
- Banas SM, Doly S, Boutourlinsky K, Diaz SL, Belmer A, Callebort J, Collet C, Launay JM, Maroteaux L. Deconstructing Antiobesity Compound Action: Requirement of Serotonin 5-HT_{2B} Receptors for Dexfenfluramine Anorectic Effects. *Neuropsychopharmacology.* 2011; 36:423–433. DOI: 10.1038/npp.2010.173 [PubMed: 20927048]
- Bassareo V, De Luca MA, Di Chiara G. Differential Expression of Motivational Stimulus Properties by Dopamine in Nucleus Accumbens Shell versus Core and Prefrontal Cortex. *J Neurosci Off J Soc Neurosci.* 2002; 22:4709–4719. 20026445.
- Bassareo V, Di Chiara G. Differential influence of associative and nonassociative learning mechanisms on the responsiveness of prefrontal and accumbal dopamine transmission to food stimuli in rats fed ad libitum. *J Neurosci Off J Soc Neurosci.* 1997; 17:851–861.
- Bendotti C, Samanin R. 8-Hydroxy-2-(di-n-propylamino) tetrinalin (8-OH-DPAT) elicits eating in free-feeding rats by acting on central serotonin neurons. *Eur J Pharmacol.* 1986; 121:147–150. [PubMed: 2937644]
- Berridge KC. The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology (Berl).* 2007; 191:391–431. DOI: 10.1007/s00213-006-0578-x [PubMed: 17072591]

- Berridge KC, Ho CY, Richard JM, DiFeliceantonio AG. The tempted brain eats: pleasure and desire circuits in obesity and eating disorders. *Brain Res.* 2010; 1350:43–64. DOI: 10.1016/j.brainres.2010.04.003 [PubMed: 20388498]
- Berthoud HR, Lenard NR, Shin AC. Food reward, hyperphagia, and obesity. *Am J Physiol - Regul Integr Comp Physiol.* 2011; 300:R1266–R1277. DOI: 10.1152/ajpregu.00028.2011 [PubMed: 21411768]
- Bonhaus DW, Flippin LA, Greenhouse RJ, Jaime S, Rocha C, Dawson M, Van Natta K, Chang LK, Pulido-Rios T, Webber A, Leung E, Eglen RM, Martin GR. RS-127445: a selective, high affinity, orally bioavailable 5-HT_{2B} receptor antagonist. *Br J Pharmacol.* 1999; 127:1075–1082. DOI: 10.1038/sj.bjp.0702632 [PubMed: 10455251]
- Bonhaus DW, Weinhardt KK, Taylor M, DeSouza A, McNeeley PM, Szczepanski K, Fontana DJ, Trinh J, Rocha CL, Dawson MW, Flippin LA, Eglen RM. RS-102221: a novel high affinity and selective, 5-HT_{2C} receptor antagonist. *Neuropharmacology.* 1997; 36:621–629. [PubMed: 9225287]
- Bovetto S, Richard D. Functional assessment of the 5-HT 1A-, 1B-, 2A/2C-, and 3-receptor subtypes on food intake and metabolic rate in rats. *Am J Physiol.* 1995; 268:R14–20. [PubMed: 7840314]
- Bubar MJ, Cunningham KA. Distribution of serotonin 5-HT_{2C} receptors in the ventral tegmental area. *Neuroscience.* 2007; 146:286–297. DOI: 10.1016/j.neuroscience.2006.12.071 [PubMed: 17367945]
- Bubar MJ, Stutz SJ, Cunningham KA. 5-HT(2C) receptors localize to dopamine and GABA neurons in the rat mesoaccumbens pathway. *PLoS One.* 2011; 6:e20508.doi: 10.1371/journal.pone.0020508 [PubMed: 21687728]
- Clissold KA, Choi E, Pratt WE. Serotonin 1A, 1B, and 7 receptors of the rat medial nucleus accumbens differentially regulate feeding, water intake, and locomotor activity. *Pharmacol Biochem Behav.* 2013; 112:96–103. DOI: 10.1016/j.pbb.2013.10.002 [PubMed: 24125784]
- Conn PJ, Sanders-Bush E. Serotonin-stimulated phosphoinositide turnover: mediation by the S₂ binding site in rat cerebral cortex but not in subcortical regions. *J Pharmacol Exp Ther.* 1985; 234:195–203. [PubMed: 2989504]
- Currie PJ, Coiro CD, Niyomchai T, Lira A, Farahmand F. Hypothalamic paraventricular 5-hydroxytryptamine: receptor-specific inhibition of NPY-stimulated eating and energy metabolism. *Pharmacol Biochem Behav.* 2002; 71:709–716. [PubMed: 11888562]
- Currie PJ, Coscina DV. 5-Hydroxytryptaminergic receptor agonists: effects on neuropeptide Y potentiation of feeding and respiratory quotient. *Brain Res.* 1998; 803:212–217. [PubMed: 9729393]
- Currie PJ, Coscina DV. Stimulation of 5-HT(2A/2C) receptors within specific hypothalamic nuclei differentially antagonizes NPY-induced feeding. *Neuroreport.* 1997; 8:3759–3762. [PubMed: 9427365]
- Currie PJ, Fletcher PJ, Coscina DV. Administration of 8-OH-DPAT into the midbrain raphe nuclei: effects on medial hypothalamic NE-induced feeding. *Am J Physiol.* 1994; 266:R1645–1651. [PubMed: 8203645]
- Currie PJ, Saxena N, Tu AY. 5-HT(2A/2C) receptor antagonists in the paraventricular nucleus attenuate the action of DOI on NPY-stimulated eating. *Neuroreport.* 1999; 10:3033–3036. [PubMed: 10549818]
- Curzon G. Effects of tryptophan and of 5-hydroxytryptamine receptor subtype agonists on feeding. *Adv Exp Med Biol.* 1991; 294:377–388. [PubMed: 1837683]
- Danielli B, Scheggi S, Grappi S, Marchese G, De Montis MG, Tagliamonte A, Gambarana C. Modifications in DARPP-32 phosphorylation pattern after repeated palatable food consumption undergo rapid habituation in the nucleus accumbens shell of non-food-deprived rats. *J Neurochem.* 2010; 112:531–541. DOI: 10.1111/j.1471-4159.2009.06483.x [PubMed: 19895662]
- Di Matteo V, Cacchio M, Di Giulio C, Esposito E. Role of serotonin(2C) receptors in the control of brain dopaminergic function. *Pharmacol Biochem Behav.* 2002; 71:727–734. [PubMed: 11888564]
- Díaz-Mataix L, Scorza MC, Bortolozzi A, Toth M, Celada P, Artigas F. Involvement of 5-HT_{1A} receptors in prefrontal cortex in the modulation of dopaminergic activity: role in atypical

