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Differential Involvement of M1-type and M4-type Muscarinic Cholinergic Receptors in the Dorsomedial Striatum in Task Switching

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Abstract

Previous experiments have demonstrated that the rat dorsomedial striatum is one brain area that plays a crucial role in learning when conditions require a shift in strategies. Further evidence indicates that muscarinic cholinergic receptors in this brain area support adaptations in behavioral responses.

Unknown is whether specific muscarinic receptor subtypes in the dorsomedial striatum contribute to a flexible shift in response patterns. The present experiments investigated whether blockade of M1-type and/or M4-type cholinergic receptors in the dorsomedial striatum underlie place reversal learning. Experiment 1 investigated the effects of the M1-type muscarinic cholinergic antagonist, muscarinic-toxin 7 (MT-7) infused into the dorsomedial striatum in place acquisition and reversal learning. Experiment 2 investigated the effects of the M4-type muscarinic cholinergic antagonist, muscarinic-toxin 3 (MT-3) injected into the dorsomedial striatum in place acquisition and reversal learning. All testing occurred in a modified cross-maze across two consecutive sessions. Bilateral injections of MT-7 into the dorsomedial striatum at 1 or 2 μg , but not 0.05 μg impaired place reversal learning. Analysis of the errors revealed that MT-7 at 1 and 2 μg significantly increased regressive errors, but not perseverative errors. An injection of MT-7 2 μg into the dorsomedial striatum prior to place acquisition did not affect learning. Experiment 2 revealed that dorsomedial striatal injections of MT-3 (0.05, 1 or 2 μg) did not affect place acquisition or reversal learning. The findings suggest that activation of M1-type muscarinic cholinergic receptors in the dorsomedial striatum, but not M4-type muscarinic cholinergic receptors facilitate the flexible shifting of response patterns by maintaining or learning a new choice pattern once selected.

Introduction

Several experiments have demonstrated that the rat dorsomedial striatum, is part of a larger neural circuit that supports a shift in strategies or choice patterns (Divac, Rosvold & Schwarcbart, 1967; Kolb, 1977; Kirkby, 1969; Pisa & Cyr, 1990; Ragozzino, Jih & Tzavos, 2002a; Ragozzino, Ragozzino, Mizumori & Kesner, 2002b; Ragozzino & Choi, 2004). Furthermore, there is substantial evidence that cholinergic interneurons within the dorsomedial striatal area support the flexible shifting of choice patterns. More specifically, acetylcholine

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(ACh) output from the dorsomedial striatum selectively increases during different reversal learning tests, but not during initial learning of a discrimination (Palencia & Ragozzino, 2006; Ragozzino & Choi, 2004). Other studies have demonstrated that muscarinic cholinergic receptors, but not nicotinic cholinergic receptors mediate the effects of ACh within the dorsomedial striatum to facilitate a shift in strategies (Ragozzino et al., 2002a; Tzavos, Jih, and Ragozzino, 2004). In particular, infusions of scopolamine, a non-specific muscarinic cholinergic antagonist infused into the dorsomedial striatum does not impair acquisition of a response discrimination, but does impair response reversal learning (Ragozzino et al., 2002a). In contrast, mecamylamine, a nicotinic cholinergic antagonist, infused into the dorsomedial striatum does not impair acquisition or reversal learning (Ragozzino et al., 2002a). A subsequent experiment demonstrated that pirenzepine, a M1-type muscarinic antagonist, injected into the dorsomedial striatum does not impair response acquisition, but does impair response reversal learning in a dose-dependent fashion (Tzavos et al., 2004). These findings suggest that M1-type muscarinic cholinergic receptors in the dorsomedial striatum may facilitate learning when conditions require a shift in response patterns.

Even though pirenzepine is considered to be a selective M1-type muscarinic cholinergic antagonist, its selectivity for M1-type vs. M4-type muscarinic cholinergic receptors is considered to be only 4-6 times greater (Madison et al. 1987; Nasman et al., 2000). Both M1-type and M4-type muscarinic cholinergic receptors are expressed in the rat striatum and reported to have the highest density postsynaptically on striatal neurons compared to the other muscarinic cholinergic receptor subtypes (Weiner, Levey & Brann, 1990; Yan, Flores-Hernandez & Surmeier, 2001). Therefore, one possibility is that a pirenzepine-induced reversal learning deficit may have resulted from either blockade of M4-type muscarinic cholinergic receptors or combined M1-type and M4-type muscarinic cholinergic receptor blockade.

Recent studies suggest muscarinic toxins purified from the venom of *Dendroaspis* snakes are much more selective for specific muscarinic receptors subtypes (Nasman et al., 2000; Karlsson et al., 2000). For example, Karlsson and colleagues (2000) report that muscarinic toxin 7 (MT-7) has more than 20,000 times higher selectivity for the M1 muscarinic cholinergic receptor than for any other muscarinic receptor subtype. In a comparable manner, muscarinic toxin 3 (MT-3) has more than 200 times higher selectivity for the M4 muscarinic cholinergic receptor than for the M1 muscarinic cholinergic receptor subtype with negligible activity at the other muscarinic cholinergic receptor subtypes (Jerusalinsky, Kornisiuk, Alfaro, Quillfeldt., Alonso, Verde, Cervenansky & Harvey, 1998). Thus, the use of specific muscarinic toxins, e.g. MT-7 and MT-3, can better determine whether specific muscarinic cholinergic receptor subtypes in the dorsomedial striatum support behavioral flexibility.

