

RUNNING HEAD: Nucleus accumbens acetylcholine and feeding

Nucleus accumbens acetylcholine and food intake:

Decreased muscarinic tone reduces feeding but not food-seeking

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This is a revised personal edition of a manuscript that has been published as noted below:

Pratt, W. E., & Blackstone, K. (2009). Nucleus accumbens acetylcholine and food intake: Decreased muscarinic tone reduces feeding but not food-seeking. Behavioural Brain Research, *198*(1), 252-257. ([doi:10.1016/j.bbr.2008.11.008](https://doi.org/10.1016/j.bbr.2008.11.008))

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Acknowledgements:

This work was supported by a Wake Forest University Summer Undergraduate Fellowship (to KB) and the Wake Forest University Department of Psychology. We would like to thank Dr. Federico Bermúdez-Rattoni for advice on preparing AFDX-116, and Dr. Terry D. Blumenthal for his comments on the manuscript.

Separate groups of food-deprived rats were given 2-hr access to food after receiving bilateral nucleus accumbens infusions of the muscarinic antagonist scopolamine methyl bromide (at 0, 1.0, and 10.0 micrograms/side), the M2-preferring agonist oxotremorine sesquifumarate (Oxo-S; at 0, 1.0, or 10.0 micrograms/side) or the M2 antagonist AFDX-116 (at 0, 0.2, or 1.0 micrograms/side). Injections of scopolamine or Oxo-S, but not AFDX-116, reduced food consumption across the two hours. These experiments confirm a critical role for Acb acetylcholine in promoting food ingestion, and suggest that decreased acetylcholine tone at post-synaptic muscarinic receptors disrupts normal consummatory behavior.

Keywords: Nucleus accumbens; acetylcholine; muscarinic receptors; feeding; food intake; scopolamine; AFDX-116; oxotremorine sesquifumarate.

The nucleus accumbens (Acb) is known for its role in coordinating goal-directed behaviors, including those aimed at acquiring natural (i.e., food) and drug reinforcement. Separate neurotransmitter systems within the Acb serve complementary but distinct roles to coordinate the appetitive and consummatory phases of goal acquisition. For instance, intra-Acb injections of dopaminergic agonists influence effort and incentive motivation directed toward food-associated cues in rats, while having limited effects on free-feeding. In contrast, Acb opioid receptor stimulation increases food ingestion, particularly on palatable diets, but does not increase lever-pressing for conditioned cues associated with food delivery [3, 12, 25].

The Acb regulates motivated behavior via projections from its medium spiny neurons to other structures within basal ganglia motor pathways. These neurons integrate inputs from extrastriatal limbic regions, but are also heavily influenced by acetylcholine output from striatal giant aspiny interneurons. Acetylcholine modulates striatal function via its actions on muscarinic and nicotinic receptors on striatal neurons and incoming axons from the cortex, thalamus, and tegmentum [28]. Recent research suggests that Acb acetylcholine plays an important role in learning and motivational processes. For instance, striatal cholinergic neurons respond to primary rewards and cues that predict them [1]. Rats self-administer acetylcholine agonists directly into the Acb [9], and selective lesions of Acb cholinergic interneurons alter food intake patterns and impair learning directed toward food reinforcement [6, 13]. Furthermore, antagonism of Acb muscarinic receptors with scopolamine methyl bromide impairs learning and performance of a lever-pressing task in the rat, reduces progressive ratio breakpoint for obtaining sucrose reinforcement, lessens 15-min sucrose intake in hungry animals, and diminishes feeding on rat chow over a 24-hour period [19, 20]. Acb muscarinic receptor

antagonism also decreases the ingestion of palatable diets that is induced by Acb opioid mu receptor activation [26].

Although Acb muscarinic receptors appear to serve a critical role in coordinating food-directed behavior, it is not yet clear what the specific mechanisms are for these behavioral effects. Global muscarinic receptor antagonism of the Acb with scopolamine may serve to reduce feeding behavior and food motivation by blocking post-synaptic muscarinic signaling on axonal processes within the striatum and the cell bodies of medium spiny neurons. This would be consistent with prior research suggesting that increased Acb acetylcholine receptor activation (or “tone”) is rewarding, and that acetylcholine is necessary for food-reinforced learning and normal food intake (see above). Alternatively, the effects of scopolamine infusions into the Acb may be mediated by antagonizing acetylcholine autoreceptors, causing a corresponding increase in Acb acetylcholine outflow that has been argued to be fundamental for initiating satiety mechanisms. This would be consistent with reports by Hoebel and colleagues, who have demonstrated that Acb acetylcholine rises during the course of a meal, peaking as rats begin to reduce their rate of feeding [15].

