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Muscarinic Receptor Antagonism of the Nucleus Accumbens Core
Causes Avoidance to Flavor and Spatial Cues

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Abstract

Pharmacological blockade of muscarinic receptors in the nucleus accumbens reduces food intake and instrumental behaviors that are reinforced by food delivery. Nucleus accumbens muscarinic antagonism may specifically suppress the hedonic or reinforcing effects of food, thus blocking its capacity to direct behavior. Alternatively, muscarinic receptor blockade may cause a negative hedonic state that interferes with appetitive learning and food intake. In these experiments, rats received infusions of scopolamine methyl bromide (10 $\mu\text{g}/0.5 \mu\text{l}$) into the nucleus accumbens core, following exposure to a novel flavor of liquid diet (Experiment 1) or prior to being placed into a place preference apparatus (Experiment 2). In both experiments, nucleus accumbens muscarinic receptor antagonism caused subsequent avoidance of the paired cue (flavor or spatial location). This effect was specific to cholinergic manipulation; no conditioned taste avoidance was observed after pairing the novel flavor with nucleus accumbens core antagonism of NMDA, D1, or opioid receptors (Experiment 3). These experiments confirm previous reports of a critical role for striatal acetylcholine in modulating goal-directed behaviors, but suggest caution when interpreting behavioral effects of pharmacological manipulation of striatal acetylcholine.

Keywords: Nucleus accumbens, Acetylcholine, Muscarinic Receptors, Food Intake, Appetitive behaviors

Nucleus Accumbens Muscarinic Receptor Antagonism

Causes Avoidance to Flavor and Spatial Cues

The nucleus accumbens is known to be involved in promoting goal-directed behavior based on reinforcement history, including that derived from both natural and drug rewards. Although much of the inquiry regarding the pharmacological mechanisms by which the nucleus accumbens modulates appetitive behavior has focused on the integration of glutamatergic, dopaminergic, and opioid signals onto the ventral striatum's medium spiny output neurons (Dalley et al., 2005; Kelley, 2004; Levine & Billington, 2004; Pecina, Smith, & Berridge, 2006; Salamone, Correa, Mingote, & Weber, 2003; West, Floresco, Charara, Rosenkranz, & Grace, 2003), recent evidence has suggested a powerful role for acetylcholine in modulating striatal function. For instance, blockade of cholinergic receptors of the striatum reduce the plasticity exhibited in the striatal slice, and lesions of striatal cholinergic interneurons impair the learning of appetitively-reinforced tasks (Calabresi, Centonze, Gubellini, Pisani, & Bernardi, 1998; Centonze, Gubellini, Bernardi, & Calabresi, 1999; Kitabatake, Hikida, Watanabe, Pastan, & Nakanishi, 2003; Partridge, Apparsundaram, Gerhardt, Ronesi, & Lovinger, 2002).

The high level of cholinergic tone within the striatum arises from a relatively small population (~2% of striatum) of interneurons that release acetylcholine (Zhou, Wilson, & Dani, 2002). Acetylcholine is known to presynaptically regulate neurotransmitter output from both glutamatergic and dopaminergic terminals arriving from extrinsic inputs to the striatum (Hersch, Gutekunst, Rees, Heilman, & Levey, 1994; Rawls, McGinty, & Terrian, 1999; Wonnacott, Kaiser, Mogg, Soliakov, & Jones, 2000). Post-synaptic muscarinic receptors are also found on medium spiny output neurons, and are fundamentally important

for promoting neuronal plasticity in striatal slice preparations (Calabresi, Centonze, Gubellini, & Bernardi, 1999; Calabresi, Centonze, Gubellini, Pisani, & Bernardi, 1998; Centonze, Gubellini, Bernardi, & Calabresi, 1999; Meredith & Chang, 1994). Activation of muscarinic receptors also modulates the levels of striatal preproenkephalin gene expression in normal and drug-treated rats (Nisenbaum, Kitai, & Gerfen, 1994; Pratt & Kelley, 2005; Wang & McGinty, 1996, 1997; Weisinger, DeCristofaro, & LaGamma, 1992; Weisinger, Zinder, DeCristofaro, & LaGamma, 1998). Enkephalin signaling within the nucleus accumbens has been implicated in promoting the intake of highly palatable foods (Kelley, Baldo, Pratt, & Will, 2005; MacDonald, Billington, & Levine, 2004; Pecina & Berridge, 2005); acetylcholine may play an important role in modulating food-directed behavior that is directed by nucleus accumbens opioid receptor stimulation (Kelley, Baldo, & Pratt, 2005).