- antipsychotic action. *J Neurosci Off J Soc Neurosci*. 2005; 25:10831–10843. DOI: 10.1523/JNEUROSCI.2999-05.2005
- Doherty MD, Pickel VM. Targeting of serotonin 1A receptors to dopaminergic neurons within the parabrachial subdivision of the ventral tegmental area in rat brain. *J Comp Neurol*. 2001; 433:390–400. [PubMed: 11298363]
- Doherty MD, Pickel VM. Ultrastructural localization of the serotonin 2A receptor in dopaminergic neurons in the ventral tegmental area. *Brain Res*. 2000; 864:176–185. [PubMed: 10802024]
- Dourish CT, Cooper SJ, Gilbert F, Coughlan J, Iversen SD. The 5-HT_{1A} agonist 8-OH-DPAT increases consumption of palatable wet mash and liquid diets in the rat. *Psychopharmacology (Berl)*. 1988; 94:58–63. [PubMed: 2964672]
- Dourish CT, Hutson PH, Kennett GA, Curzon G. 8-OH-DPAT-induced hyperphagia: its neural basis and possible therapeutic relevance. *Appetite*. 1986; 7(Suppl):127–140. [PubMed: 2943219]
- Ebenezer IS. Effects of the 5-HT_{1A} agonist 8-OH-DPAT on food intake in food-deprived rats. *Neuroreport*. 1992; 3:1019–1022. [PubMed: 1482760]
- Ebenezer IS, Parrott RF, Vellucci SV. Effects of the 5-HT_{1A} receptor agonist 8-OH-DPAT on operant food intake in food-deprived pigs. *Physiol Behav*. 1999; 67:213–217. [PubMed: 10477052]
- Ebenezer IS, Surujbally A. The effects of 8-hydroxy-2-(di-n-propylamino)-tetralin (8-OH-DPAT) on food intake in non-deprived C57BL6 mice. *Eur J Pharmacol*. 2007; 559:184–188. DOI: 10.1016/j.ejphar.2007.01.010 [PubMed: 17300778]
- Egashira N, Yano A, Ishigami N, Mishima K, Iwasaki K, Fujioka M, Matsushita M, Nishimura R, Fujiwara M. Investigation of mechanisms mediating 8-OH-DPAT-induced impairment of spatial memory: involvement of 5-HT_{1A} receptors in the dorsal hippocampus in rats. *Brain Res*. 2006; 1069:54–62. DOI: 10.1016/j.brainres.2005.10.103 [PubMed: 16386718]
- Ellis ES, Byrne C, Murphy OE, Tilford NS, Baxter GS. Mediation by 5-hydroxytryptamine_{2B} receptors of endothelium-dependent relaxation in rat jugular vein. *Br J Pharmacol*. 1995; 114:400–404. [PubMed: 7881740]
- Filip M, Cunningham KA. Serotonin 5-HT_{2C} receptors in nucleus accumbens regulate expression of the hyperlocomotive and discriminative stimulus effects of cocaine. *Pharmacol Biochem Behav*. 2002; 71:745–756. [PubMed: 11888566]
- Filip M, Papla I, Nowak E, Czepiel K, Przeglasiński E. Effects of 5-HT_{1B} receptor ligands microinjected into the ventral tegmental area on cocaine discrimination in rats. *Eur J Pharmacol*. 2003; 459:239–245. [PubMed: 12524152]
- Fletcher PJ. Dopamine receptor blockade in nucleus accumbens or caudate nucleus differentially affects feeding induced by 8-OH-DPAT injected into dorsal or median raphe. *Brain Res*. 1991; 552:181–189. [PubMed: 1833034]
- Fletcher PJ, Azampanah A, Korth KM. Activation of 5-HT_{1B} receptors in the nucleus accumbens reduces self-administration of amphetamine on a progressive ratio schedule. *Pharmacol Biochem Behav*. 2002; 71:717–725. [PubMed: 11888563]
- Fletcher PJ, Chintoh AF, Sinyard J, Higgins GA. Injection of the 5-HT_{2C} receptor agonist Ro60-0175 into the ventral tegmental area reduces cocaine-induced locomotor activity and cocaine self-administration. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol*. 2004; 29:308–318. DOI: 10.1038/sj.npp.1300319
- Fletcher PJ, Coscina DV. Injecting 5-HT into the PVN does not prevent feeding induced by injecting 8-OH-DPAT into the raphe. *Pharmacol Biochem Behav*. 1993; 46:487–491. [PubMed: 8265706]
- Fletcher PJ, Davies M. A pharmacological analysis of the eating response induced by 8-OH-DPAT injected into the dorsal raphe nucleus reveals the involvement of a dopaminergic mechanism. *Psychopharmacology (Berl)*. 1990; 100:188–194. [PubMed: 2137632]
- Fletcher PJ, Korth KM. Activation of 5-HT_{1B} receptors in the nucleus accumbens reduces amphetamine-induced enhancement of responding for conditioned reward. *Psychopharmacology (Berl)*. 1999; 142:165–174. [PubMed: 10102769]
- Fletcher PJ, Ming ZH, Zack MH, Coscina DV. A comparison of the effects of the 5-HT₁ agonists TFMPP and RU 24969 on feeding following peripheral or medial hypothalamic injection. *Brain Res*. 1992; 580:265–272. [PubMed: 1387034]

