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Overlapping striatal sites mediate scopolamine-induced feeding suppression and mu-opioid-mediated hyperphagia in the rat

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Abstract

Rationale—Intra-striatal infusions of the muscarinic antagonist, scopolamine, markedly suppress feeding; however, the underlying mechanisms are unclear. Recent findings suggest that scopolamine influences opioid-dependent mechanisms of feeding modulation. Robust mu-opioid-mediated feeding responses are obtained in anterior, ventral sectors of the striatum with progressively weaker effects posteriorly and dorsally. One might therefore expect the effects of scopolamine to conform to similar boundaries, but a systematic mapping of scopolamine-induced feeding suppression has not yet been undertaken.

Objective—This study aimed to assess the overlap between the striatal sites mediating scopolamine-induced feeding suppression and mu-opioid-induced hyperphagia.

Methods—Dose–effect functions for scopolamine (0, 1, 5, and 10 μ g) were obtained in the nucleus accumbens (Acb), anterior dorsal striatum (ADS), and posterior dorsal striatum (PDS) in three different groups of rats. In the same subjects, the mu-opioid receptor agonist (*D*-Ala2-*N*-MePhe4, Glyol)-enkephalin (DAMGO; 0.25 μ g) was infused on a separate test day. The dependent variables were food and water intake, ambulation, and rearing.

Results—The greatest dose sensitivity for scopolamine-induced feeding suppression was observed in the Acb. Only the highest dose was effective in the ADS, and no effects were seen in the PDS. Water intake and general motor activity were not altered by scopolamine in any site. DAMGO infusions produced hyperphagia only in the Acb.

Conclusions—These results support a model in which the behavioral effects of muscarinic blockade are limited by the same anatomical constraints that govern mu-opioid receptor-mediated control of feeding. These constraints are likely imposed by the topographic arrangement of feeding-related afferent inputs and efferent projections of the striatum.

Keywords

Scopolamine; Nucleus accumbens; Striatum; Opioid; Feeding; Anorexia; Acetylcholine; Motivation; Rat; Muscarinic receptor

Introduction

It has been shown that the striatal control of motivational processes is heterogeneous across anatomical subregions. This heterogeneity is thought to derive from the partial segregation of functionally complementary cortical and thalamic inputs to distinct striatal zones, and from regional differences in the content of neurotransmitters, peptides, and other markers. An influential model proposes that functionally related striatal territories are arranged in ventromedial-to-dorsolateral bands progressing posteriorly, with the proportionately greatest compliment of allocortical and frontal neocortical inputs innervating anterior ventral sectors (Berendse et al. 1992; Groenewegen et al. 1999; Haber et al. 2006; McGeorge and Faull 1989; Voorn et al. 2004). This model echoes Mogenson's original proposal that the ventral striatum (nucleus accumbens, Acb) represents a functionally specialized limbic-motor interface, accounting for its crucial role in appetitive motivation (Mogenson et al. 1980).

Numerous studies using localized intra-striatal drug infusions have confirmed that these regional heterogeneities correspond to functional differences in the modulation of appetitively motivated behaviors. Relevant to the present work, intra-striatal infusion of mu-opioid receptor-selective opioid agonists produce intense hyperphagia; the strongest effects are found in the Acb, particularly in the medial shell subregion where opioid agonists also enhance positive unconditioned taste reactions (Bakshi and Kelley 1993a; b; Pecina and Berridge 2005; Zhang and Kelley 2000). Opioid-mediated hyperphagia is not, however, limited to the Acb; nearby anterior dorsal striatal sites also support mu-opioid receptor-driven feeding, with progressively diminishing effects as infusions are placed more dorsally and caudally (Bakshi and Kelley 1993b; Di Feliceantonio et al. 2012). Thus, the distribution of active sites for opiate modulation of feeding behavior maps well onto the striatal zones (including those adjacent to but outside of the Acb) receiving the greatest proportion of limbic input according to the models described above.