Lesions of striatal cholinergic neurons have been shown to affect both food intake and metabolism in chronically tested rats (Hajnal, Szekely, Galosi, & Lenard, 2000), consistent with a hypothesized role of the striatum (and particularly the nucleus accumbens), in translating learned and homeostatic signals into adaptive behavior (Kelley, Baldo, Pratt, & Will, 2005; Mogenson, Jones, & Yim, 1980). Pharmacological blockade of nucleus accumbens muscarinic receptors has been shown to have a broad impact on food consumption. Hungry rats reduce their intake of freely available sucrose pellets immediately following such treatment (Pratt & Kelley, 2004). Additionally, rats that have been accustomed to 2-hr daily access to a highly palatable fat diet (in addition to ad libitum chow access) reduce their consumption of the fat diet following nucleus accumbens muscarinic blockade (Will, Pratt, & Kelley, 2006). Scopolamine infusions into either the nucleus accumbens core or the anterior dorsal striatum result in a dramatic reduction in 24-hour food

consumption in rats fed rat chow *ad libitum* (Pratt & Kelley, 2005). These feeding effects do not appear to be due to an overt reduction in gross locomotor behavior; in fact, rats increase their overall locomotion for a short time following the infusion of the drug. Rats that have learned to bar press for sucrose pellets also remain capable of lever pressing and retrieving food pellets as efficiently as untreated animals (Pratt & Kelley, 2004, 2005). Thus, muscarinic receptor antagonism of the striatum causes both acute and long-term (up to 24 hours) reductions in food consumption.

Although such evidence suggests an important role for striatal cholinergic interneurons in the processing of natural rewards and their impact on behavior, the nature of the motivational change that occurs following muscarinic receptor antagonism of the nucleus accumbens remains to be determined. Cholinergic signaling within the nucleus accumbens may play a critical role in linking behavioral outcomes to their consequences, specifically by supporting the striatal plasticity that has been argued to be fundamental in modifying instrumental behavior (Kelley, 2004; Mizumori, Pratt, & Ragozzino, 1999). Alternatively, it is possible that muscarinic receptor antagonism of the nucleus accumbens may result in a general negative hedonic state that directly and globally interferes with the ability of the reinforcer (in our case, food) to modify behavior. These possibilities are not mutually exclusive; however, determining the extent to which the latter may be the case has substantial implications with regard to the feasibility of striatally-targeted cholinergic agents as possible targets for drug treatments of obesity and/or drug addiction. This is particularly relevant as evidence suggests that cholinergic manipulations of striatum may also play an important role in drug reward (Crespo, Sturm, Saria, & Zernig, 2006; Hikida et al., 2001; Hikida, Kitabatake, Pastan, & Nakanishi, 2003).

The current experiments specifically sought to determine whether the blockade of nucleus accumbens muscarinic receptors would result in the development of a negative hedonic state that could be associated with flavor or spatial cues. Individual groups of rats were treated with intra-accumbens infusions of scopolamine methyl bromide following exposure to a novel flavor (in a conditioned taste avoidance paradigm; Experiment 1), or immediately preceding placement into one chamber of a Y-maze (in a conditioned place preference paradigm; Experiment 2). Furthermore, to be certain that the effects seen following muscarinic receptor antagonism could not be interpreted as a non-specific consequence of pharmacological manipulation of the nucleus accumbens, we further tested whether antagonism of NMDA, D1, and opioid receptors would result in taste avoidance, at drug doses which have been previously shown to affect appetitive behaviors (Experiment 3).

Method

Subjects and Housing

A total of 51 male Sprague-Dawley rats (Harlan, Madison, WI) were dually housed in clear acrylic cages (in one case a single cage housed three rats). The colony room was maintained at approximately 21°C on a 12-hr light-dark cycle (lights on at 7 am). During acclimation to the laboratory prior to surgery, and during surgical recovery, standard rat chow (Harlan, Madison, WI) and water were available ad libitum. All procedures and animal care were performed in accordance with National Institute of Health guidelines for the ethical use of animals in research, following approval from the University of Wisconsin-Madison Medical School Animal Care and Use Committee.

Surgery

Rats were anesthetized with a Ketamine-Xylazine cocktail (100 mg/kg-10mg/kg). Standard aseptic procedures were used to implant indwelling stainless steel guide cannulas (23 gauge) bilaterally above the nucleus accumbens core (flat skull; 1.3 mm anterior and 1.7 mm lateral to bregma, 5.1 mm ventral to the skull surface). Guide cannulas were affixed to the skull with the use of screws and dental acrylic, and stylets were placed within the cannulas to prevent obstruction. Rats recovered for at least 7 days prior to food restriction and/or behavioral testing.

Drugs

Scopolamine methyl bromide (10 µg/0.5 µl; Sigma), SCH 23390 (1 µg/0.5 µl; Sigma), 2-amino-5-phosphonopentanoic acid (AP-5; 1 µg/0.5 µl; Sigma), and naltrexone (20 µg/0.5 µl; Sigma) were dissolved in sterile saline for these experiments. Scopolamine and naltrexone solutions were mixed immediately prior to the experiments and stored in 60 µl aliquots at 4°C until used. SCH 23390 and AP-5 were prepared and frozen in 60 µl aliquots at -20° until used. All doses were chosen on the basis of data in the literature and from experiments in this laboratory showing clear behavioral effects in various learning or food intake paradigms (Baldo, Sadeghian, Basso, & Kelley, 2002; Di Ciano, Cardinal, Cowell, Little, & Everitt, 2001; Kelley, Bless, & Swanson, 1996; Pratt & Kelley, 2004).