- Francis HM, Kraushaar NJ, Hunt LR, Cornish JL. Serotonin 5-HT₄ receptors in the nucleus accumbens are specifically involved in the appetite suppressant and not locomotor stimulant effects of MDMA ('ecstasy'). *Psychopharmacology (Berl)*. 2011; 213:355–363. DOI: 10.1007/s00213-010-1982-9 [PubMed: 20740276]
- Glazer. Long-term pharmacotherapy of obesity 2000: A review of efficacy and safety. *Arch Intern Med*. 2001; 161:1814–1824. DOI: 10.1001/archinte.161.15.1814 [PubMed: 11493122]
- Grignaschi G, Fanelli E, Scagnol I, Samanin R. Studies on the role of serotonin receptor subtypes in the effect of sibutramine in various feeding paradigms in rats. *Br J Pharmacol*. 1999; 127:1190–1194. DOI: 10.1038/sj.bjp.0702633 [PubMed: 10455265]
- Guan XM, McBride WJ. Serotonin microinfusion into the ventral tegmental area increases accumbens dopamine release. *Brain Res Bull*. 1989; 23:541–547. [PubMed: 2575444]
- Hajnal A, Norgren R. Accumbens dopamine mechanisms in sucrose intake. *Brain Res*. 2001; 904:76–84. DOI: 10.1016/S0006-8993(01)02451-9 [PubMed: 11516413]
- Halford JCG, Harrold JA, Boyland EJ, Lawton CL, Blundell JE. Serotonergic drugs : effects on appetite expression and use for the treatment of obesity. *Drugs*. 2007; 67:27–55. [PubMed: 17209663]
- Hayes MR, Covasa M. Dorsal hindbrain 5-HT₃ receptors participate in control of meal size and mediate CCK-induced satiation. *Brain Res*. 2006a; 1103:99–107. DOI: 10.1016/j.brainres.2006.05.058 [PubMed: 16793030]
- Hayes MR, Covasa M. Gastric distension enhances CCK-induced Fos-like immunoreactivity in the dorsal hindbrain by activating 5-HT₃ receptors. *Brain Res*. 2006b; 1088:120–130. DOI: 10.1016/j.brainres.2006.03.018 [PubMed: 16630589]
- Heisler LK, Cowley MA, Kishi T, Tecott LH, Fan W, Low MJ, Smart JL, Rubinstein M, Tatro JB, Zigman JM, Cone RD, Elmquist JK. Central serotonin and melanocortin pathways regulating energy homeostasis. *Ann N Y Acad Sci*. 2003; 994:169–174. [PubMed: 12851313]
- Heisler LK, Jobst EE, Sutton GM, Zhou L, Borok E, Thornton-Jones Z, Liu HY, Zigman JM, Balthasar N, Kishi T, Lee CE, Aschkenasi CJ, Zhang CY, Yu J, Boss O, Mountjoy KG, Clifton PG, Lowell BB, Friedman JM, Horvath T, Butler AA, Elmquist JK, Cowley MA. Serotonin reciprocally regulates melanocortin neurons to modulate food intake. *Neuron*. 2006; 51:239–249. DOI: 10.1016/j.neuron.2006.06.004 [PubMed: 16846858]
- Hutson PH, Dourish CT, Curzon G. Evidence that the hyperphagic response to 8-OH-DPAT is mediated by 5-HT_{1A} receptors. *Eur J Pharmacol*. 1988; 150:361–366. [PubMed: 2970974]
- Jackson HC, Bearham MC, Hutchins LJ, Mazurkiewicz SE, Needham AM, Heal DJ. Investigation of the mechanisms underlying the hypophagic effects of the 5-HT and noradrenaline reuptake inhibitor, sibutramine, in the rat. *Br J Pharmacol*. 1997; 121:1613–1618. DOI: 10.1038/sj.bjp.0701311 [PubMed: 9283694]
- Jean A, Conduetier G, Manrique C, Bouras C, Berta P, Hen R, Charnay Y, Bockaert J, Compan V. Anorexia induced by activation of serotonin 5-HT₄ receptors is mediated by increases in CART in the nucleus accumbens. *Proc Natl Acad Sci U S A*. 2007; 104:16335–16340. DOI: 10.1073/pnas.0701471104 [PubMed: 17913892]
- Kaye WH, Frank GK, Bailer UF, Henry SE, Meltzer CC, Price JC, Mathis CA, Wagner A. Serotonin alterations in anorexia and bulimia nervosa: new insights from imaging studies. *Physiol Behav*. 2005; 85:73–81. DOI: 10.1016/j.physbeh.2005.04.013 [PubMed: 15869768]
- Kelley AE, Baldo BA, Pratt WE, Will MJ. Corticostriatal-hypothalamic circuitry and food motivation: integration of energy, action and reward. *Physiol Behav*. 2005; 86:773–795. DOI: 10.1016/j.physbeh.2005.08.066 [PubMed: 16289609]
- Kennett GA, Ainsworth K, Trail B, Blackburn TP. BW 723C86, a 5-HT_{2B} receptor agonist, causes hyperphagia and reduced grooming in rats. *Neuropharmacology*. 1997; 36:233–239. [PubMed: 9144661]
- Kennett GA, Bright F, Trail B, Baxter GS, Blackburn TP. Effects of the 5-HT_{2B} receptor agonist, BW 723C86, on three rat models of anxiety. *Br J Pharmacol*. 1996; 117:1443–1448. [PubMed: 8730737]