Another key striatal transmitter involved in the control of feeding behavior is acetylcholine. Blockade of cholinergic muscarinic receptors in the striatum produces a profound, long-lasting anorexia (Perry et al. 2009; Pratt and Kelley 2005), which may result from a functional impairment of opioid transmission. Blockade of Acb-localized muscarinic receptors with the antagonist, scopolamine, produces a decrease in food intake induced by the stimulation of Acb-localized mu-opioid receptors (Perry et al. 2009; Will et al. 2006). In fact, scopolamine was the only drug out of several dopamine, glutamate, or nicotinic receptor antagonists tested to block mu-opioid receptor-driven feeding (Will et al. 2006). Moreover, scopolamine infusions produced a long-lasting decrease in expression of the peptide precursor gene, preproenkephalin, throughout the striatum (Pratt and Kelley 2005). These findings indicate that muscarinic blockade interacts with the striatal opioid system, suggesting that manipulations of mu-opioid and muscarinic receptors would influence feeding behavior within overlapping striatal territories.

In this regard, it is important to note that striatal acetylcholine is localized in interneurons with extensive processes that communicate with other cholinergic interneurons (Zhou et al. 2002). It has been hypothesized that this arrangement results in a "reticular" mode of network control that results in the functional coordination of wide areas of striatum (Kelley et al. 2005a; Phelps et al. 1985; Zhou et al. 2002). Alternatively, the functional coordination may occur on a smaller scale. To date, intra-striatal microinfusion studies have failed to identify a null site for scopolamine's effect (Pratt and Kelley 2005). Nevertheless, because striatal sites tested to date fall within or near the active zones for opioid-driven feeding, the two possibilities (i.e., reticular control vs. more localized modulation) cannot yet be distinguished.

Based on these considerations, we conducted dose–response analyses of scopolamine-induced anorexia in three striatal sites: the Acb, the anterior dorsal striatum (ADS), and the posterior dorsal striatum (PDS). The ADS is strongly innervated by sensorimotor cortex, but more ventral sectors receive some degree of limbic input. The more ventral aspects of ADS also support mu-opioid receptor-mediated hyperphagia (Bakshi and Kelley 1993b). The PDS receives input predominantly from sensorimotor cortex (McGeorge and Faull 1987); this site does not support mu-opioid receptor-driven feeding behavior. After scopolamine infusions were completed, the mu-selective opioid agonist, (D-Ala2-N-MePhe4, Glyol)-enkephalin (DAMGO), was infused into each site at an active (but submaximal) dose known to elicit hyperphagia. Our main objective was to determine whether or not the anatomical gradient of dose sensitivity to scopolamine would map onto the active striatal sites for opioid-driven feeding, as a means of evaluating the broader issue regarding the anatomical limits of muscarinic network control.

Methods

Experimental subjects

One hundred and six male Sprague–Dawley rats (Harlan, Madison, WI, USA) were used in these studies. Rats were housed in pairs in clear plastic cages kept in a temperature- and light-controlled vivarium (12 h light/dark cycle, lights on at 07:00 h). Subjects weighed 275–300 g upon arrival in the laboratory and were initially maintained on an ad libitum feeding schedule. Prior to the start of experiments, rats were placed on a food-restriction regimen resulting in maintenance at 85 % of free-feeding body weight. Subjects were handled daily to minimize stress. All procedures related to experimental manipulations and animal care were performed according to NIH guidelines on the use of animals in research and regulated by the University of Wisconsin-Madison Medical School Animal Care and Use Committee.