Of the five different muscarinic subtypes, both the M2 and M4 receptor have been argued to function as autoreceptors [4, 27]. Microdialysis studies have shown that M2 receptor stimulation reduces cholinergic levels in both cortex and striatum, while M2 receptor antagonism increases acetylcholine outflow [4, 23, 24]. These experiments were designed to characterize the time course of the acute food intake deficit of rats with Acb muscarinic receptor antagonism, and test whether the known behavioral effects of global muscarinic receptor blockade of the Acb core could be replicated by antagonism or stimulation of the muscarinic M2 receptor subtype. If the effects of intra-Acb scopolamine on food motivation are predominantly due to increases in

acetylcholine outflow, then M2-specific antagonism with AFDX-116 (at drug doses that have been shown to increase acetylcholine levels in brain) should lead to a similar feeding reduction. Alternatively, if scopolamine's effects are the result of diminished cholinergic tone on post-synaptic sites, stimulation of the M2 autoreceptor (with oxotremorine sesquifumarate) should cause decreased food intake.

All experiments were conducted in accordance to NIH animal care guidelines and were approved by the Wake Forest University Animal Care and Use Committee. 30 adult male Sprague-Dawley rats (Harlan, Madison, WI) 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.). Standard aseptic procedures were used to implant indwelling stainless steel guide cannulas (23 gauge) bilaterally above the Acb core (flat skull; 1.3 mm anterior and 1.7 mm lateral to bregma, 5.0 mm ventral to skull surface), as described previously [19]. After one week of recovery, rats were food restricted and gradually reduced to approximately 90% of their *ad libitum* body weight. Water was available at all times.

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 standard rat chow 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). The weights of the food

monitors were recorded at 10-sec intervals throughout each feeding session. A speaker maintained an ambient level of white noise at 65 dB in the experimental room.

Rats received six days of habituation to the feeding chambers prior to pharmacological treatments. Each session consisted of 2 hours of free access to rat chow and water. On the final two days of habituation, rats received mock infusions to allow acclimation to microinfusion procedures, as previously described [26]. Experimental treatments began 48 hrs after the last mock infusion. During vehicle and drug infusions, injection cannulas (30 gauge) were lowered into the Acb 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 to allow for diffusion, and rats were then immediately placed in the feeding chambers. Dependent measures included the amount of chow eaten over the 2-hr period, the number of approaches to the food chamber, 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 during the feeding session. 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. Locomotion, water intake, and head entry measures were analyzed with one-way repeated measures ANOVAs with drug dose as the independent variable; Bonferroni-corrected paired t-tests were conducted as post-hoc analysis when appropriate, comparing drug doses with behavior on vehicle days.

Three groups of rats ($N = 10$ per group) were used in these experiments. Each group received three bilateral Acb infusions of the muscarinic receptor antagonist scopolamine methyl bromide (at 0, 1.0, and 10.0 micrograms/0.5 microliter/side), the M2 receptor antagonist AFDX-116 (at 0, 0.2, and 1.0 micrograms/side), or the M2 preferential agonist oxotremorine

sesquifumarate (Oxo-S, at 0, 1.0, and 10.0 micrograms/side). Scopolamine and Oxo-S were mixed in 0.9% sterile saline; AFDX-116 was dissolved into 0.1% DMSO in saline. Oxo-S drug solutions were Ph-balanced to the saline vehicle. Drug doses were at or above those previously shown to be physiologically effective [4, 19, 22-24]. Each rat received all three doses of drug within its drug group (scopolamine, Oxo-S, or AFDX-116), the order of which was randomly determined for each animal. Experimental treatments were separated by at least 48 hours. Two scopolamine and three AFDX animals were omitted from analysis due to misplacement of cannulas, necrosis surrounding the infusion site, or equipment failure.