- Kennett GA, Curzon G. Potencies of antagonists indicate that 5-HT_{1C} receptors mediate 1-(3-chlorophenyl)piperazine-induced hypophagia. *Br J Pharmacol.* 1991; 103:2016–2020. [PubMed: 1912990]
- Koe BK, Lebel LA, Fox CB, Macor JE. Binding and uptake studies with [3H]CP-93, 129, a radiolabeled selective 5-HT_{1B} receptor ligand. *Drug Dev Res.* 1992; 25:67–74. DOI: 10.1002/ddr.430250107
- Lam DD, Przydzial MJ, Ridley SH, Yeo GSH, Rochford JJ, O'Rahilly S, Heisler LK. Serotonin 5-HT_{2C} receptor agonist promotes hypophagia via downstream activation of melanocortin 4 receptors. *Endocrinology.* 2008; 149:1323–1328. DOI: 10.1210/en.2007-1321 [PubMed: 18039773]
- Lee MD, Aloyo VJ, Fluharty SJ, Simansky KJ. Infusion of the serotonin_{1B} (5-HT_{1B}) agonist CP-93,129 into the parabrachial nucleus potently and selectively reduces food intake in rats. *Psychopharmacology (Berl).* 1998; 136:304–307. [PubMed: 9566817]
- Lee MD, Kennett GA, Dourish CT, Clifton PG. 5-HT_{1B} receptors modulate components of satiety in the rat: behavioural and pharmacological analyses of the selective serotonin_{1B} agonist CP-94,253. *Psychopharmacology (Berl).* 2002; 164:49–60. DOI: 10.1007/s00213-002-1162-7 [PubMed: 12373419]
- Lee MD, Simansky KJ. CP-94, 253: a selective serotonin_{1B} (5-HT_{1B}) agonist that promotes satiety. *Psychopharmacology (Berl).* 1997; 131:264–270. [PubMed: 9203237]
- Lee MD, Somerville EM, Kennett GA, Dourish CT, Clifton PG. Reduced hypophagic effects of d-fenfluramine and the 5-HT_{2C} receptor agonist mCPP in 5-HT_{1B} receptor knockout mice. *Psychopharmacology (Berl).* 2004; 176:39–49. DOI: 10.1007/s00213-004-1864-0 [PubMed: 15138762]
- Lenard NR, Berthoud HR. Central and Peripheral Regulation of Food Intake and Physical Activity: Pathways and Genes. *Obes Silver Spring Md.* 2008; 16:S11–S22. DOI: 10.1038/oby.2008.511
- López-Alonso VE, Mancilla-Díaz JM, Rito-Domingo M, González-Hernández B, Escartín-Pérez RE. The effects of 5-HT_{1A} and 5-HT_{2C} receptor agonists on behavioral satiety sequence in rats. *Neurosci Lett.* 2007; 416:285–288. DOI: 10.1016/j.neulet.2007.02.026 [PubMed: 17324511]
- Lucas JJ, Yamamoto A, Searce-Levie K, Saudou F, Hen R. Absence of fenfluramine-induced anorexia and reduced c-Fos induction in the hypothalamus and central amygdaloid complex of serotonin 1B receptor knock-out mice. *J Neurosci Off J Soc Neurosci.* 1998; 18:5537–5544.
- MacDonald AF, Billington CJ, Levine AS. Alterations in food intake by opioid and dopamine signaling pathways between the ventral tegmental area and the shell of the nucleus accumbens. *Brain Res.* 2004; 1018:78–85. DOI: 10.1016/j.brainres.2004.05.043 [PubMed: 15262208]
- MacDonald AF, Billington CJ, Levine AS. Effects of the opioid antagonist naltrexone on feeding induced by DAMGO in the ventral tegmental area and in the nucleus accumbens shell region in the rat. *Am J Physiol Regul Integr Comp Physiol.* 2003; 285:R999–R1004. DOI: 10.1152/ajpregu.00271.2003 [PubMed: 12907414]
- Martin JR, Bös M, Jenck F, Moreau J, Mutel V, Sleight AJ, Wichmann J, Andrews JS, Berendsen HH, Broekkamp CL, Ruigt GS, Köhler C, Delft AM. 5-HT_{2C} receptor agonists: pharmacological characteristics and therapeutic potential. *J Pharmacol Exp Ther.* 1998; 286:913–924. [PubMed: 9694950]
- McLean TH, Parrish JC, Braden MR, Marona-Lewicka D, Gallardo-Godoy A, Nichols DE. 1-Aminomethylbenzocycloalkanes: conformationally restricted hallucinogenic phenethylamine analogues as functionally selective 5-HT_{2A} receptor agonists. *J Med Chem.* 2006; 49:5794–5803. DOI: 10.1021/jm060656o [PubMed: 16970404]
- McMahon LR, Filip M, Cunningham KA. Differential regulation of the mesoaccumbens circuit by serotonin 5-hydroxytryptamine (5-HT)_{2A} and 5-HT_{2C} receptors. *J Neurosci Off J Soc Neurosci.* 2001; 21:7781–7787.
- Mena JD, Sadeghian K, Baldo BA. Induction of hyperphagia and carbohydrate intake by μ -opioid receptor stimulation in circumscribed regions of frontal cortex. *J Neurosci Off J Soc Neurosci.* 2011; 31:3249–3260. DOI: 10.1523/JNEUROSCI.2050-10.2011
- Mucha RF, Iversen SD. Increased food intake after opioid microinjections into nucleus accumbens and ventral tegmental area of rat. *Brain Res.* 1986; 397:214–224. [PubMed: 3026557]