Conditioned taste avoidance procedure (Experiments 1 & 3)

To maintain consistency with our earlier studies examining cholinergic influences on food motivation, we developed a hunger-motivated experimental design for conditioned taste avoidance that is comparable to traditional thirst-driven two-bottle choice tasks for conditioned taste avoidance. The primary differences between this procedure and commonly utilized avoidance procedures were that the rats were motivated to drink the Ensure® by food

restriction (rather than water deprivation), and that multiple pairings were made between the novel flavor and the treatment to assure adequate opportunity to learn a possible association between the flavor and any potential negative hedonic aspect of the drug treatment.

Following recovery from surgery, daily food availability was restricted to gradually reduce the body weight of each rat to 85-90% of free feeding levels. Behavioral testing was conducted in a room distinct from the animal colony, in individual hanging wire cages that measured 25 x 20 x 20 cm. All experiments were conducted at or shortly after 1 p.m.

For the first phase of the experiment, rats were individually placed into a wire cage and given 30 min of free access to Vanilla Ensure® for five sessions, each separated by at least 24 hours. The amount of liquid diet consumed was measured to the closest ml. On experimental days 3-5, the rats were habituated to microinfusion procedures following liquid diet exposure. On days 3 and 4, mock infusions consisted of lowering 10 millimeter injection cannula flush to the end of the guide cannula; on day 5, a final mock injection utilized a cannula that was lowered to the targeted infusion site, 2.5 mm below the end of the guide cannulas. Rats were gently handled with the mock cannula in place for 2 min 33 sec.

On days 6, 7, and 8 of experimental testing, the liquid diet was changed to Chocolate-flavored Ensure®. Following 30 min access, rats were removed from the wire cages and received intra-accumbens injections of drug or vehicle. For experimental vehicle and drug infusions, microinfusion cannulas (30 gauge) were lowered into the nucleus accumbens. These cannulas were connected to a syringe via polyethylene tubing, 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 (infusion time: 1.33 min). The injection cannulas were removed one minute later, to allow for drug diffusion away from the injection site, and the wire stylets

were replaced. All drug infusions were completed within half an hour following exposure to the chocolate-flavored diet. Individual rats received three experimental infusion sessions, each separated by at least 48 hr. For experiment 1, the drug treatment group (N = 8) was infused with 10 µg/0.5 µl/side scopolamine into the nucleus accumbens on days 6-8 of the experiment. The vehicle group (N = 8) received 0.5 µl/side of sterile physiological saline. For experiment 3, the treatment groups received intra-accumbens infusions of 1.0 µg/0.5 µl/side SCH 23390 (N = 8), 1.0 µg/0.5 µl/side AP-5 (N = 7), or 20 µg/0.5 µl/side naltrexone (N = 7), and were compared to a separate vehicle control group (N = 5).

Four days after the final chocolate-treatment pairing for each experiment, rats were returned to the testing chambers for a final test session, and offered simultaneous free access to both vanilla- and chocolate-flavored Ensure® for 30 minutes. The amount of vanilla and chocolate diet consumed by each rat was recorded to the nearest ml. Mixed design ANOVAs were used to compare the pattern of liquid diet intake between experimental groups during initial exposure of the Vanilla (days 1-5; within-subject repeated measure) diet, as well as across the three days of Chocolate-treatment pairing (Days 6-8; within-subject repeated measure). Independent group t-tests were used to compare the total amount of Chocolate diet consumed on the test day by each group, as well as the proportion of overall intake on the test day that was chocolate (chocolate consumed/total consumption of both Vanilla and Chocolate diets).

Conditioned Place Preference (Experiment 2).

For the conditioned place preference experiment, eight rats were maintained on rat chow with *ad libitum* access to both food and water. Rats were acclimated to the injection procedure for two days, beginning no less than one week after surgery. On the first

habituation day, a cannula was lowered for the length of the guide and left in place for 2 min 33 seconds. On the second day, an injection cannula was lowered to the injection site and vehicle was infused (0.5 μ l/side, as above). Rats were then immediately placed into a custom-built three-arm place preference apparatus. The maze was constructed from black Plexiglas, with three corridors (12.7 x 12.7 x 79cm) radiating 120 degrees apart from a center equilateral triangle (12.7 cm per side). Each segment (including the center) was covered with removable clear Plexiglas during the time the animals were on the maze. The floor of each of the three arms was surfaced with a different textured rectangle of acrylic cut to size from industrial florescent lighting covers. Each runway was furthermore distinguished with a 12.7 x 12.7 cm printed pattern placed behind clear Plexiglas at the end of each arm. The patterns consisted of 1 cm black and white stripes placed vertically, horizontally, or in 1 cm squares placed in a checkered pattern. Unique extra-maze cues were hung at various locations about the walls of the room (4 x 4 m). Between each individual session, the maze was wiped down with 50% alc/vol. Prior to the current experiment, in separate groups of animals, the maze arms were tested to make certain that no arm was significantly preferred over the others.