The present experiment examined whether M1-type and/or M4-type muscarinic cholinergic receptors in the dorsomedial striatum play a role in behavioral flexibility by determining whether the selective M1-type muscarinic receptor antagonist, MT-7 or selective M4-type muscarinic receptor antagonist, MT-3 affects place reversal learning.

2. Materials & Methods

Subjects

Male Long-Evans rats (Harlan, Indianapolis, IN) weighing between 350-400 grams at the start of the experiment served as subjects. Rats were singly housed in plastic cages (26.5 cm wide × 50 cm long × 20 cm high) in a humidity (30%) and temperature (22°C) controlled room with a 12-h light/dark cycle (lights on at 0700 h). Each rat received surgery approximately 5-7 days after arriving at the colony. Animal care and use was in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals, and was approved by the

Institutional Laboratory Animal Care and Use Committee at the University of Illinois at Chicago.

Surgery

Each rat received stereotaxic surgery to bilaterally implant cannula into the dorsomedial striatum. Before surgery each rat received an intraperitoneal (i.p.) injection of atropine sulfate (0.2 ml of a 250 µg/ml solution) followed 10 min later by an i.p. injection of sodium pentobarbital (50 mg/kg). The stereotaxic coordinates were 1.1 mm anterior to bregma and ± 2.8 mm lateral to the midline. Eight mm stainless steel guide cannulae (Plastics One, Roanoke, VA) were implanted at a 10° angle aimed medially and placed 4.0 mm below dura. The coordinates were based on Paxinos and Watson's (1996) rat brain atlas. Four jeweler's screws were positioned in the skull surrounding the guide cannulae. Dental acrylic (Stoelting, Wood Dale, IL) was used to secure the guide cannula. After the dental acrylic dried, stylets (Plastics One, Roanoke, VA) were placed in the guide cannulae. After surgery, the rats received 6 ml of saline subcutaneously (s.c.). In addition, each rat received a single injection of 2.5 mg/kg of carprofen Sq (ip) following surgery as a post-operative analgesic. Subsequently, each rat was fed ground rat chow and sugar mixed with water for 2 days. For 5-7 days after surgery the rats were allowed to recover and handled for 5 min each day. After 2 days of recovery all rats were food-restricted to maintain their weight at about 85% of their free-feed weight. Each rat had free access to water throughout the study. Behavioral training commenced 5-7 days after surgery.

Apparatus

A black plastic four-arm cross maze was used for behavioral testing. The height of the maze walls was 15.0 cm and each of the arms measured 55 cm long × 10 cm wide. There was a food well (3.2 cm diameter × 1.6 cm high) in each arm located 3 cm away from the end wall. The hole in the food well measured 2.3 cm in diameter and was 1.6 cm deep. The maze was elevated 72 cm above the floor in a room with extra-maze cues.

Pretraining

On the first day of pretraining all arms of the maze were baited with a half piece of Froot Loops cereal (Kelloggs, Battle Creek, MI). Each rat was placed at the end of a maze arm and allowed to explore and consume the Froot Loop pieces. One pretraining trial was completed after a rat consumed all the cereal pieces. Between trials a rat was placed in a holding cage while the maze was rebaited with Froot Loops pieces. The pretraining session was terminated after 15 min had elapsed and the number of trials completed was recorded. If a rat did not complete 1 pretraining trial within 15 min, it remained in the maze until it consumed all cereal pieces or 20 min had elapsed. During subsequent pretraining sessions, when a rat had consumed a piece of Froot Loops cereal from two baited arms it was picked up and placed in an unbaited arm. After eating from a third baited arm it was picked up and placed in another unbaited arm. After consuming cereal from the fourth arm, a rat was removed, the arms were rebaited and another trial was started. This procedure was used to acclimate a rat to being picked up in the maze after consuming a cereal piece. This procedure was continued until the rat was able to complete 5-7 trials in 15 min across 2 consecutive days. After reaching this criterion, a final day of pretraining occurred in which a black plastic block (9 cm wide × 13 cm high × 1 cm thick) was placed at the entrance of one arm so that it prevented entry, giving the maze a T-shape. Therefore, there were only two arm choices available to a rat. A rat was placed in the stem arm and allowed to enter either choice arm to obtain a cereal piece. After the initial choice, a rat was placed back in the stem arm. If a rat chose the same arm as the initial choice, it was returned to the stem arm until it chose the other arm. When a rat had chosen both arms it was placed in the holding cage while the two choice arms were re-baited. The session ended after a rat had

completed 7 of these trials. After the last pretraining session, the stylets of a rat were removed and injection cannulae were inserted through the guide cannulae for 1 min. However, there was no infusion at this point. This was done to acclimate the rat to the insertion of the injection cannulae through the guide cannulae and prevent clogging of the microinfusion through the guide cannulae on test days.