Surgery

Rats were anesthetized with isoflurane (1.5–2.5 %) using an inhalant anesthesia system (Summit, Bend, OR) with oxygen being delivered at all times during the procedure (0.9 l/min). Standard aseptic procedures were utilized to implant indwelling stainless steel guide cannulae (23 gauge) bilaterally in multiple areas of the striatum using different groups of rats for each site: Acb (1.3 mm anterior to Bregma; 1.7 mm lateral to Bregma; and 5.3 mm ventral to skull surface), posterior dorsal striatum (PDS, 0.9 mm posterior to Bregma; 3.3 mm lateral to Bregma; and 3.2 mm ventral to skull surface), and anterior dorsal striatum (ADS, 1.3 mm anterior to Bregma; 2.8 mm lateral to Bregma; and 3.0 mm ventral to skull surface). The toothbar was set at –4 mm below interaural zero. These coordinates were selected based on the flat-skull coordinate system of Paxinos and Watson. Guide cannulae were affixed to the skull with the use of screws and dental acrylic (Lang, Henry Schein Inc, Melville, NY) and wire stylets were placed in the cannulae to prevent occlusion. Rats received an IM injection of ketoprofen (5 mg/kg of 5 mg/mL) for pain and recovered for at least 7 days prior to behavioral testing.

Drugs and microinfusion procedures

The following drugs were dissolved in sterile 0.9 % saline: muscarinic antagonist scopolamine methyl bromide (1, 5, 10 µg/0.5 µl/side, Sigma Aldrich) and (D-Ala2-N-MePhe4, Glyol)-enkephalin (0.25 µg/0.5 µl/side, Sigma Aldrich, St. Louis, MO). Scopolamine and DAMGO doses were chosen on the basis of data from previous experiments in this laboratory showing clear behavioral effects (Bakshi and Kelley 1993a; Cunningham and Kelley 1992; Perry et al. 2009; Will et al. 2006; Zhang et al. 2003).

Drugs were administered bilaterally through stainless steel injectors (30 gauge) connected via polyethylene tubing (PE-10) to a microdrive pump (Harvard Apparatus, South Natick, MA). Injectors protruded 2.5 mm (Acb) or 1.2 mm (ADS and PDS) below the guide cannulas to the final injection sites (Acb, 7.8 mm below skull surface; PDS, 4.4 mm below skull surface; and ADS, 4.2 mm below skull surface). Rats were gently hand-held during the infusion process. Habituation to drug infusion began with a mock infusion during which injectors were lowered to the bottom of the guide cannulas and the infusion pump was activated, but no infusions were made (habituation to infusion-related auditory stimuli). The next day saline was infused using 30 gauge injectors extending beyond the end of the guide cannula into the target site. The rate of injection was 0.32 $\mu\text{l}/\text{min}$ for all drugs, and the total volume infused was 0.5 μl . An additional minute was allowed for diffusion of injectate into the tissue. Injectors were then removed, stylets were replaced, and rats were returned to their home cage. The same procedure was used to administer drug on testing days.

Experimental design

The same apparatus was used in each experiment to examine changes in feeding behavior following scopolamine or DAMGO administration into various striatal regions. All behavioral testing began 1 week following surgery, in a room separate from the animal colony containing automated activity/feeding chambers (Med Associates, St. Albans, VT). The sides and top of the activity/feeding chambers were made of clear Plexiglas, and the chambers had wire grid floors. Food intake monitors (Med Associates) were mounted on the sides of the chambers and were able to measure food weight with an accuracy of 0.1 g. Two arrays of infrared photobeams were mounted on the front and back of the chambers to measure locomotor activity of the rats (bottom row: three beams, 3.8 cm above floor, 11.4 cm apart; top row: four beams, 15.3 cm above floor, 12.7 cm apart). Pre-weighed water bottles were attached to the side of the chamber and the tub attached to the food intake monitor was filled with sucrose pellets. On the test day, rats received an intra-striatal infusion of saline or scopolamine (0, 1, 5, or 10 $\mu\text{g}/0.5 \mu\text{l}$) 3 h prior to the 45-min test session. At the end of the testing period, the rats were returned to their cages, and the experimenter recorded uneaten food, food spillage, and remaining water while ambulations and rears were recorded via the accompanying MedPC software package.