During initial exposure to the maze, rats were placed in the center triangle and allowed to explore the entire maze for 15 minutes before being removed. Each session was recorded using a mini-DV camcorder placed 2 meters above the center of the maze. The resulting videos were analyzed using behavioral analysis programs contained in TopScan Suite by Cleversys (Reston, VA) to determine the length of time 80% or more of the rat's body was in each arm during this preconditioning phase. Using the results from this initial exposure, the experimenter identified the two arms that each individual rat spent the most

equivalent amount of time in. One was randomly assigned as the “drug” arm, and the other the “vehicle” arm.

On each of six treatment days, separated by at least 48 hours, rats were given intra-accumbens infusions of either vehicle or scopolamine (10 µg/0.5 µl/side) and immediately placed in and restricted to the designated arm for 20 minutes (three times for each treatment). 48 hours after the last treatment, each rat was placed back in the maze, and allowed to explore the entire apparatus for 15 min. The TopScan suite was used to quantify the amount of time the animals had 80% or more of their body mass within each arm. T-tests were used to compare the time spent in the vehicle-paired arm with the time spent in the drug-paired arm during the final session.

Histology

Once the experiments were complete, rats were deeply anesthetized with sodium pentobarbital and perfused through the heart with a 0.9% buffered NaCl solution, followed by 10.0% formalin. Brains were removed and allowed to sink in 10.0% sucrose formalin. The brains were then frozen and sliced into 60-µm sections with a cryostat. Sections were stained with cresyl violet. The tips of the cannulas were confirmed by light microscopy and charted in reference to an atlas (Paxinos & Watson, 1998). Representative histological placements are shown in Figure 1.

Results

Experiment 1

The purpose of the first experiment was to test whether the pairing of a novel flavor with muscarinic receptor blockade of the nucleus accumbens (10 µg scopolamine/0.5 µl/side) would result in conditioned taste avoidance. Both the saline and drug-treated groups

exhibited a significant increase in consumption across the first five days of vanilla Ensure® exposure, consistent with known patterns of neophobia in rats consuming novel diets ($F_{4,56} = 94.985$, $p < .001$). As can be seen in Figure 2A, both groups increased their daily intake of the diet by equivalent amounts across the first five days of the experiment (main effect of group: $F_{1,14} = .038$, $p = .85$; interaction of Group X Day: $F_{4,56} = 1.389$, $p = .25$).

The two groups continued to consume similar amounts of the liquid diet upon the switch to the chocolate-flavored Ensure® during treatment days (Figure 2A). During the course of the three treatment sessions, there was no difference in diet consumption between the groups ($F_{1,14} = 1.133$, $p = .31$), no effect across days ($F_{2,28} = 2.410$, $p = .11$), and no treatment x day interaction ($F_{2,28} = 2.410$, $p = .11$).

On the test day, with both flavored diets available, rats that had experienced pairings of the chocolate flavor with nucleus accumbens muscarinic receptor antagonism showed a marked decrease in the amount of chocolate diet consumed, relative to the control animals (Figure 2B). This was evident both in the absolute amount of chocolate Ensure® consumed ($t_{14} = 2.91$, $p = .01$), and in the proportion of diet intake on the final day that was chocolate flavored ($t_{14} = 3.127$, $p = .007$).

Experiment 2

The results of Experiment 1 suggest that muscarinic receptor blockade of the nucleus accumbens results in a negative hedonic state that can be associable with food cues. However, it has been previously shown that many drugs of abuse that are self-administered and that result in conditioned place preference to spatial cues (presumably due to reinforcing properties of the drug) can also cause conditioned taste avoidance (Parker, 2003). The purpose of Experiment 2 was to determine if the hedonic state elicited by nucleus accumbens

muscarinic receptor blockade (10 µg scopolamine/0.5 µl/side) would also be associable to spatial cues, and if so, whether the pattern would be a preference or an avoidance response.

Figure 3A shows that on the test day rats spent less time in the arm paired with muscarinic antagonism of the nucleus accumbens (Mean = 125.9 sec) than in the vehicle-paired arm (Mean = 194.6 sec; $t_{14} = 2.14$, $p = .05$). To be certain of the reliability of the measure, two days later the rats were returned to the maze and granted free access to the saline- and drug-paired arms, but not the third “unpaired” arm. When the rats’ choices were limited to the treatment arms, they showed a stronger, more significant avoidance response to the drug-paired arm ($T_{14} = 4.49$, $p < .001$; Figure 3B). On average, each rat spent 359.2 sec in the vehicle-paired arm, and 172.8 seconds in the drug-paired arm.

Experiment 3

Experiments 1 & 2 suggest that nucleus accumbens muscarinic receptors play an important role in modulating the hedonic state of the rat. The purpose of experiment 3 was determine if the effect is derived from cholinergic mechanisms *per se*, or whether such effects might be derived from any pharmacological manipulation of the nucleus accumbens that has been shown to reduce appetitive behaviors. Therefore, the third experiment tested whether pharmacological blockade of nucleus accumbens NMDA, D1, or opioid receptors would also lead to conditioned taste avoidance of a novel flavor.

As in Experiment 1, there were no differences between groups in terms of their Vanilla Ensure® consumption across the first five days of the experiment (Figure 4A). All groups increased their consumption during those five days ($F_{4,92} = 112.472$, $p < .001$), but there was no difference in intake between groups ($F_{3,23} = 1.197$, $p = .33$), nor was a day by group interaction observed ($F_{12,92} = .548$, $p = .88$).