- Navailles S, Moison D, Cunningham KA, Spampinato U. Differential regulation of the mesoaccumbens dopamine circuit by serotonin_{2C} receptors in the ventral tegmental area and the nucleus accumbens: an in vivo microdialysis study with cocaine. *Neuropsychopharmacol Off Publ Am Coll Neuropsychopharmacol*. 2008; 33:237–246. DOI: 10.1038/sj.npp.1301414
- Nilsson BM. 5-Hydroxytryptamine _{2C} (5-HT_{2C}) receptor agonists as potential antiobesity agents. *J Med Chem*. 2006; 49:4023–4034. DOI: 10.1021/jm058240i [PubMed: 16821762]
- Noel MB, Wise RA. Ventral tegmental injections of a selective mu or delta opioid enhance feeding in food-deprived rats. *Brain Res*. 1995; 673:304–312. [PubMed: 7606445]
- Oades RD, Halliday GM. Ventral tegmental (A10) system: neurobiology. 1 Anatomy and connectivity. *Brain Res*. 1987; 434:117–165. [PubMed: 3107759]
- O'Dell LE, Parsons LH. Serotonin_{1B} receptors in the ventral tegmental area modulate cocaine-induced increases in nucleus accumbens dopamine levels. *J Pharmacol Exp Ther*. 2004; 311:711–719. DOI: 10.1124/jpet.104.069278 [PubMed: 15226384]
- Pauwels PJ, Gouble A, Wurch T. Activation of constitutive 5-hydroxytryptamine_{1B} receptor by a series of mutations in the BBXXB motif: positioning of the third intracellular loop distal junction and its G_o protein interactions. *Biochem J*. 1999; 343:435–442. DOI: 10.1042/bj3430435 [PubMed: 10510311]
- Paxinos, G.; Watson, C. *The Rat Brain in Stereotaxic Coordinates*, Fourth Edition. 4. Academic Press; San Diego: 1998.
- Pazos A, Palacios JM. Quantitative autoradiographic mapping of serotonin receptors in the rat brain. I. Serotonin-1 receptors. *Brain Res*. 1985; 346:205–230. DOI: 10.1016/0006-8993(85)90856-X [PubMed: 4052776]
- Perry ML, Baldo BA, Andrzejewski ME, Kelley AE. Muscarinic receptor antagonism causes a functional alteration in nucleus accumbens mu-opiate-mediated feeding behavior. *Behav Brain Res*. 2009; 197:225–229. DOI: 10.1016/j.bbr.2008.08.002 [PubMed: 18761381]
- Perry ML, Pratt WE, Baldo BA. Overlapping striatal sites mediate scopolamine-induced feeding suppression and mu-opioid-mediated hyperphagia in the rat. *Psychopharmacology (Berl)*. 2014; 231:919–928. DOI: 10.1007/s00213-013-3317-0 [PubMed: 24190586]
- Pobbe RLH, Zangrossi H. 5-HT(1A) and 5-HT(2A) receptors in the rat dorsal periaqueductal gray mediate the antipanic-like effect induced by the stimulation of serotonergic neurons in the dorsal raphe nucleus. *Psychopharmacology (Berl)*. 2005; 183:314–321. DOI: 10.1007/s00213-005-0196-z [PubMed: 16231166]
- Pranzatelli MR, Pluchino RS. The relation of central 5-HT_{1A} and 5-HT₂ receptors: low dose agonist-induced selective tolerance in the rat. *Pharmacol Biochem Behav*. 1991; 39:407–413. [PubMed: 1835098]
- Pratt WE, Blackstone K. Nucleus accumbens acetylcholine and food intake: decreased muscarinic tone reduces feeding but not food-seeking. *Behav Brain Res*. 2009; 198:252–257. DOI: 10.1016/j.bbr.2008.11.008 [PubMed: 19041901]
- Pratt WE, Blackstone K, Connolly ME, Skelly MJ. Selective serotonin receptor stimulation of the medial nucleus accumbens causes differential effects on food intake and locomotion. *Behav Neurosci*. 2009; 123:1046–1057. DOI: 10.1037/a0016882 [PubMed: 19824770]
- Pratt WE, Schall MA, Choi E. Selective serotonin receptor stimulation of the medial nucleus accumbens differentially affects appetitive motivation for food on a progressive ratio schedule of reinforcement. *Neurosci Lett*. 2012; 511:84–88. DOI: 10.1016/j.neulet.2012.01.038 [PubMed: 22306095]
- [accessed 3.25.16] Press Announcements - FDA approves Belviq to treat some overweight or obese adults [WWW Document]. n.d. URL <http://www.fda.gov/NewsEvents/Newsroom/PressAnnouncements/ucm309993.htm>
- Prisco S, Pagannone S, Esposito E. Serotonin-dopamine interaction in the rat ventral tegmental area: an electrophysiological study in vivo. *J Pharmacol Exp Ther*. 1994; 271:83–90. [PubMed: 7965760]
- Przegaliński E, Papla I, Siwanowicz J, Filip M. Effects of 5-HT_{1B} receptor ligands microinjected into the ventral tegmental area on the locomotor and sensitizing effects of cocaine in rats. *Eur*