A between-subjects design was used in which acute feeding responses to different doses of scopolamine were tested in separate groups of rats, using the injection methodology described above. Four days after the scopolamine test, half the rats in each scopolamine dose group were challenged with DAMGO (0.25 $\mu\text{g}/0.5 \mu\text{l}$), the other half received saline. DAMGO was injected immediately before the 45-min test session according to the injection methods described above, and rats were returned to their cages immediately after testing. Thus, the DAMGO and saline groups each contained subjects from all the scopolamine dose groups; data were pooled for statistical analysis.

The Acb experiment was run in two cohorts of rats; for this experiment the DAMGO challenge was only conducted in the first of these cohorts, yielding lower group sizes for the DAMGO manipulation compared to the ADS and PDS experiments (see Table 1). However, statistically significant effects were still obtained (see Results section) indicating that the study had adequate statistical power, given the large effect size.

Statistical analysis

For all scopolamine-feeding experiments, one-way ANOVAs (with scopolamine dose as the between-subjects variable) were used to analyze food and water intake, ambulations, and rears. Dunnett's method used for post hoc analysis, contingent upon significant effects in the

ANOVA. For experiments where DAMGO was administered, *t* tests for analysis of food and water intake, rears, and ambulations were performed. The alpha value was set at $p < 0.05$.

Results

Nucleus accumbens

Following scopolamine infusions into this site, rats displayed a significant decrease in sucrose-pellet intake, shown in Fig. 1. Analysis of variance indicated a main effect of scopolamine treatment ($F_{3,36}=6.39$, $p < 0.001$); post hoc analysis with Dunnett's test showed that intake levels produced by the 5- μ g and 10- μ g doses of scopolamine were significantly lower than saline control values ($p < 0.05$). Water intake was unaffected by scopolamine administration into the Acb; furthermore, ambulations and rears were unaltered by drug treatment (see Table 1).

To explore possible differences between placements in rostral versus caudal levels of the Acb, sucrose intake values were grouped according to whether the corresponding placements occurred in the anterior half or posterior half of all Acb placements, and an ANOVA was conducted using anteroposterior (AP) placement as an additional factor. This analysis indicated neither a significant effect of AP level, nor an AP level $\times$ dose interaction.

For two rats, one side of the bilateral placement was in the Acb shell instead of the core; these rats were in the 1 and 10 μ g scopolamine dose groups. These placements yielded values within the general range of other subjects in their respective dose groups.

DAMGO infusion into the Acb resulted in an augmentation of sucrose pellet intake ($t_{(8)}=3.37$, $p < 0.010$) as depicted in the Fig. 1 inset. Statistical analysis of water intake, ambulations, and rearing indicated no effect of drug (see Table 1). Thus, the Acb, through its anteroposterior extent is a sensitive site for both scopolamine-induced anorexia and opioid-driven hyperphagia.

Anterior dorsal striatum

Scopolamine infused into ADS resulted in a decrease in sucrose-pellet intake, but only at the highest dose ($F_{3,22}=2.94$, $p < 0.05$; Fig. 1). Hence, scopolamine's effect on food intake in the ADS appeared to exhibit less dose sensitivity compared to its effects in the Acb. Table 1 shows the lack of effect on water intake and locomotor activity after scopolamine infusion into the ADS.

DAMGO infusion into the ADS resulted in no change in any variable measured. The inset in Fig. 1 shows the lack of effect on feeding behavior. General motor activity and water intake were also unaffected (see Table 1). Therefore, relative to the Acb, the ADS is a less-sensitive site for the feeding-related effects of both DAMGO and scopolamine.

Posterior dorsal striatum

There were no changes in sucrose-pellet intake following scopolamine administration into this striatal region (Fig. 1). Also, no change was found in water intake, or in ambulatory or rearing behavior after scopolamine infusion (Table 1).

Similar to DAMGO infusion into the ADS, DAMGO in the PDS had no effect on food or water intake or general motor activity. The inset in Fig. 1 depicts the lack of effect on sucrose-pellet intake. General motor activity and water intake are shown in Table 1. Thus, the PDS was a null site for both scopolamine-induced anorexia and DAMGO-induced hyperphagia.