All groups continued to consume similar amounts of the liquid diet during the three chocolate-treatment days (Figure 4A). There were no significant differences between the groups ($F_{3,23} = 0.042$, $p = .98$), no effect across days ($F_{2,46} = 1.730$, $p = .19$), and no treatment by day interaction ($F_{6,46} = .579$, $p = .75$).

When both the vanilla and chocolate diets were presented on the test day, all groups drank similar absolute amounts of chocolate ($F_{3,23} = .413$, $p = .75$), as well as similar proportions of chocolate relative to the entire amount of liquid diet consumed ($F_{3,23} = .358$, $p = .78$; Figure 4B).

Discussion

The current experiments demonstrate that muscarinic receptor antagonism of the nucleus accumbens core causes a depression in the general hedonic or motivational state of the rat. Specifically, blocking muscarinic receptors within the nucleus accumbens core after exposure to a novel-flavored (chocolate) liquid diet resulted in a strong conditioned taste avoidance when rats were later given a choice between the drug-paired flavor and a familiar, non-paired flavor. Similarly, pairing a spatial location with scopolamine methyl bromide infusion into the nucleus accumbens core caused conditioned place avoidance. Thus, across two paradigms that test drug-stimulus associations, nucleus accumbens muscarinic antagonism caused substantial, significant avoidance of paired cues. In contrast, conditioned taste avoidance was not observed following antagonism of glutamate NMDA, dopamine D1, or opioid receptors at drug doses that have consistently yielded behavioral effects in other appetitive paradigms (Baldo, Sadeghian, Basso, & Kelley, 2002; Di Ciano, Cardinal, Cowell, Little, & Everitt, 2001; Kelley, Bless, & Swanson, 1996). This suggests that the hedonic

effect was specific to the blockade of cholinergic muscarinic receptors, and is not merely a consequence of pharmacological manipulation of the nucleus accumbens core.

As outlined in the introduction, previous studies have shown that muscarinic antagonism of the nucleus accumbens, as well as lesions targeting the cholinergic interneurons of the ventral striatum, alter food intake and impair the learning and performance of appetitive behaviors that are motivated by food (Hajnal, Szekely, Galosi, & Lenard, 2000; Hikida, Kitabatake, Pastan, & Nakanishi, 2003; Pratt & Kelley, 2004, 2005). The reduction of ventral striatal cholinergic tone may either specifically block the reinforcing attributes of food on behavior, or cause a general hedonic suppression that interferes with the ability of food to direct behavior. Although these possibilities are not mutually exclusive, the present experiments suggest that these effects are mediated, at least in part, by the latter. Acute muscarinic receptor antagonism of the nucleus accumbens core caused a general negative hedonic state within the rat that was associable to both flavor and non-food cues. Such a state is likely to interfere with the ability of the food (and possibly other reinforcers) to subsequently direct the animal's behavior. With regard to our own experimental work, a general hedonic depression following nucleus accumbens muscarinic receptor antagonism is sufficient to explain impaired learning to lever press for food reward, reduced lever-pressing performance following the learning of the response, reduced short (15 min) and long term (24 hours) food intake, as well as the conditioned taste avoidance and place avoidance observed in the current experiments (Pratt & Kelley, 2004, 2005).

Nucleus accumbens acetylcholine, reward, and conditioned taste avoidance

Conditioned taste avoidance alone is not sufficient to argue that a drug produces a negative, rather than a positive, affective response in a rat. Many drugs of abuse cause taste

avoidance when paired with a novel flavor, even though they support place preferences when paired with spatial cues (for a review, see Parker, 2003). One theoretical explanation for the contrasting effects in taste avoidance and place preference paradigms has been the attribution of both positive and negative states following drug intake that are differentially associable with spatial and flavor cues (Goudie, 1979; Hunt & Amit, 1987). Another account is that the conditioned taste aversion to drugs of abuse may result from reward contrast effects between the flavor and the upcoming, expected drug infusion (Grigson, 1997; Grigson & Freet, 2000). Regardless of the correct interpretation of such studies, nucleus accumbens muscarinic blockade did not vary across the two paradigms, suggesting that the overall result of such treatment is one of negative hedonic impact. Acetylcholine may then serve an important facilitative role in the rewarding functions of the nucleus accumbens and other reward circuitry. This suggestion is supported by the work of Ikemoto and colleagues (Ikemoto, Glazier, Murphy, & McBride, 1998; Ikemoto & Wise, 2002, 2004), who have shown that infusion of the acetylcholine agonist carbachol into either the nucleus accumbens or the interconnected ventral tegmentum supports active self-administration in the rat.