The results from the scopolamine group confirm and expand upon previous reports examining the effects of muscarinic receptor blockade on feeding behavior. As shown in Figure 1, broad acetylcholine muscarinic receptor antagonism of the Acb dose-dependently reduced feeding in food-deprived animals across the 2-hour experimental session (drug effect: $F_{2,14} = 3.38$, $p = .006$; drug x time interaction: $F_{46,322} = 1.5$, $p = .025$). This reduction of food intake was accompanied by a corresponding decrease in drinking, with total water consumption reduced significantly on days that rats received scopolamine treatment ($F_{2,14} = 21.554$, $p < .001$). Reduced feeding was not likely the result of rats avoiding the food or being unable to visit the food intake chamber, as 1.0 microgram of scopolamine did not alter locomotion but did cause a significant decrease in feeding behavior. Additionally, although the high drug dose significantly increased rearing behavior ($F_{2,14} = 8.86$, $p = .003$) and ambulation across the chamber ($F_{2,14} = 22.8$, $p < .001$), the rats also dose-dependently increased the number of approaches to the food, as measured by head entries into the food intake monitor ($F_{2,14} = 13.8$, $p < .001$; see figure 1). Thus, muscarinic receptor blockade preserved food-seeking behavior, but reduced overall ingestion.

M2 receptor inactivation did not cause the same reduction of consummatory activity that follows antagonism of all muscarinic receptor subtypes with scopolamine. Infusions of AFDX-116, at concentrations equal to or higher than those shown to promote acetylcholine output in cortex and striatum [4, 23, 24], did not affect food intake when injected into the Acb (drug effect: $F_{2,10} = 0.43$, $p = .66$; drug x time interaction: $F_{46,230} = .71$, $p = .92$; see Figure 2). Additionally, there was no effect of M2 receptor antagonism on ambulation ($F_{2,12} = 1.48$, $p = .27$), rearing ($F_{2,12} = 0.09$, $p = .91$), water intake ($F_{2,12} = 1.18$, $p = .34$), or the number of approaches made to the feeding chamber ($F_{2,12} = 1.73$, $p = .22$).

Rats treated with the high dose of Oxo-S, a preferential M2 receptor agonist, significantly reduced food consumption across the 2-hr feeding session (see Figure 3). Intra-Acb Oxo-S treatment resulted in a significant drug effect ($F_{2,18} = 35.06$, $p < .001$) and drug x time interaction effect ($F_{46,414} = 8.63$, $p < .001$) on food intake. As with scopolamine, this reduction was accompanied by a decrease in drinking; total water consumption was significantly smaller on days that rats received the high dose of the drug ($F_{2,18} = 13.41$, $p < .001$). Unlike the locomotor increases observed with scopolamine, Oxo-S treatment caused an overall decrease in rearing ($F_{2,18} = 7.13$, $p = .005$) and ambulation across the chamber ($F_{2,18} = 4.73$, $p = .022$). However, reduced locomotor output did not lead to a parallel decrease in the approaches to the food intake monitor ($F_{2,18} = 0.57$, $p = .57$). Thus, similar to global muscarinic blockade with scopolamine, Oxo-S treatment of the Acb reduced food consumption without affecting food approaches.

Altogether, these experiments confirm a critical role for Acb acetylcholine in regulating ingestive behaviors. Intra-accumbens injections of both scopolamine, which antagonizes muscarinic receptors, and Oxo-S, which stimulates the M2 autoreceptor and lowers striatal cholinergic outflow [17, 23], reduced feeding within the experimental chambers in a time- and

dose-dependent manner. AFDX-116, at doses previously shown to increase brain acetylcholine output [4, 23], did not affect food intake in this paradigm. These data suggest that intra-Acb scopolamine blocks feeding by decreasing, rather than increasing, acetylcholine tone within ventral striatal feeding circuitry.