Histology

Following behavioral testing, rats were overdosed with sodium pentobarbital and perfused transcardially with saline (200 ml), followed immediately by 500 ml of a 10 % buffered formalin solution. The brains were then removed and placed in 10 % buffered formalin–10 % sucrose solution overnight. Frozen serial sections (60 μ m) were collected through the entire extent of the injection sites, mounted on gelatinized slides, stained with Cresyl violet, and cover slipped. Cannulae placements were then assessed with light microscopy by an observer blind to the behavioral results of the rats. Photomicrographs of representative acceptable placements, and line charts with all subjects' injection site locations, are shown in Fig. 2. One rat was removed from the PDS group and six from the ADS experiment due to inaccurate placements. Sample sizes reported for behavioral data reflect the omission of rats with inaccurate placements.

Discussion

The present study provides support for anatomical heterogeneity of the striatum with regard to muscarinic receptor modulation of feeding behavior. No effects were seen with any dose of scopolamine in the PDS, and the middle dose (5 μ g) produced a significant decrease only when infused into the Acb, demonstrating a ventral–dorsal dose-sensitivity gradient similar to the previously published opioid-induced feeding gradient (Bakshi and Kelley 1993b). DAMGO infusions produced hyperphagia only within the Acb, suggesting an overlapping anatomical localization of the area most sensitive to scopolamine-induced anorexia and opioid-induced hyperphagia. The current study also confirms scopolamine's previously published specificity for food consumption (Perry et al. 2009), as water intake and locomotor activity were unaltered. It should be noted that scopolamine was administered 3 h prior to behavioral testing. Previous studies have demonstrated hyperactivity following acute drug administration (Joyce and Koob 1981; Pratt and Kelley 2004); this hyperactivity could interfere with the interpretation of decreases in feeding. Previously, we have shown that scopolamine-induced anorexia greatly outlasts this acute hyperactivity (Perry et al. 2010; Perry et al. 2009), and the present results confirm that the use of a 3-h post-injection delay permits an analysis of scopolamine-induced effects on feeding devoid of confounding effects on general motor activity. As well, use of a 3-h post-injection delay in the present study enabled a direct comparison to our previous analyses of the motivational effects of intra-striatal scopolamine (Perry et al. 2010).

Striatal acetylcholine is found within intrinsic interneurons, which represent only 1–2 % of the total complement of striatal neurons (Zhou et al. 2002). Nevertheless, these interneurons have the capability of influencing large areas by virtue of their extensive axonal and dendritic processes (Hersch et al. 1994; Izzo and Bolam 1988). It has been proposed that these extensive processes allow distant cholinergic interneurons to interact and to coordinate activity between the striatal matrix and mu-opioid receptor-enriched striosomes (Aosaki et al. 1995; Miura et al. 2008; Sullivan et al. 2008). One theory posits that this arrangement allows a perturbation of cholinergic transmission in one striatal sector to coordinate activity throughout the striatal complex, in conjunction with appetitive drive states (Kelley et al. 2005a). Accordingly, infusions of scopolamine in either the Acb core or ADS have been shown to negatively modulate proenkephalin expression throughout wide expanses of the striatum (Pratt and Kelley 2005; Wang and McGinty 1996), and there is evidence that striatal tonically active neurons (TANs), presumably cholinergic interneurons, display synchronous firing across different regions of the caudate during learning (Aosaki et al. 1995). Considering these findings, an important question arises regarding the regional limits of cholinergic control within the striatum.