Nucleus accumbens muscarinic receptors have recently been implicated in the formation of taste memory. Of particular interest to the current experiment, Ramirez-Lugo and colleagues have demonstrated that muscarinic antagonism of the nucleus accumbens shell (60 μ g scopolamine hydrobromide/1 μ l/side) *prior* to a flavor (saccharin) – illness (LiCl) pairing reduces the avoidance of the saccharin solution during intake tests performed four hours or three days later (Ramirez-Lugo, Zavala-Vega, & Bermudez-Rattoni, 2006). In a separate group of thirst-motivated rats, they also demonstrated that shell infusions of scopolamine prior to initial saccharin exposure reduced the normal attenuation of neophobia

that rats exhibit upon the second presentation of a novel flavor. This latter finding has particular relevance to the current study, as it could be argued that the conditioned taste avoidance observed on the test day occurred because the drug treatment into the nucleus accumbens core stopped an attenuation of neophobia to the novel chocolate flavor. This interpretation is unlikely for several reasons. First, in the same study, Ramirez-Lugo and colleagues did not report an attenuation of neophobia to the saccharin solution when the drug was injected directly into nucleus accumbens core, rather than the shell. Secondly, in the current experiments there was no obvious neophobic response to the chocolate flavor upon the first presentation of the chocolate diet (see Figure 2). The composition of the vanilla and chocolate diets consisted of the same base ingredients, which offer flavor components that likely became familiar to the animals over the first five days of the experiment. The switch to the chocolate flavored diet may not have been as pronounced as a switch from water to saccharin solution in the Ramirez-Lugo et al. study. This also may explain why the conditioned taste avoidance was not detectable until the choice test, as the development of the avoidance response may have been slowed or masked by the familiar flavor components of the diet. Nonetheless, the dramatic avoidance of the chocolate-flavored diet during the final two-bottle test demonstrated the rats' abilities to discriminate the two flavors, as well as their relative incentive value.

Ramirez-Lugo et al. (2006) also reported that nucleus accumbens core infusions of scopolamine hydrobromide (30 $\mu\text{g}/0.5 \mu\text{l}/\text{side}$) did not block conditioned taste avoidance in their thirst-motivated paradigm, when given either before or after a taste-illness pairing. The authors did not explicitly test whether pairing a novel flavor with intra-accumbens infusions might itself result in taste avoidance in core or shell. Nevertheless, Ramirez-Lugo and

colleagues demonstrated that, in addition to any hedonic effects that result from striatal muscarinic antagonism, taste memory formation is dependent upon muscarinic activation of the medial nucleus accumbens. That nucleus accumbens muscarinic receptors support a role in learning is consistent with work from other laboratories studying the role of acetylcholine on neural plasticity and behavior within the dorsal striatum (Apicella, Ravel, Sardo, & Legallet, 1998; Calabresi, Centonze, Gubellini, & Bernardi, 1999; Calabresi, Centonze, Gubellini, Pisani, & Bernardi, 1998; Centonze, Gubellini, Bernardi, & Calabresi, 1999; Chang & Gold, 2003; Matsumoto, Minamimoto, Graybiel, & Kimura, 2001; Ragozzino, Jih, & Tzavos, 2002; Sardo, Ravel, Legallet, & Apicella, 2000).

Nucleus accumbens acetylcholine and food intake

The present data suggest an important role for cholinergic outflow within the nucleus accumbens core in modulating hedonic responses to food. Striatal cholinergic interneurons receive their most prominent input from midline thalamic regions which themselves have a substantial projection from lateral hypothalamic regions involved in the homeostatic regulation of food intake (Baldo, Daniel, Berridge, & Kelley, 2003; Lapper & Bolam, 1992; Meredith & Wouterlood, 1990). Thus, it seems likely that cholinergic output of these neurons may be modulated by homeostatic information arising from hypothalamic regions. Mark and colleagues have demonstrated that striatal cholinergic output significantly increases during the course of a meal in hungry rats (Mark, Rada, Pothos, & Hoebel, 1992). The authors have suggested that *increased* nucleus accumbens acetylcholine reflects a component of satiety, an argument strengthened by the recent demonstration that sham-feeding (which eliminates the satiety response to food intake) blocks meal-induced increases in nucleus accumbens acetylcholine output (Avena, Rada, Moise, & Hoebel, 2006).

Interestingly, presenting a flavor cue that predicts lithium chloride illness also results in a rise of striatal acetylcholine output (Mark, Weinberg, Rada, & Hoebel, 1995), even as the rats reject the solution. Although such data may seem at odds with the current results, as well as previous reports in which muscarinic cholinergic receptor blockade reduces food intake, scopolamine hydrobromide infusion into the accumbens increases striatal acetylcholine output, presumably by blocking the M2 autoreceptor (Chau, Rada, Kosloff, Taylor, & Hoebel, 2001). To our knowledge, the only other study that has reported a taste avoidance following a pharmacological treatment of the ventral striatum was reported by Shoaib and Stolerman (1995; although see Reynolds & Berridge, 2002), in which a taste avoidance was reported following nucleus accumbens nicotinic acetylcholine receptor stimulation. Thus, while it may seem most parsimonious to attribute intra-accumbens scopolamine's observed effects to muscarinic blockade, they could also be due to compensatory *increases* in extracellular acetylcholine. Future work will be needed to address this issue, and to delineate the specificity of individual muscarinic receptors on mechanisms underlying food intake and satiety mechanisms.