Interestingly, these data also provide an explanation for an apparent discrepancy between results derived from behavioral pharmacological work and that which has been observed utilizing microdialysis in feeding rats. As noted above, Hoebel and colleagues have shown that Acb acetylcholine rises during meal ingestion, reaching a peak as rats slow their food intake [15]. Furthermore, subsequent work has shown that Acb acetylcholine levels do not rise in sham-fed rats (that are presumably not sated), and hypothalamic manipulations that reduce feeding also promote Acb acetylcholine release [2, 8]. The authors have suggested that a rise in ventral striatum acetylcholine may serve as a defining marker for behavioral satiety. Thus, it could be argued that reduced activity at post-synaptic acetylcholine receptors in the Acb might block satiety and therefore increase, rather than reduce, overall feeding. The current data do not support this prediction, but neither do they suggest that muscarinic receptor blockade impacts feeding due to enhancing satiety mechanisms. Although we did not specifically measure the behaviorally-defined satiety sequence as others have done [7], one would expect that any manipulation that advanced satiety would cause a normal pattern of food intake followed by a drug-dependent early cessation of feeding. Furthermore, treated rats should approach the feeding chamber fewer times during the session, as food-approach behavior would be replaced by behaviors that are characteristic of satiety (e.g., grooming, resting). Neither scopolamine nor Oxo-S treatment reduced entries to the food intake monitor (see Figs 1 and 3), nor did the drug-induced feeding patterns demonstrate normal food intake early in the session followed by an

early cessation. Thus, although heightened Acb acetylcholine outflow may be a characteristic physiological feature of satiety, reductions of the normally high cholinergic tone on Acb muscarinic receptors (caused by scopolamine or Oxo-S) disrupts feeding at some point prior to satiety onset.

Despite the similar patterns of feeding results, the behavioral profiles of scopolamine and Oxo-S were not identical; Acb scopolamine infusions increased ambulatory and rearing behaviors while Oxo-S decreased locomotor behavior. Although the affinity of Oxo-S is highest at the M2 receptor, its actions are preferential, rather than selective [5]. It is possible that the doses utilized here caused some activation at other muscarinic receptor subtypes, which may have affected motor output and reduced food motivation. Alternatively, reduced Ach influence on dopamine-enhancing nicotinic receptors may explain the locomotor inhibition. In either case, the current food intake reduction following Oxo-S treatment is most plausibly explained by decreased muscarinic receptor activation at post-synaptic sites within the Acb, as it has been previously shown that direct stimulation of Acb cholinergic receptors (with an Ach/physostigmine cocktail) has no effect upon rats' feeding on a palatable diet [26]. This is consistent with the results of the current study, as M2 receptor antagonism with AFDX-116 (at doses known to increase brain Ach concentrations), also did not affect ingestion in hungry rats. Together, these data suggest that although reduced Acb muscarinic receptor tone decreases feeding, enhanced Acb acetylcholine receptor activation does not cause a corresponding increase in food intake.

The current experiments specifically targeted the nucleus accumbens core, to be consistent with much of our previous work examining the role of Acb acetylcholine on food-motivated behavior [19-21, 26]. The Acb is divided into both core and shell subregions based

upon anatomical connectivity and cellular morphology, and there is substantial evidence that the two areas serve distinct roles in directing appetitive behaviors [10, 16]. However, muscarinic receptor blockade of either the shell or the core of the Acb cause comparable reductions in instrumental learning and responding for sucrose reinforcement, and scopolamine injections into both regions reduce short-term (15-min) intake of freely-available sucrose [19]. It seems likely that muscarinic receptors of the Acb core and shell serve similar functional roles in promoting food-directed behavior, although additional research is needed to verify this claim.

These current data are consistent with the hypothesis that Acb muscarinic receptor activity is required to direct normal consummatory behavior. What then is the nature of the feeding deficit observed? Previous experiments targeting the role of Acb glutamate, dopamine, opiate, and cannabinoid receptors have suggested that each plays a complementary but unique function in modulating the appetitive and consummatory phases of food intake [3, 12, 14]. Dopaminergic antagonism reduces the vigilance in appetitive processes directed at cues and behaviors leading to the availability of food as a reinforcer, while leaving ingestion of freely-available foods intact. Cannabinoid and mu-opioid receptors within the Acb promote hedonic reactions to palatable foods, and stimulation of their receptors increase consummatory behavior accordingly. In this experiment, although food consumption was reduced following M2 receptor stimulation with Oxo-S or muscarinic receptor antagonism with scopolamine, appetitive food-seeking (as measured by head entries into the food intake monitor) was unchanged (for Oxo-S) or increased (for scopolamine). Reduced muscarinic receptor tone may therefore selectively block the consummatory phase of feeding behavior, perhaps by reducing the hedonic or reinforcing properties of the food itself. We have previously shown that reduced 24-hr feeding following scopolamine infusions into the Acb is accompanied by reduced preproenkephalin

expression throughout the striatum [20], and that antagonism of muscarinic receptors reduces palatable diet intake that follows Acb opioid stimulation [26]. Thus, Acb cholinergic and opioid systems likely serve as part of an interconnected neural system that regulates the consummatory phase of food ingestion, possibly by modulating feeding in response to the hedonic/reinforcing value of the diet [11].