- Neuropsychopharmacol J Eur Coll Neuropsychopharmacol. 2004; 14:217–225. DOI: 10.1016/S0924-977X(03)00106-8
- Rodríguez JJ, Doherty MD, Pickel VM. N-methyl-D-aspartate (NMDA) receptors in the ventral tegmental area: subcellular distribution and colocalization with 5-hydroxytryptamine(2A) receptors. *J Neurosci Res*. 2000; 60:202–211. [PubMed: 10740225]
- Rothman RB, Baumann MH. Appetite Suppressants, Cardiac Valve Disease and Combination Pharmacotherapy: *Am. J Ther*. 2009; 16:354–364. DOI: 10.1097/MJT.0b013e31817fde95
- Saadoun A, Cabrera MC. Hypophagic and dipsogenic effect of the 5-HT_{1A} receptor agonist 8-OH-DPAT in broiler chickens. *J Anim Physiol Anim Nutr*. 2008; 92:597–604. DOI: 10.1111/j.1439-0396.2007.00754.x
- Salamone JD, Correa M. Motivational views of reinforcement: implications for understanding the behavioral functions of nucleus accumbens dopamine. *Behav Brain Res*. 2002; 137:3–25. [PubMed: 12445713]
- Schechter LE, Simansky KJ. 1-(2,5-Dimethoxy-4-iodophenyl)-2-aminopropane (DOI) exerts an anorexic action that is blocked by 5-HT₂ antagonists in rats. *Psychopharmacology (Berl)*. 1988; 94:342–346. [PubMed: 3128809]
- Schreiber R, Selbach K, Asmussen M, Hesse D, de Vry J. Effects of serotonin(1/2) receptor agonists on dark-phase food and water intake in rats. *Pharmacol Biochem Behav*. 2000; 67:291–305. [PubMed: 11124393]
- Sharf R, Lee DY, Ranaldi R. Microinjections of SCH 23390 in the ventral tegmental area reduce operant responding under a progressive ratio schedule of food reinforcement in rats. *Brain Res*. 2005; 1033:179–185. DOI: 10.1016/j.brainres.2004.11.041 [PubMed: 15694922]
- Shepherd JK, Rodgers RJ. 8-OH-DPAT specifically enhances feeding behaviour in mice: evidence from behavioural competition. *Psychopharmacology (Berl)*. 1990; 101:408–413. [PubMed: 2141947]
- Simansky KJ. Serotonergic control of the organization of feeding and satiety. *Behav Brain Res*. 1996; 73:37–42. [PubMed: 8788474]
- Simansky KJ, Nicklous DM. Parabrachial infusion of D-fenfluramine reduces food intake. Blockade by the 5-HT_{1B} antagonist SB-216641. *Pharmacol Biochem Behav*. 2002; 71:681–690. [PubMed: 11888559]
- Skelly MJ, Guy EG, Howlett AC, Pratt WE. CB₁ receptors modulate the intake of a sweetened-fat diet in response to μ -opioid receptor stimulation of the nucleus accumbens. *Pharmacol Biochem Behav*. 2010; 97:144–151. DOI: 10.1016/j.pbb.2010.05.024 [PubMed: 20562021]
- Stanguini LA, Resstel LBM, Corrêa FMA, Joca SRL, Scopinho AA. Prelimbic cortex 5-HT_{1A} and 5-HT_{2C} receptors are involved in the hypophagic effects caused by fluoxetine in fasted rats. *Pharmacol Biochem Behav*. 2015; 136:31–38. DOI: 10.1016/j.pbb.2015.06.011 [PubMed: 26143050]
- Steffens SM, Beckman D, Faria MS, Marino-Neto J, Paschoalini MA. WAY100635 blocks the hypophagia induced by 8-OH-DPAT in the hypothalamic nuclei. *Physiol Behav*. 2010; 99:632–637. DOI: 10.1016/j.physbeh.2010.01.024 [PubMed: 20138904]
- Steffens SM, da Cunha IC, Beckman D, Lopes APF, Faria MS, Marino-Neto J, Paschoalini MA. The effects of metergoline and 8-OH-DPAT injections into arcuate nucleus and lateral hypothalamic area on feeding in female rats during the estrous cycle. *Physiol Behav*. 2008; 95:484–491. DOI: 10.1016/j.physbeh.2008.07.019 [PubMed: 18694771]
- Stice E, Figlewicz DP, Gosnell BA, Levine AS, Pratt WE. The contribution of brain reward circuits to the obesity epidemic. *Neurosci Biobehav Rev*. 2013; 37:2047–2058. DOI: 10.1016/j.neubiorev.2012.12.001 [PubMed: 23237885]
- Tricklebank MD, Forler C, Fozard JR. The involvement of subtypes of the 5-HT₁ receptor and of catecholaminergic systems in the behavioural response to 8-hydroxy-2-(di-n-propylamino)tetrinalin in the rat. *Eur J Pharmacol*. 1984; 106:271–282. [PubMed: 6241568]
- Vickers SP, Dourish CT, Kennett GA. Evidence that hypophagia induced by d-fenfluramine and d-norfenfluramine in the rat is mediated by 5-HT_{2C} receptors. *Neuropharmacology*. 2001; 41:200–209. [PubMed: 11489456]