The present results suggest that the effects of scopolamine correspond to the same anatomical constraints that govern mu-opioid receptor-mediated effects, implying that putative functional integration across cholinergic interneurons (at least, as relevant to feeding) occurs within these topographic limits. It is important to note that, just as with opioid-mediated feeding, there are sites outside the striatum that subserve feeding-modulatory scopolamine actions. Prior work (Pratt WE, personal communication) tested the effects of a high scopolamine dose (10 μ g) on 24-h food intake, when infused into the dorsal hippocampus, thalamus, basolateral amygdala, medial prefrontal cortex, and premotor cortex. Significant effects (% feeding suppression; scopolamine relative to saline) were found in thalamus (50.4 %, on the order of the effect seen here in the Acb) and dorsal hippocampus (32.8 %), and basolateral amygdala (20.1 %), with a small suppression (14.1 %) in premotor cortex. Of these extra-striatal sites, the ones showing the strongest effects (dorsal hippocampus and thalamus) are closest to our inactive site (PDS), and furthest from our active site (Acb). Moreover, several studies examining the effects of scopolamine infusion (in the dose range used here) into a site even closer to the Acb, the medial prefrontal cortex, have shown deficits in cognitive processes such as social learning, temporal discrimination, and spatial working memory but without impairments in motivation or consumption of the food reinforcers used in these studies (Ragozzino and Kesner 1998; Hata and Okaichi 2004; Boix-Trelis et al. 2007). Collectively, these data suggest that the striatal gradient of sensitivity to scopolamine's feeding-related effects seen here is unlikely the result of diffusion to extra-striatal sites, but instead reflects the organization of intra-striatal opiate- and acetylcholine-sensitive zones.

It has been proposed that functional heterogeneity across striatal territories derives, in part, from the information-processing domains of the allocortical, neocortical, and thalamic sites that supply convergent glutamatergic input to a given striatal zone (Haber and Calzavara 2009; Voorn et al. 2004). Specializations relevant to mu-opioid and scopolamine-modulated feeding may be conferred by gustatory inputs from insular cortex, by amygdalar or hippocampal inputs that convey contextual or cue-related learned associations with food reward, or by inputs from midline thalamic structures that are interposed between feeding-related hypothalamic zones and the Acb. All of these inputs preferentially target the ventral striatum, broadly defined (Baldo et al. 2003; Kelley et al. 2005a; McGeorge and Faull 1989; Peyron et al. 1998; Reynolds and Zahm 2005; Voorn et al. 2004). It is somewhat surprising that scopolamine's feeding-related effects are roughly equivalent in the Acb core and shell (shown here and also in Pratt and Kelley 2004); among striatal subregions the medial Acb shell possesses arguably the greatest degree of specialized input/output circuitry related to hypothalamic feeding systems. Thus, the Acb shell projects most strongly among striatal sites to the lateral hypothalamus (LH) (Heimer et al. 1991; Zahm and Heimer 1993), and receives, in turn, the greatest density among striatal sites of peptide-coded input from arousal- and feeding-related hypothalamic systems, such as the orexin-hypocretin and melanin-concentrating hormone systems, and from leptin receptor-bearing projection neurons (Baldo et al. 2003; Bittencourt et al. 1992; Patterson et al. 2011; Peyron et al. 1998). This specialized anatomical relationship between the Acb shell and LH has been hypothesized to represent the functional basis for certain anatomically circumscribed pharmacological effects, notably the hyperphagic effects of GABA receptor stimulation or AMPA-type glutamate receptor antagonism, which are restricted to the Acb shell (Kelley and Swanson 1997; Stratford and Kelley 1997). The fact that scopolamine and DAMGO's effects on food intake do not respect a sharp core-shell boundary (Pratt and Kelley 2004; Zhang and Kelley 2000) suggests that the feeding-related modulatory actions of these drugs are subserved by networks beyond the tightly circumscribed Acb shell efferent circuitry (Thompson and Swanson 2010), but which still respect a ventral-to-dorsal striatal gradient. This could occur if scopolamine manipulations in ventral striatal areas outside the Acb shell engage a broader, recurrent network (with loops through "limbic" allocortical and

neocortical sites) that modulates the positive affective components of sampled food, a mechanism that has been suggested to subserve μ -opioid actions (Baldo and Kelley 2007; Baldo et al. 2010; Gosnell and Levine 2009; Kelley et al. 2005b; Woolley et al. 2006).