Place Discrimination Test

Rats were tested on acquisition and reversal learning of a place discrimination across two consecutive days. Rats were started pseudorandomly from two different arms such that any start arm was not used more than 3 consecutive trials. The two start arms used were always opposite each other. A black plastic block was placed in the entrance of the maze arm opposite to that of the start arm, giving the maze a T-shape. Thus, the same two choice arms were used no matter what start arm was used. A rat was started in the stem arm with only one of the two choice arms baited. In the acquisition phase, one choice arm was designated the reinforced arm which contained a ½ piece of cereal reinforcement on each trial. In this phase, a rat was required to enter the reinforced arm containing a ½ piece of cereal. If a rat chose the correct arm, the trial was terminated after a rat consumed the cereal piece. If a rat chose the incorrect arm, the trial was terminated after a rat reached the unbaited food well. Between trials, a rat was placed in a metal grid holding cage which sat by the maze. The maze was then wiped down and re-baited if necessary. The inter-trial interval was approximately 15 seconds. To minimize the use of intramaze cues the maze was rotated 90° every fourth trial. The criterion for acquisition of the place discrimination was 10 consecutive correct trials.

On the second day of testing (reversal learning session), the rat was required to enter the arm not reinforced in acquisition, but that was now reinforced in reversal learning. Thus, the same start arms and choice arms were used as in acquisition, but the choice arm not reinforced on acquisition was now reinforced on reversal learning. Criterion for reversal learning was also 10 consecutive correct trials. Additional measures including perseverative and regressive errors were later analyzed for the reversal learning session as in previous studies (Ragozzino et al., 2002 a,b; Ragozzino, Kim, Hassert, Minniti & Kiang, 2003; Ragozzino & Choi, 2004). Perseverative errors involve initially entering the same arm that was reinforced during the acquisition session. Perseveration was operationally defined as the numbers of trials a rat initially made an incorrect choice following the first incorrect choice until choosing the new, currently reinforced arm. For example, if a rat initially made four incorrect choices before making a correct choice on the fifth trial, a rat would receive a perseverative score of three. After a rat made the first correct arm choice during reversal learning, all subsequent entries into the previously reinforced arm were scored as regressive errors.

Microinfusion

Each rat received a bilateral infusion into the dorsomedial striatum either 10 or 60 minutes prior to testing. The specific drug used in a particular experiment determined whether behavioral testing occurred 10 minutes post-injection (MT-3) or 60 minutes post-injection (MT-7). The injection was carried out via an internal cannulae (28 gauge) that extend 1.0 mm below the guide cannulae. Polyethylene tubing (PE-20) connected the cannulae to 10 µl Hamilton syringes. The syringes were driven by a microinfusion pump (74900 Series, Cole-Parmer, Vernon Hills, IL). Solutions were infused at a rate of 0.25 µl/min and the total volume injected on each side was 0.5 µl. The cannulae remained in the guide cannula for approximately 1 min after the injection to allow for diffusion. In the experiment investigating the effects of MT-7, rats received saline or MT-7 at one of the following doses: 0.05, 1.0 or 2.0 µg (prepared in saline). These doses were chosen based on previous studies that used comparable doses to examine behavioral effects following intracranial infusions (Potter et al., 2001; Romanelli et al., 2003). Sixty minutes prior to each test session a rat received an intracranial injection. A 60

minute post-injection test time was chosen based on a previous study by Potter and colleagues (2001) that demonstrated a significant blockade of M1 muscarinic receptors in the rat striatum within a couple of hours following injection of M1-toxin. Group assignment was determined by the treatment administered during each test phase with the final sample size for each group represented in brackets: 1) acquisition – saline and reversal learning – saline (n = 7); 2) acquisition – MT-7 2 µg and reversal learning – saline (n = 6); 3) acquisition – saline and reversal learning – MT-7 0.05 µg (n = 7); 4) acquisition – saline and reversal learning – MT-7 1 µg (n = 8); 5) acquisition – saline and reversal learning – MT-7 2 µg (n = 7). Group 1 served as the control group. Group 2 determined whether MT-7 impaired initial learning of a place discrimination. Groups 3-5 determined whether MT-7 affected reversal learning in a dose-dependent manner.