Conclusions

Antagonism of nucleus accumbens core muscarinic receptors, at doses that have been shown to effectively reduce food consumption and impair instrumental responding for food reward, also induces a negative hedonic or emotional state that can be associated with both flavor and spatial cues. In contrast, nucleus accumbens antagonism of glutamate NMDA receptors, dopamine D1 receptors, or opioid receptors does *not* result in conditioned taste avoidance, even in drug doses shown to reduce instrumental learning for food reward (for NMDA and D1 receptor antagonism) or reduce palatable food intake (opioid receptor

antagonism). These experiments confirm previous reports of a critical role for nucleus accumbens acetylcholine in modulating goal-directed behaviors, but suggest the use of caution in interpreting the behavioral effects of muscarinic acetylcholine antagonism within the ventral striatum. Specifically, in addition to direct effects that inhibitory cholinergic manipulations may have on striatal plasticity and goal-directed behavior, the current experiments suggest that cholinergic interventions of the striatum have motivational consequences that are yet to be fully characterized.

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Figure Captions

Figure 1. Representative histology for these experiments. The top panel shows the location of the tip of the infusion cannulas for the rats in Experiment 1. Filled circles represent cannula placement in the scopolamine treatment group; + symbols denote the locations of infusions for the vehicle control group. The bottom photomicrograph shows a representative cannula placement within the nucleus accumbens, in this case from one of the rats in Experiment 2. The top panels were adapted from *The Rat Brain in Stereotaxic Coordinates*, 4th ed., G. Paxinos and C. Watson, Figures 10, 11, and 13, Copyright 1998.

Figure 2. Daily liquid diet intake for Experiment 1. Rats in both groups consumed equivalent amounts of the vanilla- and chocolate-flavored diet offered during the first eight days of the experiment (A). However, rats that had received intra-accumbens scopolamine infusions following chocolate exposure avoided the chocolate-flavored diet on the test day, when both diets were available (B). * $p < .05$.

Figure 3. Nucleus accumbens muscarinic blockade results in conditioned place avoidance. Several days following the last of three three exposures each of vehicle-place pairing and scopolamine-place pairing (top panel), rats chose to spend less time in the scopolamine-paired arm relative to the saline paired arm during a test exposure to the entire maze (A). In a subsequent test, when animals were permitted access only to the paired arms, this effect is even more robust (B). * $p = .05$, ** $p < .01$.

Figure 4. Daily liquid diet intake for Experiment 3. Rats in all groups consumed equivalent amounts of the vanilla- and chocolate-flavored diet offered during the first eight days of the experiment (A) None of the treatment groups significantly differed in their consumption of the chocolate diet on the test day (B).