References

- [1] Apicella P. Tonically active neurons in the primate striatum and their role in the processing of information about motivationally relevant events. *Eur J Neurosci*, 2002;16:2017-2026.
- [2] Avena NM, Rada P, Moise N, Hoebel BG. Sucrose sham feeding on a binge schedule releases accumbens dopamine repeatedly and eliminates the acetylcholine satiety response. *Neuroscience*, 2006;139:813-820.
- [3] Berridge KC. The debate over dopamine's role in reward: the case for incentive salience. *Psychopharmacology (Berl)*, 2007;191:391-431.
- [4] Billard W, Binch H, 3rd, Crosby G, McQuade RD. Identification of the primary muscarinic autoreceptor subtype in rat striatum as m2 through a correlation of in vivo microdialysis and in vitro receptor binding data. *J Pharmacol Exp Ther*, 1995;273:273-279.
- [5] Brauner-Osborne H, Brann MR. Pharmacology of muscarinic acetylcholine receptor subtypes (m1-m5): high throughput assays in mammalian cells. *Eur J Pharmacol*, 1996;295:93-102.
- [6] Hajnal A, Szekely M, Galosi R, Lenard L. Accumbens cholinergic interneurons play a role in the regulation of body weight and metabolism. *Physiol Behav*, 2000;70:95-103.
- [7] Halford JC, Wanninayake SC, Blundell JE. Behavioral satiety sequence (BSS) for the diagnosis of drug action on food intake. *Pharmacol Biochem Behav*, 1998;61:159-168.
- [8] Helm KA, Rada P, Hoebel BG. Cholecystokinin combined with serotonin in the hypothalamus limits accumbens dopamine release while increasing acetylcholine: a possible satiation mechanism. *Brain Res*, 2003;963:290-297.

- [9] Ikemoto S, Glazier BS, Murphy JM, McBride WJ. Rats self-administer carbachol directly into the nucleus accumbens. *Physiol Behav*, 1998;63:811-814.
- [10] Kelley AE. Functional specificity of ventral striatal compartments in appetitive behaviors. *Ann N Y Acad Sci*, 1999;877:71-90.
- [11] Kelley AE, Baldo BA, Pratt WE. A proposed hypothalamic-thalamic-striatal axis for the integration of energy balance, arousal, and food reward. *J Comp Neurol*, 2005;493:72-85.
- [12] 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.
- [13] Kitabatake Y, Hikida T, Watanabe D, Pastan I, Nakanishi S. Impairment of reward-related learning by cholinergic cell ablation in the striatum. *Proc Natl Acad Sci U S A*, 2003;100:7965-7970.
- [14] Mahler SV, Smith KS, Berridge KC. Endocannabinoid hedonic hotspot for sensory pleasure: anandamide in nucleus accumbens shell enhances 'liking' of a sweet reward. *Neuropsychopharmacology*, 2007;32:2267-2278.
- [15] Mark GP, Rada P, Pothos E, Hoebel BG. Effects of feeding and drinking on acetylcholine release in the nucleus accumbens, striatum, and hippocampus of freely behaving rats. *J Neurochem*, 1992;58:2269-2274.
- [16] Meredith GE, Baldo BA, Andrezjewski ME, Kelley AE. The structural basis for mapping behavior onto the ventral striatum and its subdivisions. *Brain Struct Funct*, 2008;213:17-27.
- [17] Murakami Y, Matsumoto K, Ohta H, Watanabe H. Effects of oxotremorine and pilocarpine on striatal acetylcholine release as studied by brain dialysis in anesthetized rats. *Gen Pharmacol*, 1996;27:833-836.