In the experiment investigating the effects of MT-3, rats received saline or MT-3 at one of the following doses: 0.05, 1.0 or 2.0 µg (prepared in saline). The doses were chosen based on previous experiments using similar concentrations to examine learning and memory effects following intracranial infusions (Diehl, Furstenu de Oliveira, Sanchez, Camboim, de Oliveira Alvares, Lanzotti, Cervenansky, Kornisiuk, Jerusalinky & Quillfeldt, 2007; Ferreira, Furstenu, Blanco, Kornisiuk, Sanchez, Daroit, Castro e Silva, Cervenansky, Jerusalinsky & Quillfeldt, 2003; Jerusalinsky et al., 1998). Ten minutes prior to each test session a rat received an intracranial injection. A 10-minute post-injection test time was chosen based on previous experiments demonstrating that a comparable post-injection time with MT-3 affected learning and memory (Diehl et al., 2007; Ferreira et al., 2003; Jerusalinsky et al., 1998). Group assignment was determined by the treatment administered during each test phase with the final sample size for each group represented in brackets: 1) acquisition – saline and reversal learning – saline (n = 7); 2) acquisition – MT-3 2 µg and reversal learning – saline (n = 5); 3) acquisition – saline and reversal learning – MT-3 0.05 µg (n = 7); 4) acquisition – saline and reversal learning – MT-3 1 µg (n = 6); 5) acquisition – saline and reversal learning – MT-3 2 µg (n = 9). To determine whether post-injection test time influenced the effects of MT3, two additional groups were tested one that received saline (n = 6) and one that received MT-3 2 µg (n = 6) 60 minutes prior to reversal learning.

Histology

Following the completion of behavioral testing all rats received a lethal dose of sodium pentobarbital followed by a 0.5 µl injection of 2.5% Chicago blue stain through each of the guide cannulae. The stain was used to highlight the placement of the cannulae. The rats were perfused intracardially with 0.9% phosphate-buffered saline followed by a 4% formaldehyde solution. The brains of the rats were removed and stored in a 4% formaldehyde solution until being sectioned. Using a cryostat, the brains were frozen and sliced into coronal sections (40 µm). The brain sections were mounted on slides and let to dry overnight before being stained with cresyl violet and cover-slipped. The sections were then examined to determine the placement of the cannula tips.

In previous studies (Palencia & Ragozzino, 2004; Tzavos et al., 2004; Ragozzino et al., 2002a) a rat was considered to have a misplacement if one or both of the cannulae were found dorsal in the corpus callosum, ventral in the nucleus accumbens or medial in the lateral ventricles. This same criterion was used in the present study.

Statistical Analysis

An analysis of variance (ANOVA) was used to determine whether treatment groups differ on the number of trials to criterion for the acquisition and reversal learning sessions. A separate analysis was conducted on the trials to criterion for the acquisition and reversal learning phases. Separate ANOVAs were conducted to determine differences between treatment groups in

perseverative and regressive errors. Any significant group effect test was followed up Newman-Keuls *post-hoc* tests.

3. Results

3.1 Experiment 1: The effects of MT-7 infusions into the dorsomedial striatum on the acquisition and reversal learning of a place discrimination

3.1.1. Histology—The location of the cannula tip placements are shown in Figure 1. The histological analysis indicated that the injection tips were concentrated in the dorsomedial sector of the striatum ranging from 0.48 to 1.7 mm anterior to bregma.

The data from fifteen rats were excluded from the behavioral analyses because of a cannula misplacement. The cannula misplacements were located in the following areas: bilaterally in nucleus accumbens ($N = 6$); bilaterally in the corpus callosum ($N = 1$); bilaterally in the forceps minor of the corpus callosum ($N = 2$); bilaterally in the lateral ventricles ($N = 2$) or unilaterally in the lateral ventricles ($N = 4$).

3.1.2 Place Acquisition and Reversal Learning—Figure 2A illustrates the results on acquisition and reversal learning of a place discrimination. All the groups receiving a saline injection, as well as the group receiving MT-7 2 μg achieved criterion in the acquisition phase in a comparable manner. The difference in trials to criterion on acquisition among the groups was not significant ($F_{4,30} = 0.54, p > 0.05$). In place reversal learning, the saline-saline, MT-7 2 μg - saline and saline- MT-7 0.05 μg groups required a mean trials to criterion of 36.6 ± 4.1 (s.e.m.), 40.5 ± 2.2 and 42.7 ± 3.2 , respectively. In contrast, the saline- MT-7 1 μg and saline-MT-7 2 μg groups required a mean trials to criterion of 58.3 ± 5.8 and 63.9 ± 4.0 , respectively. The difference in trials to criterion among the groups was significant ($F_{4,30} = 7.34, p < 0.01$). Newman-Keuls tests indicated that the groups receiving either MT-7 1 or 2 μg required significantly more trials to criterion compared to that of the groups that received saline or MT-7 0.05 μg during reversal learning (p 's < 0.05).

The results on the number of perseverative and regressive errors committed during reversal learning are illustrated in Figure 2B. An analysis of the perseverative errors revealed that there was not a significant difference among the groups ($F_{4,30} = 0.55, p > 0.05$). However, the difference in the number of regressive errors among the groups was significant ($F_{4,30} = 6.25, p < 0.01$). Newman-Keuls tests indicated that the MT-7 1 and 2 μg groups made significantly more regressive errors than saline-treated controls or the MT-7 0.05 μg group (p 's < 0.05).