In general support of this hypothesis, feeding-related effects of intra-striatal μ -opioid and scopolamine manipulations are expressed most consistently in behavioral tests where food is encountered and eaten, as opposed to tests that assay the control over goal-seeking behavior of food expectation and/or food-predictive cues (Barbano et al. 2009; Perry et al. 2010; Pratt and Blackstone 2009). For example, intra-Acb muscarinic blockade decreases progressive ratio responding (in which the food pellet is eaten after each completed ratio), but produces no effect on conditioned reinforcement responding (CR, in which lever pressing is reinforced by a food-associated Pavlovian cue in the absence of food itself) or amphetamine-induced potentiation of CR responding (Perry et al. 2010; Pratt and Kelley 2004). Intra-Acb muscarinic blockade also suppresses food intake without altering hyperactivity associated with food expectation (Perry et al. 2010), and, correspondingly, opioid receptor blockade reduces palatable food intake, but not running speed, in a food-reinforced runway paradigm (Barbano et al. 2009). Finally, μ -opioid receptor stimulation specifically within the medial Acb shell enhance unconditioned palatable taste reactions to a sucrose solution infused into the oral cavity (Pecina and Berridge 2005) indicating a modulation of the hedonic impact of sucrose currently being tasted. Collectively, these results suggest that μ -opioid receptor stimulation facilitates, while muscarinic blockade attenuates, the rewarding properties of food during the experience of eating.

This conclusion may at first seem inconsistent with insights garnered from the study of striatal acetylcholine efflux during feeding. Acetylcholine is released during the peak of the meal and parallels the onset of satiety; this response is largely dependent on the muscarinic receptor (Avena et al. 2006; Mark et al. 1992). It has been suggested that striatal cholinergic transmission acts to counterbalance dopamine transmission, dampening dopamine-mediated processes, and perhaps signaling an aversive state (Hoebel et al. 2007; Hoebel et al. 1989). One might therefore predict that scopolamine would enhance dopamine-mediated motivational processes, seemingly in contrast with the demonstrations of scopolamine-induced suppression of food reward here and elsewhere (Perry et al. 2010; Perry et al. 2009; Pratt and Blackstone 2009; Pratt and Kelley 2004). Studies with dopamine receptor antagonists, dopamine-depleting lesions, and catecholamine-deficient mice have, however, provided converging evidence that Acb-based modulation of taste hedonics is neuropharmacologically dissociable from modulation of the motivationally arousing or activating properties of food, with dopamine playing a prominent role in the latter process (for reviews, see Baldo and Kelley 2007; Berridge 2007; Palmiter 2008; Salamone et al. 2007). Findings regarding acetylcholine release and acetylcholine/dopamine interactions may therefore be more relevant to understanding the acute motor-enhancing actions of scopolamine, whereas the long-lasting suppression of food intake may be linked to the protracted suppression of striatal enkephalin expression. Additionally, considering its enduring time-course, scopolamine-induced feeding suppression may result from a postsynaptic counteradaptation that outlasts acute scopolamine-mediated muscarinic receptor blockade. Either way, the cholinergic perturbation may act to “reset” the modulation of food reward in striatal networks via its downregulation of opioid function (Kelley et al. 2005a; Pratt and Kelley 2005).

Recent studies with genetically altered mice lacking μ -opioid receptors support the idea that this receptor mediates functions generally consistent with “non-homeostatic” food intake and energy storage (see in particular discussion in Tabarin et al. 2005). Accordingly, μ -opioid receptor-deficient mice show deficits in operant responding in food-reinforced operant tasks (Kas et al. 2004; Papaleo et al. 2007), and, remarkably, diminished fat