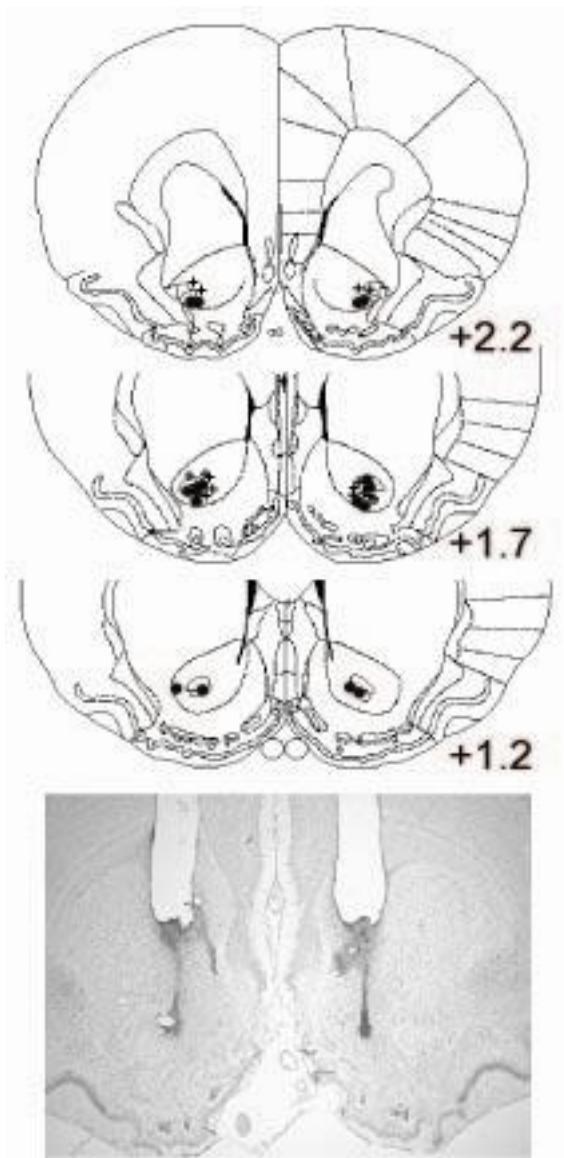


Figure 1

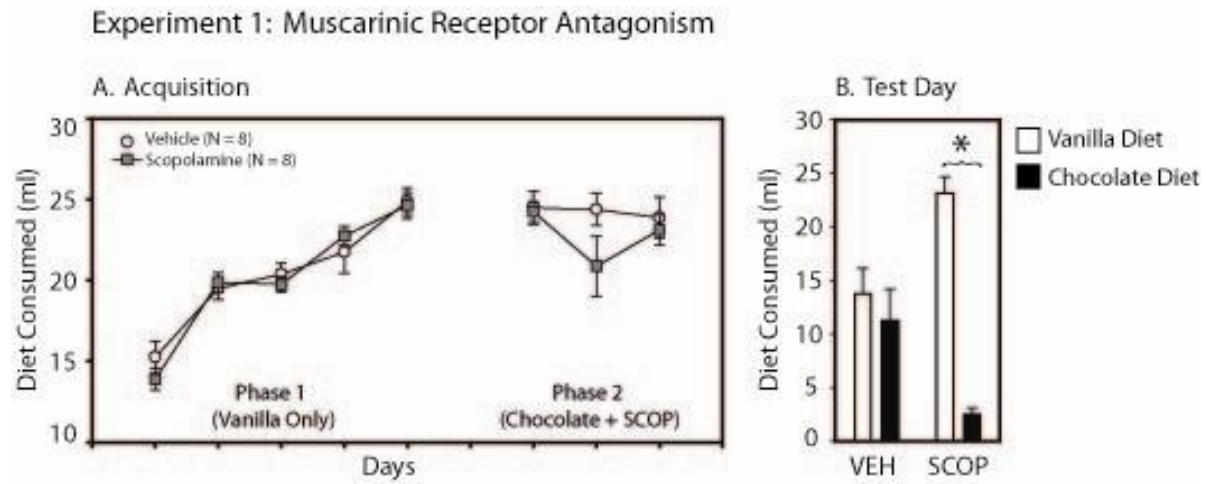
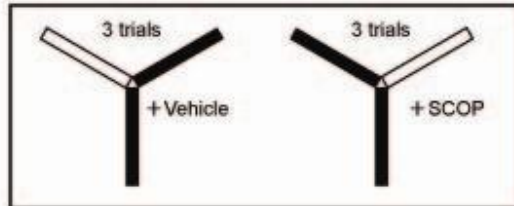
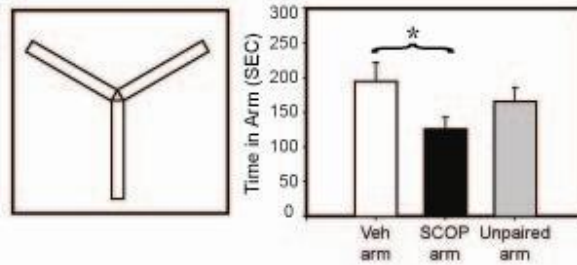


Figure 2

Experiment 2: Acquisition Phase



A. 3-arm test (access to all maze arms)



B. 2-arm test (vehicle- and drug-paired arm access only)

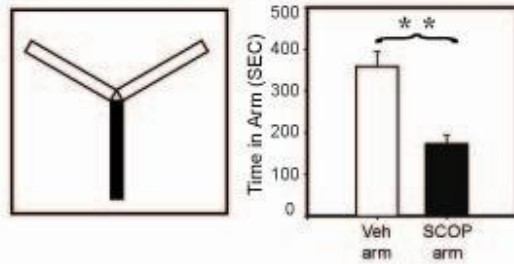


Figure 3

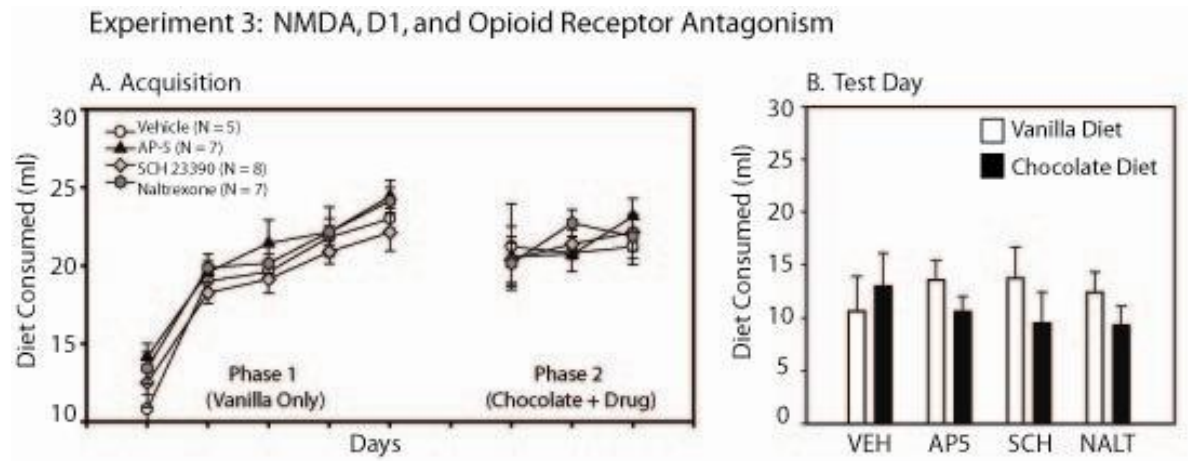


Figure 4