All the groups with the exception of the MT-7 2 μg - saline group had rats with cannula misplacements. Each treatment group with misplacements had rats with different types of cannula misplacements, e.g. a rat with a bilateral placement in nucleus accumbens, as well as another rat with a unilateral ventricular placement. The results were comparable independent of the treatment received and cannula location. To demonstrate this, data from rats were collapsed across either cannula placement or treatment group. Collapsing the data based on cannula location led to the following means to reach criterion for acquisition: corpus callosum = 39.3 ± 2.9 ; nucleus accumbens = 45.5 ± 3.1 and ventricles = 43.0 ± 6.2 . The difference in criterion scores among the groups was not significant ($F_{2,12} = 0.31, p > 0.05$). The mean scores for the groups during reversal learning were as follows: corpus callosum = 39.0 ± 4.7 ; nucleus accumbens = 51.5 ± 9.8 and ventricles = $38.2.0 \pm 4.0$. The difference in trials to criterion among the groups was not significant ($F_{2,12} = 1.46, p > 0.05$).

A similar pattern of results was observed when analyzing the data by treatment group. The mean trials to criterion during acquisition was as follows: saline-saline ($n = 3$) = 49 ± 3.1 ; saline - MT-7 0.05 μg ($n = 5$) = 41.6 ± 3.7 ; saline - MT-7 1 μg ($n = 5$) = 39 ± 6.4 ; saline-MT-7 2

μg ($n = 2$) = 50 ± 3.2 . There was not a significant difference to reach criterion among the groups ($F_{3,11} = 0.68, p > 0.05$). The mean trials to criterion during reversal learning was as follows: saline-saline = 42.3 ± 5.2 ; saline – MT-7 0.05 μg = 48.4 ± 13.3 ; saline – MT-7 1 μg = 39.8 ± 3.1 ; saline-MT-7 2 μg = 41 ± 11.0 . There was not a significant treatment effect in reversal learning ($F_{3,11} = 0.18, p > 0.05$).

3.2 Experiment 2: The effects of MT-3 infusions into the dorsomedial striatum on the acquisition and reversal learning of a place discrimination

3.2.1. Histology—The location of the cannula tip placements are shown in Figure 3. The histological analysis indicated that the injection tips were concentrated in the dorsomedial sector of the striatum ranging from 0.48 to 1.7 mm anterior to bregma.

The data from four rats were excluded from the behavioral analyses because of a cannula misplacement. All four rats had a unilateral placement in the lateral ventricles.

3.2.2 Place Acquisition and Reversal Learning—All the groups obtained criterion in acquisition of the place discrimination in a comparable manner (see Figure 4A). The difference in the trials to criterion among the groups was not significant, ($F_{4, 28} = 1.00, p > 0.05$). Similar to acquisition, all the groups exhibited comparable scores during place reversal learning. There was not a significant difference in trials to criterion among the groups, ($F_{4, 28} = 0.97, p > 0.05$).

Because a reversal learning deficit was observed 60 minutes following an infusion of MT-7, but not following an infusion of MT-3 10 minutes post-injection, another group receiving MT-3 2 μg 60 minutes post-injection along with a control group that received saline 60 minutes post-injection were tested on reversal learning (see Figure 4B). The findings indicated that the MT-3 group and saline group tested 60 minutes post-injection achieved criterion in 39.6 ± 2.9 and 40.7 ± 4.1 , respectively. The difference between the groups was not significant, $t(10) = 0.17, p > 0.05$.

4. Discussion

The present experiments demonstrate that blockade of M1-type muscarinic cholinergic receptors in the dorsomedial striatum dose-dependently impairs place reversal learning. In contrast, blockade of M4-type muscarinic cholinergic receptors in the dorsomedial striatum does not impair place reversal learning. The findings are comparable to a previous study demonstrating that pirenzepine infusions into the dorsomedial striatum, a proposed M1-like muscarinic cholinergic antagonist, dose-dependently impairs reversal learning (Tzavos et al., 2004). Even though pirenzepine is considered to be a selective M1-like muscarinic cholinergic antagonist, its selectivity for M1 over M4 muscarinic cholinergic receptors is considered to be only 4-6 times greater (Madison et al. 1987; Nasman et al., 2000). In contrast, MT-7 has a high selectivity for the M1-type muscarinic receptor with no or minimal affinity for the other muscarinic receptor subtypes, including the M4-type receptor (Karlsson, Jolkkonen, Mulugeta, Onali & Adem, 2000). MT-3 has a high selectivity for the M4-type receptor which is 100 to 200-fold greater than for the M1-type receptor (Jolkkonen et al., 1994; Max et al., 1993; Jerusalinsky et al., 1998). Because these different muscarinic toxins are highly selective for specific muscarinic receptor subtypes the present findings more definitely indicate that M1-type muscarinic receptors in the dorsomedial striatum are important for a shift in response patterns.