deposition under fat-enriched diets (Tabarin et al. 2005). Genetically altered mice also show evidence that some degree of gustatory reward evaluation is preserved; for example, mice lacking mu-opioid receptors demonstrate preference for sucrose over chow even though operant responding for both is depressed (Papaleo et al. 2007). Hence, although numerous pharmacological studies have shown that stimulation of striatal mu-opioid receptors enhances taste hedonics and certain flavor or macronutrient preferences (Pecina and Berridge 2005; Woolley et al. 2007; Zhang and Kelley 2000; 2002), the ability of a preferred taste to guide basic choice behavior is determined by additional, redundant influences (including post-ingestive factors, see Azzara et al. 2000). The affective state engendered by ventral striatal mu-opioid transmission may thus have a particularly important role in amplifying the reward properties of already-preferred foods (Baldo et al. 2010; Pecina and Berridge 2005; Woolley et al. 2006). If so, the insight that muscarinic receptors reciprocally modulate opioid-dependent processes within similar striatal zones may guide the development of pharmacological interventions that selectively attenuate excessive palatable feeding during a meal, perhaps by rendering highly palatable food less rewarding than expected, but without engendering a global motivational deficit. Such drugs may have great utility for body weight control in our contemporary environment of readily available, calorie-dense palatable food, particularly given current epidemiological trends regarding obesity prevalence. Cholinergic drugs may also have utility in the treatment of binge eating disorder, in which mu-opioid transmission has been implicated (Davis et al. 2009; Cambridge et al. 2013).

In summary, the current study, representing the first systematic mapping of intra-striatal scopolamine infusions on feeding, adds new insight into the functional heterogeneity of the striatum with regard to its modulation of discrete motivational components of feeding behavior. The results demonstrate anatomical overlap between cholinergic and opiate control of feeding, providing an anatomical framework for understanding functional interactions between these two systems.

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Fig. 1.

Effects of scopolamine infusions into the nucleus accumbens (*Acb*), anterior dorsal striatum (*ADS*), or posterior dorsal striatum (*PDS*) on sucrose intake. *Insets* depict DAMGO-induced feeding (*hatched bars*) compared to saline (*white bars*) in the same groups of rats; see text for procedural details. All graphs show group means + SEMs. * $P < 0.05$; ** $P < 0.01$, compared to respective saline groups

Fig. 2.

Line drawings and photomicrographs showing injector placements in the nucleus accumbens, anterior dorsal striatum, and posterior dorsal striatum. *Darkened circles* on line drawings depict injector placements for the rats used in these studies. Stereotaxic coordinates (in millimeters anterior to Bregma) are given for the most rostral and most caudal of the line drawings in each series

Table 1

Effects of intra-striatal infusions of scopolamine or DAMGO on water intake and general motor activity

Treatment		Water intake (mL)	Ambulation (beam breaks)	Rearing (beam breaks)
Scopolamine				
Acb				
Saline	(N=10)	3.4±0.8	162±26	50±11
1 µg	(N=10)	3.4±0.7	147±28	25±6
5 µg	(N=10)	2.7±0.8	172±34	31±5
10 µg	(N=10)	1.9±0.6	182±28	37±9
ADS				
Saline	(N=6)	3.0±1.3	111±22	32±8
1 µg	(N=7)	1.3±0.3	138±28	37±12
5 µg	(N=6)	2.3±0.5	129±22	39±12
10 µg	(N=7)	2.4±0.4	146±11	33±6
PDS				
Saline	(N=7)	3.4±0.9	96±10	27±4
1 µg	(N=8)	4.8±1.0	72±10	30±9
5 µg	(N=8)	2.5±0.8	99±15	17±3
10 µg	(N=8)	3.4±0.9	100±16	26±6
DAMGO				
Acb				
Saline	(N=5)	3.0±0.7	121±34	38±19
2.5 µg	(N=5)	1.4±0.4	95±30	20±8
ADS				
Saline	(N=12)	1.3±0.4	144±21	39±7
2.5 µg	(N=12)	2.5±0.5	180±22	31±4
PDS				
Saline	(N=15)	3.4±0.7	100±11	26±4
2.5 µg	(N=18)	3.0±0.5	101±7	31±5

Values represent group means ± SEM

Acb nucleus accumbens, *ADS* anterior dorsal striatum, *PDS* posterior dorsal striatum