- Walsh DM, Beattie DT, Connor HE. The activity of 5-HT1D receptor ligands at cloned human 5-HT1D alpha and 5-HT1D beta receptors. *Eur J Pharmacol.* 1995; 287:79–84. [PubMed: 8666030]
- Will MJ, Pratt WE, Kelley AE. Pharmacological characterization of high-fat feeding induced by opioid stimulation of the ventral striatum. *Physiol Behav.* 2006; 89:226–234. DOI: 10.1016/j.physbeh.2006.06.008 [PubMed: 16854442]
- Wood M, Chaubey M, Atkinson P, Thomas DR. Antagonist activity of meta-chlorophenylpiperazine and partial agonist activity of 8-OH-DPAT at the 5-HT7 receptor. *Eur J Pharmacol.* 2000; 396:1–8. DOI: 10.1016/S0014-2999(00)00213-2 [PubMed: 10822046]
- Woods SC, D'Alessio DA. Central Control of Body Weight and Appetite. *J Clin Endocrinol Metab.* 2008; 93:S37–S50. DOI: 10.1210/jc.2008-1630 [PubMed: 18987269]
- Yamada C, Saegusa Y, Nakagawa K, Ohnishi S, Muto S, Nahata M, Sadakane C, Hattori T, Sakamoto N, Takeda H. Rikkunshito, a Japanese kampo medicine, ameliorates decreased feeding behavior via ghrelin and serotonin 2B receptor signaling in a novelty stress murine model. *BioMed Res Int.* 2013; 2013:792940.doi: 10.1155/2013/792940 [PubMed: 24288687]
- Yan QS, Zheng SZ, Yan SE. Involvement of 5-HT1B receptors within the ventral tegmental area in regulation of mesolimbic dopaminergic neuronal activity via GABA mechanisms: a study with dual-probe microdialysis. *Brain Res.* 2004; 1021:82–91. DOI: 10.1016/j.brainres.2004.06.053 [PubMed: 15328035]
- Yoshimoto K, McBride WJ. Regulation of nucleus accumbens dopamine release by the dorsal raphe nucleus in the rat. *Neurochem Res.* 1992; 17:401–407. [PubMed: 1356241]
- Zhou L, Williams T, Lachey JL, Kishi T, Cowley MA, Heisler LK. Serotonergic pathways converge upon central melanocortin systems to regulate energy balance. *Peptides.* 2005; 26:1728–1732. DOI: 10.1016/j.peptides.2004.12.028 [PubMed: 15993514]
- Zhuang ZP, Kung MP, Kung HF. Synthesis And Evaluation of 4-(2'-Methoxyphenyl)-1-[2'-[N-(2''-pyridinyl)-p-iodobenzamido]ethyl]piperazine (p-MPPI): A New Iodinated 5-HT1A Ligand. *J Med Chem.* 1994; 37:1406–1407. DOI: 10.1021/jm00036a003 [PubMed: 8182697]

Highlights

- Feeding was assessed after stimulating or blocking ventral tegmentum 5-HT receptors
- 5-HT_{1A}, 5-HT_{1B}, & 5-HT_{2B} receptor manipulations significantly affected food intake
- 5-HT_{2A} and 5-HT_{2C} agonism or antagonism had no effects on feeding
- Effects varied between rats that were motivated by energy need or a palatable diet

Figure 1.

Effects of ventral tegmentum stimulation or blockade of the 5-HT_{1A} receptor on feeding, water intake, and locomotion. In food-restricted rats offered rat chow (top two panels), 5-HT_{1A} receptor stimulation (but not antagonism), dose-dependently reduced food intake within the first hour of the feeding session. By the end of the second hour, consumption was equivalent across all doses of the drug. Rearing behavior was also significantly inhibited at the 8.0 µg/side dose. The effects of ventral tegmental injections of 8-OH-DPAT were significant but more complex in non-restricted rats offered a palatable diet (bottom two panels): when rats were injected with 2.0 µg/side, there was a tendency for an increase in food intake, whereas the highest dose of the drug (8.0 µg/side) inhibited intake throughout the session. Follow-up ANOVAs at individual time points yielded significant effects on feeding between conditions by 15 minutes into the session, which lasted until the session was over. These drug effects appeared to be due to differences between the 2.0 and 8.0 µg/side conditions, as Tukey's HSD did not yield significant differences between the vehicle injection and either drug condition when the simple effects of drug dose was tested at 5-min intervals. 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 (1998). 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-HT_{1B} receptor on feeding, water intake, and locomotion. Stimulation, but not blockade, of 5-HT_{1B} receptors yielded a significant drug X time interaction effect on food intake in food-restricted animals. This was likely due to an inhibition of feeding early in the session after rats were treated with 4.0 µg/side of CP 93129 (top panels). This difference in food consumed was absent by the end of the 2 hr session. There was no impact of CP 93129 or GR 55562 on ambulation, rearing, or water intake in hungry animals. In contrast to the effects of the agonist on feeding in food-restricted animals, non-restricted animals offered a palatable diet showed no effects of

ventral tegmentum 5-HT_{1B} receptor stimulation on feeding. Instead, blockade of 5-HT_{1B} receptors caused a modest but significant decline in intake of the sweetened fat diet, without impacting water intake or locomotor measures. Statistical symbols as for Figure 1. The photomicrographs on the right show representative placements of the injection cannulas for these experiments.