- [18] Paxinos G, Watson C. *The Rat Brain in Stereotaxic Coordinates*. San Diego: Academic Press, 1998.
- [19] Pratt WE, Kelley AE. Nucleus accumbens acetylcholine regulates appetitive learning and motivation for food via activation of muscarinic receptors. *Behav Neurosci*, 2004;118:730-739.
- [20] Pratt WE, Kelley AE. Striatal muscarinic receptor antagonism reduces 24-h food intake in association with decreased preproenkephalin gene expression. *Eur J Neurosci*, 2005;22:3229-3240.
- [21] Pratt WE, Spencer RC, Kelley AE. Muscarinic receptor antagonism of the nucleus accumbens core causes avoidance to flavor and spatial cues. *Behav Neurosci*, 2007;121:1215-1223.
- [22] Puolivali J, Jakala P, Koivisto E, Riekkinen P, Jr. Oxotremorine suppresses thalamocortical oscillations via thalamic muscarinic acetylcholine receptors. *Psychopharmacology (Berl)*, 1998;140:285-292.
- [23] Ragozzino ME, Mohler EG, Prior M, Palencia CA, Rozman S. Acetylcholine activity in selective striatal regions supports behavioral flexibility. *Neurobiol Learn Mem*, in press.
- [24] Ramirez-Lugo L, Miranda MI, Escobar ML, Espinosa E, Bermudez-Rattoni F. The role of cortical cholinergic pre- and post-synaptic receptors in taste memory formation. *Neurobiol Learn Mem*, 2003;79:184-193.
- [25] 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.
- [26] 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.

[27] Yan Z, Surmeier DJ. Muscarinic (m2/m4) receptors reduce N- and P-type Ca²⁺ currents in rat neostriatal cholinergic interneurons through a fast, membrane-delimited, G-protein pathway. *J Neurosci*, 1996;16:2592-2604.

[28] Zhou FM, Wilson CJ, Dani JA. Cholinergic interneuron characteristics and nicotinic properties in the striatum. *J Neurobiol*, 2002;53:590-605.

Figure Captions

Figure 1. Nucleus accumbens (Acb) muscarinic receptor blockade with scopolamine methyl bromide dose-dependently decreases intake of rat chow during a 2-hr feeding session. Left panels depict the location of the injector tips and a representative photomicrograph for the rats included in the analysis (N = 8; adapted from [18]). Feeding on rat chow was significantly reduced on days that rats received 1.0 and 10.0 micrograms/side of scopolamine injected into the Acb (top right panel); water intake was also reduced. Muscarinic receptor antagonism also increased locomotor activity at the high dose, as assessed by ambulation across the chamber and rearing activity (bottom panels). Reduced consumption was not the result of avoidance of the food; scopolamine dose-dependently increased approaches to the food intake monitor. * $p < 0.05$, ** $p < 0.01$ for drug effects; \times $p < .01$ for drug x time interaction effect; D indicates a significant difference from vehicle infusion according to post-hoc tests (see text).

Figure 2. Antagonism of Acb M2 receptors with AFDX-116 does not affect ingestion of rat chow during a 2-hr feeding session. Left panels depict the location and provide a representative photomicrograph of the injector tips for the animals included in the analyses (N = 7). Neither food or water intake (top right panels), nor locomotor or food approach measures (bottom right panels) were affected by intra-accumbens AFDX-116.

Figure 3. Acb treatment with the M2-preferring muscarinic agonist oxotremorine sesquifumarate decreases rat chow intake during a 2-hr feeding session. Left panels depict locations of injection and provide a representative photomicrograph for rats included in analysis (N = 10). 10 micrograms of Oxo-S delayed feeding onset and reduced overall intake of food and water across the 2-hr period. Oxo-S also significantly reduced ambulation and rearing measures, although food approaches were unaffected by the drug treatment. Statistical symbols as in Fig. 1.