An infusion of MT-7 at the 2 μg dose did not impair the initial acquisition of the place discrimination, despite producing a reversal learning deficit. This finding suggests that the reversal learning deficit observed with MT-7 2 μg infused into the dorsomedial striatum is not due to a general learning deficit or a change in motor or motivational factors. Furthermore, this

result is consistent with previous studies that have shown that the muscarinic antagonists, scopolamine and pirenzepine, did not impair the initial acquisition of a response discrimination (Ragozzino et al., 2002a; Tzavos et al., 2004). These findings are also comparable to past studies demonstrating that lesions, inactivation with local anesthetics or a NMDA antagonist did not affect initial learning of discrimination tests (Kolb, 1977; Palencia & Ragozzino, 2004; Pisa & Cyr, 1990; Ragozzino et al., 2002b; Ragozzino & Choi, 2004; Thompson, Guilford & Hicks, 1980). Moreover, analysis of misplacements provides some support that the reversal learning deficit observed with M1-type muscarinic receptor blockade is due specifically to its actions in the dorsomedial striatum. Rats that had misplacements in the lateral ventricle or the corpus callosum and received a 1 or 2 μg dose of MT-7 did not show a reversal learning deficit as exhibited by the MT-7- treated rats with cannula placements in the dorsomedial striatum. Although there were four rats with cannula placements in the nucleus accumbens that received MT-7 that performed comparable to that of saline controls, all of these rats received the lowest dose of MT-7 which was ineffective when injected into the dorsomedial striatum. This leaves open the possibility that a higher dose of MT-7 into the nucleus accumbens may have affected reversal learning. However, overall the results suggest that ACh in the dorsomedial striatum may activate muscarinic cholinergic receptors to support a shift in choice patterns, but not an initial discrimination.

In both the acquisition and reversal learning phase of the discrimination task, a rat has to choose between two alternative choice patterns. The key difference between these two phases is that reversal learning requires inhibition of a previously reinforced choice pattern while learning to execute a new choice pattern. During the initial acquisition a rat has not been differentially reinforced across the two choice patterns prior to learning nor does a rat normally display a particular bias for a location in this task. Thus, cholinergic activity, particularly mediated by muscarinic cholinergic receptors in the dorsomedial striatum, may be specifically involved during tasks that require a shift in choice patterns and strategies and not during an initial discrimination.

An analysis of the errors during reversal learning can provide a better understanding of what process or processes are altered when a deficit occurs. As in the past experiments, errors were separated into perseverative and regressive. Perseverative errors are those in which a rat initially chooses the previously reinforced arm and provides a measure of the ability to initially inhibit the previously relevant choice pattern and/or generate a new choice pattern. Regressive errors are incorrect choices that occur after a rat has begun to make the new correct choice. This provides a measure of the ability to maintain or learn a new choice pattern once selected. The reversal learning deficit produced by infusions of MT-7 led to a significant increase in regressive errors, but not perseverative errors. This pattern of results is consistent with that observed following scopolamine or pirenzepine infusions into the dorsomedial striatum on reversal learning tests (Ragozzino et al., 2002a; Tzavos et al., 2004). Moreover, measuring ACh output from the dorsomedial striatum revealed that ACh output does not change during the initial trials of reversal learning when a rat is predominantly choosing the previously relevant choice pattern, but increases subsequently as a rat begins to select the new correct choice (Ragozzino & Choi, 2004). The selective increase in regressive errors following MT-7 infusions suggests that activation of M1-type muscarinic receptors in the dorsomedial striatum does not facilitate the initial inhibition of a previously relevant choice pattern or the generation of a new choice pattern, but supports the maintenance of a new choice pattern once selected. These effects in the dorsomedial striatum contrast with lesions or temporary inactivation of the orbitofrontal cortex which also impair reversal learning, but lead to perseverative errors and not regressive errors (Chudasama & Robbins, 2003; Kim & Ragozzino, 2005). The orbitofrontal cortex is known to project to the dorsomedial striatum (Berendse, Galis-de Graaf & Groenewegen, 1992). Thus, these areas may be part of a larger neural circuit that supports

behavioral flexibility by playing distinct, but complimentary roles to facilitate a shift in choice patterns.

In contrast to the effect observed with MT-7, infusions of MT-3 into the dorsomedial striatum did not affect acquisition or reversal learning. These results suggest that M4-type muscarinic receptors in the dorsomedial striatum do not support behavioral flexibility. The doses of MT-3 infused into the dorsomedial striatum and post-injection test periods used in the present study are comparable to those used in previous studies in which MT-3 was found to affect learning or memory (Diehl et al., 2007; Ferreira et al., 2003). There is evidence that a significant portion of M4-type receptors expressed in the striatum are on cholinergic interneurons acting as autoreceptors (Ding, Guzman, Tkatch, Chen, Goldberg, Ebert, Levitt, Wilson, Hamm & Surmeier, 2006). This raises the possibility that blockade of M4-type receptors in the dorsomedial striatum should facilitate ACh release and potentially lead to an enhancement of reversal learning. However, again no effect was observed with MT-3 injections into the dorsomedial striatum. Because different pharmacological treatments can enhance or impair performance on these behavioral flexibility tests (Hatcher et al., 2005; Steere & Arnsten, 1997), the inability to observe enhancement is unlikely related to a “floor” effect in the reversal learning session. Instead the results suggest that blockade of M4-type muscarinic cholinergic receptors in the dorsomedial striatum does not affect a shift in choice patterns.