Figure 3.

Neither stimulation nor blockade of 5-HT_{2A} receptors of the ventral tegmentum caused lasting changes in food intake or locomotion. 5-HT_{2A} receptor stimulation yielded a significant drug X time interaction for food-restricted rats, which was likely due to an initial slower rate of feeding during the first hour for the 10.0 µg/side dose. However, at no time during the 2-hr session did drug treatment yield significantly different intake of rat chow compared to the vehicle injection. Agonist treatment did not affect food intake of the sweetened palatable diet, and blockade of the 5-HT_{2A} receptor did not affect either hunger-

or palatability-induced feeding. Locomotion and water intake were also unaffected by these treatments. Statistical symbols and histological figures as for Figure 1.

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Figure 4.

Effects of ventral tegmentum stimulation or blockade of the 5-HT_{2B} receptor on food intake. In the case of food-restricted rats offered free access to rat chow, neither stimulation nor blockade of the 5-HT_{2B} receptor affected intake across the 2-hr session (top panels). In contrast, non-restricted rats offered 2-hr access to the sweetened fat diet showed significant drug and drug X time interaction effects following 5-HT_{2B} receptor stimulation or antagonism of the ventral tegmentum (bottom panels). Injections of BW 723C86 caused a biphasic effect on feeding on the palatable diet, with the 2.0 µg/side dose modestly increasing food intake, and the 5.0 µg/side tending to inhibit feeding. Blockade of the same

receptor with RS 127445 significantly inhibited feeding at 5.0 µg/side. Locomotion and water intake were unaffected by these treatments. Statistical symbols and histological figures as for Figure 1.

Figure 5.

Neither stimulation nor blockade of 5-HT_{2C} receptors of the ventral tegmentum caused prolonged changes in food intake. Neither RO 60-0175 nor RS 102221 impacted feeding or locomotion across the 2-hr period, regardless of whether they were motivated by food restriction (top panels) or a palatable diet (bottom panels).

Table 1

Summary of drugs and drug doses infused into the ventral tegmentum during these experiments. The fourth column shows the number of animals that contributed data to the analyses for each experimental group, after removing those with misplaced injection sites.

Drug Treatment	Pharmacological action	Doses used (in 0.5 µl/site)	N of rats/group: Food restricted (Palatable diet)	Concentration of highest dose	Drug potency or efficacy (ED/IC ₅₀ unless otherwise stated)
8-Hydroxy-DPAT hydrobromide (8-OH-DPAT)	5-HT _{1A} agonist	0, 2.0, 4.0 or 8.0 µg	6 (7)	48.7 mM	45 nM/kg ^a
WAY 100635	5-HT _{1A} antagonist	0, 2.5, 5.0 or 10.0 µg	6 (9)	37.1 mM	2.2 nM ^b
CP 93129	5-HT _{1B} agonist	0, 1.0, 2.0, or 4.0 µg	6 (9)	27.8 mM	K _D = 3.0 nM ^c
GR 55562	5-HT _{1B} antagonist	0, 2.0, 5.0, and 10 µg	8 (7)	44.6 mM	pEC ₅₀ = 7.27 ^d
TCB-2	5-HT _{2A} agonist	0, 1.0, 5.0, or 10 µg	8 (7)	56.6 mM	36 nM ^e
Ketanserin	5-HT _{2A} antagonist	0, 0.5, 1.0, and 2.5 µg	7 (5)	9.2 mM	3.6 nM ^f
BW 723C86	5-HT _{2B} agonist	0, 2.0, or 5.0 µg	7 (8)	31.0 mM	pEC ₅₀ = 7.9 ^g
RS 127445	5-HT _{2B} antagonist	0, 2.0, or 5.0 µg	7 (6)	31.5 mM	pIC ₅₀ = 10.4 ^h
RO 60-0175	5-HT _{2C} agonist	0, 2.0, or 5.0 µg	7 (8)	29.2 mM	pEC ₅₀ = 6.7; ED ₅₀ = 0.2 mg/kg ⁱ
RS 102221	5-HT _{2C} antagonist	0, 2.0, or 5.0 µg	5 (8)	15.4 mM	pK _i = 8.5 ^j

The right two columns give the highest dose given in nM and the reported potency or efficacy values of each agents as initially reported in:

^aArvidsson et al., 1981;
^bZhuang et al., 1994;
^cKoe et al., 1992;
^dPauwels et al., 1999;
^eMcLean et al., 2006;
^fConn and Sanders-Bush, 1985;
^gEllis et al., 1995;
^hBonhaus et al., 1999;
ⁱpEC₅₀ determined in choroid plexus; ED₅₀ is behavioral response to systemic injection in the rat;(Martin et al., 1998;

Bonhaus et al., 1997.

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