Effects of Nucleus Accumbens Scopolamine on Feeding and Activity

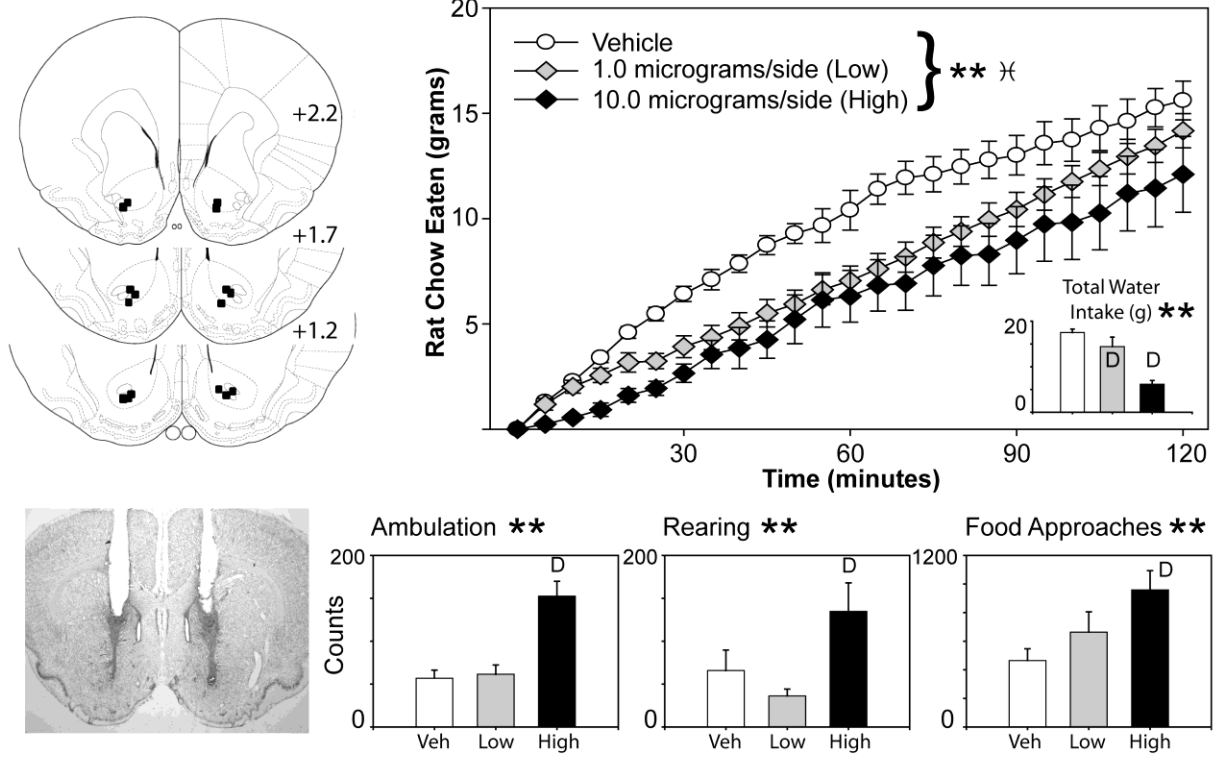


Figure 1

Effects of Nucleus Accumbens AFDX-116 on Feeding and Activity