At present, why there are dissociated effects of M1-type and M4-type muscarinic receptor blockade in the dorsomedial striatum on reversal learning is unclear. M4 muscarinic receptors are expressed in approximately half of the striatal projection neurons of the striatum while M1 muscarinic receptors are expressed in nearly all the striatal projection neurons (Bernard, Normand, & Bloch, 1992; Hersch et al., 1994). Based on these findings, one possibility is that blockade of M1-type muscarinic receptors affects activity in a greater number of output neurons than M4-type receptor blockade which led to the differential findings on reversal learning. In a somewhat related manner, blockade of M1-type muscarinic receptors may affect a different set of projection neurons, than blockade of M4-type muscarinic receptors. There is evidence that M4-type muscarinic receptors are preferentially expressed in striatonigral projections as opposed to striatopallidal neurons. (Harrison, Tissot & Wiley, 1996; Santiago & Potter, 2001; Yan et al., 2001). Therefore, another possibility is that altering activity in striatopallidal neurons or modifying activity in both striatopallidal and striatonigral projection neurons by M1-type muscarinic receptor blockade produces a reversal learning deficit as opposed to modifying activity preferentially in striatonigral neurons alone by M4-type muscarinic receptor blockade. Still another possibility is that the dissociation between M1- and M4-type muscarinic receptors is due to these receptor subtypes being coupled to different G-proteins leading to different effects on a neuron (Caulfield & Birdsall, 1998; Sanchez-Lemus, Arias-Montano, 2006). Clearly, future studies are needed to better understand the possible functional differences between M1 and M4 muscarinic cholinergic receptors in the striatum.

The present findings suggest that activity in cholinergic interneurons may increase to selectively activate M1-type muscarinic receptors in the dorsomedial striatum to enhance behavioral flexibility. At a neurophysiological level, the cholinergic interneurons are thought to be the tonically active neurons (TANs) [Aosaki et al., 1994, 1995; Graybiel, 1995, 1998; Wilson, Chang & Kitai, 1990]. These neurons commonly exhibit an initial and transient decrease in activity following a conditioned stimulus and subsequently a rebound in activation (Kimura et al., 1984; Ravel, Legallet & Apicella, 1999; Ravel, Sardo, Legallet & Apicella, 2001). In addition, there is a greater recruitment of TANs during learning and possibly an enhanced coordination of these neurons (Aosaki, Tsubokawa, Ishida, Watanabe, Graybiel & Kimura, 1994). This greater recruitment of TANs during learning would likely be observed as an increase in striatal ACh levels. This would be consistent with previous findings indicating

an increase in dorsomedial striatal ACh output during reversal learning (Ragozzino & Choi, 2004; Palencia & Ragozzino, 2006).

The dynamic changes in the activity of TANs recorded from non-human primates have been commonly reported during initial acquisition of different associative learning tests (Kimura et al., 1984; Aosaki et al., 1994). In the rat dorsomedial striatum, changes in ACh efflux or muscarinic receptor effects have not been observed in the acquisition of discrimination tests, but during tests that require a shift in choice patterns. One possible explanation for this difference is that cholinergic interneurons in the striatum play a broad role in learning and memory, but contribute to different learning and memory processes based on regional specificity. In the rat, changes in ACh output have been observed during reversal learning in the dorsomedial striatum (Palencia & Ragozzino, 2006; Ragozzino & Choi, 2004), but not from the dorsolateral striatum (Palencia & Ragozzino, unpublished observations). In the dorsolateral striatum, Gold and colleagues (2003, 2005) found that ACh output from the dorsolateral striatum gradually increases as a rat acquires a response strategy. Furthermore, measuring ACh output and examining muscarinic cholinergic receptor effects in the nucleus accumbens are important for learning related to drug and food reinforcement (Crespo, Sturm, Saria & Zernig, 2006; Mark, Hajnal, Kinney & Keys, 1999; Pratt & Kelley, 2004). Although there are differences in cholinergic actions within different regions of the rat striatum, in general, TAN recordings in non-human primates have not revealed a difference in correlated firing related to learning whether recording from the caudate or putamen (for review see Apicella, 2002). Unclear is whether this may represent a functional difference across species.

Although TANs exhibit changes during the acquisition of a task or in the execution of a task that has been learned, some of the tests used have required a shifting of responses based on the presentation of conditioned stimuli (Kimura et al., 1986; Shimo & Hikosaka, 2001). Moreover, Apicella and colleagues (1991) found that the activity of TANs within the striatum changed during a delayed go/no-go task that demands flexible responding for goal-directed behavior. TANs also exhibited changes in activity when either the timing of a predictive cue is changed or the timing of reward delivery (Apicella, Legallet & Trouche, 1997; Ravel et al., 2001). This change in TANs activity with a switch in conditions may facilitate one's ability to adapt to changing environmental conditions in order to achieve a goal.