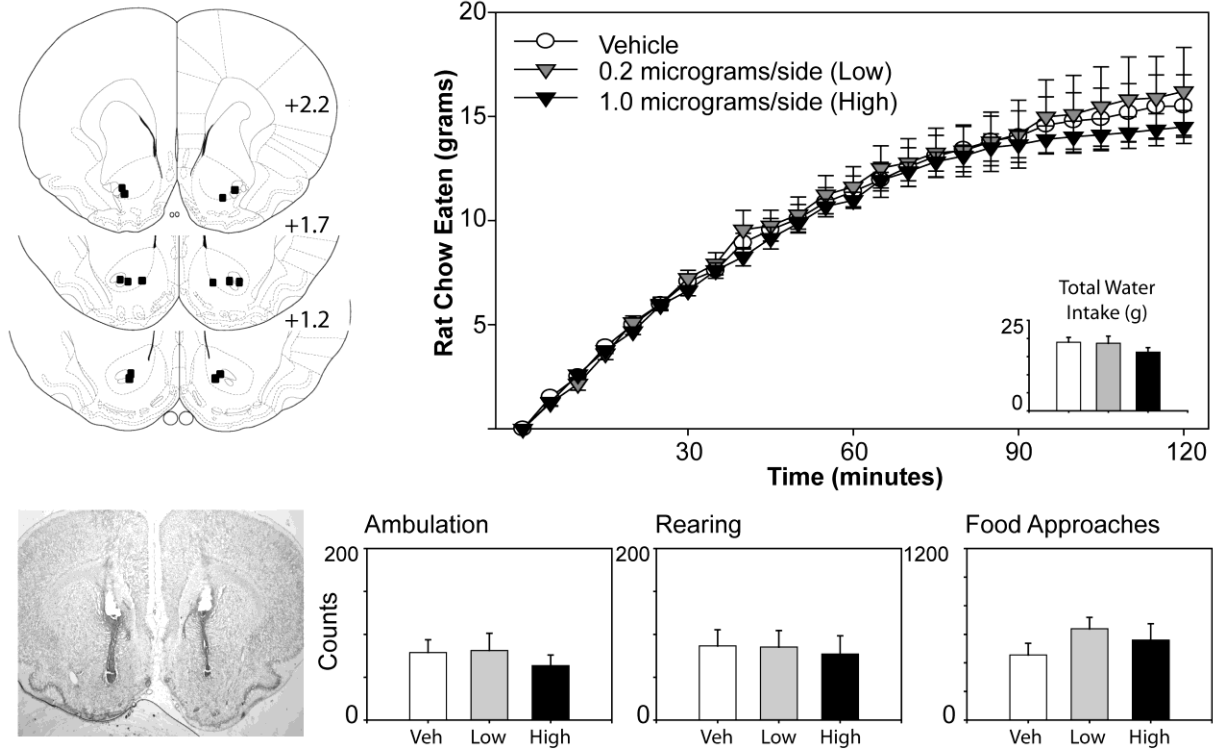


Figure 2

Effects of Nucleus Accumbens Oxotremorinine Sesquifumarate on Feeding and Activity

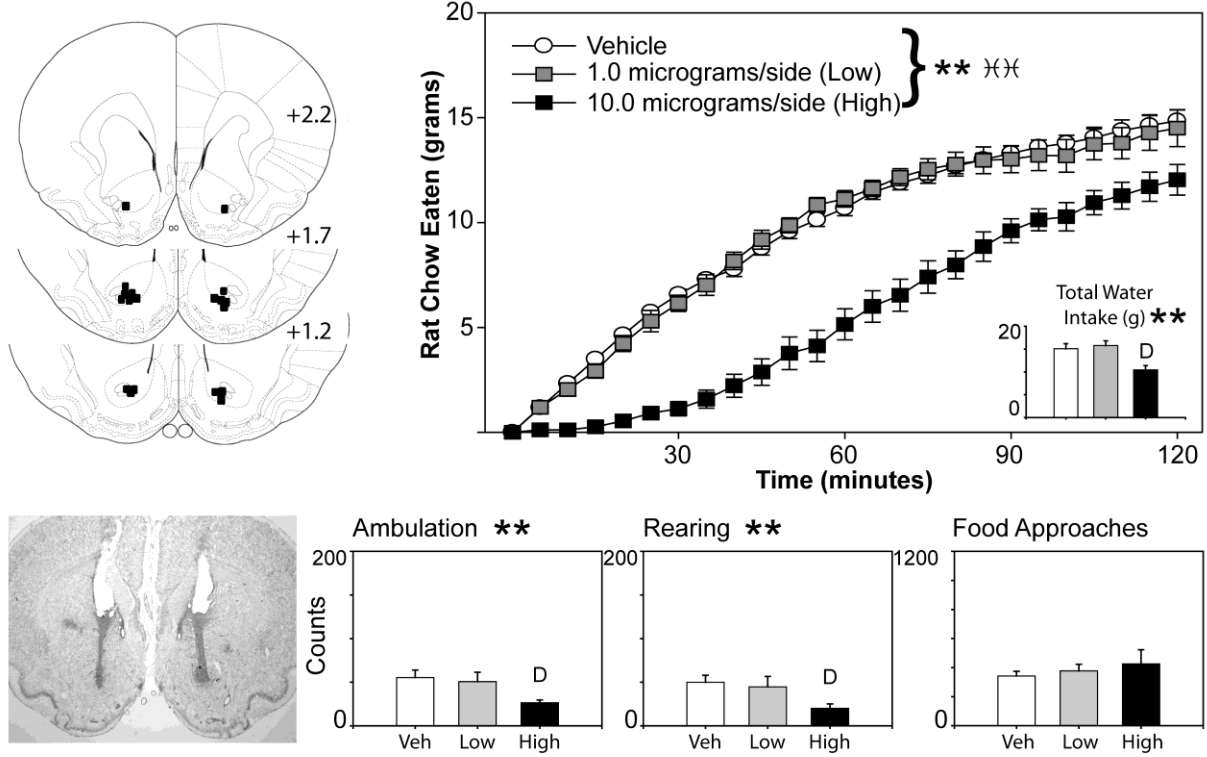


Figure 3