M1-type muscarinic receptor blockade in the dorsomedial striatum producing a reversal learning deficit raises the issue of how cholinergic interneuron activity via these receptors affects other striatal circuitry to support behavioral flexibility. Medium spiny projection neurons, which express M1-type muscarinic cholinergic receptors, are gradually activated during associative learning (Graybiel, 1998). The increase in ACh levels and the activation of M1-type cholinergic receptors may be the interface for ACh actions on medium spiny projection neurons. The change in firing of TANs and the gradual change in firing of the medium spiny output neurons may correspond to the latency to form and maintain new associations. One possibility is that a similar type of plasticity occurs in the rat dorsomedial striatum during reversal learning. Specifically, the firing pattern of TANs may change when a previously learned strategy or choice pattern must be inhibited and a new choice pattern learned. The change in TANs may lead to an increased activation of M1-type muscarinic cholinergic receptors on medium spiny neurons which leads to a changed output pattern and ultimately enables the formation and maintenance of the new association after the previously relevant one has been inhibited.

The description above raises a possible mechanism between cholinergic interneurons and medium spiny output neurons of the striatum to support behavioral flexibility. At a circuit level, cholinergic interneurons may be positioned to influence changes in goal-directed behavior. In particular these cells are often found at the striosome-matrix border and one idea is that TANs

play a key role in integrating activity in different striatal modules that influences learning (Graybiel, 1995, 1998). Thus, one possibility is that the cholinergic interneurons in the rat dorsomedial striatum may play a crucial role in coordinating cortico-striatal modules that enhance a shift in strategies.

Blockade of M1-type muscarinic receptors in the dorsomedial striatum impaired reversal learning by slowing the learning of the new task requirement. If M1-type muscarinic cholinergic receptors in the dorsomedial striatum were essential for reversal learning, then MT-7 injections should have prevented reversal learning. However, this was not the case. This result is consistent with previous studies showing that inactivation of the dorsomedial striatum or non-specific blockade of muscarinic receptors in the dorsomedial striatum did not prevent reversal learning but impaired it (Ragozzino et al., 2002a; Tzavos et al., 2004). These studies suggest that changes in muscarinic cholinergic receptor activity in the dorsomedial striatum play a role in shifting a choice pattern or strategy, however, activation of muscarinic cholinergic receptors is not essential for enabling a shift in choice patterns or strategies. Although cholinergic interneurons may not be essential for the expression of a behavioral response, cholinergic activity in the striatum may facilitate or modulate the coordination between cortico-striatal circuits that leads to an adaptive response (Graybiel., 1998).

In summary, the present experiments demonstrated that blockade of M1-type muscarinic cholinergic receptors in the dorsomedial striatum does not impair initial learning of a place discrimination, but dose-dependently impairs place reversal learning. The reversal learning deficit produced by M1-type receptor blockade results from an impairment in maintaining a new choice pattern once selected. In contrast, M4-type muscarinic receptor blockade in the dorsomedial striatum does not impair acquisition or reversal learning. Taken together, the results suggest that M1-type muscarinic, but not M4-type muscarinic receptors support behavioral flexibility.

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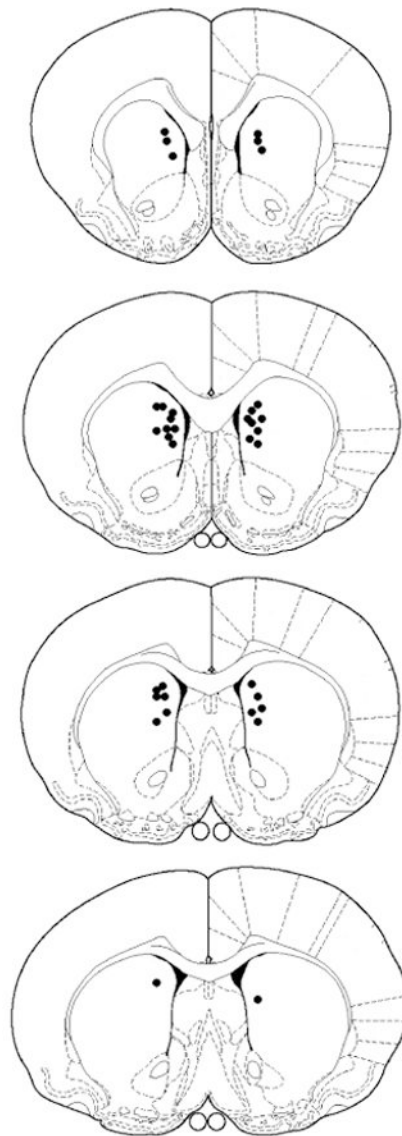


Figure 1.

Placement of cannula tips in the dorsomedial striatum for rats included in the behavioral analyses for Experiment 1. The location of the cannula tip placements ranged from 0.48 - 1.7 mm anterior to bregma. The rat brain sections were modified from the atlas of Paxinos and Watson (1996). The number of circles does not match the total number of cannula tips for rats included in the behavioral analyses because some cannula placements overlapped to such a large extent that a single circle represents more than one cannula tip placement.

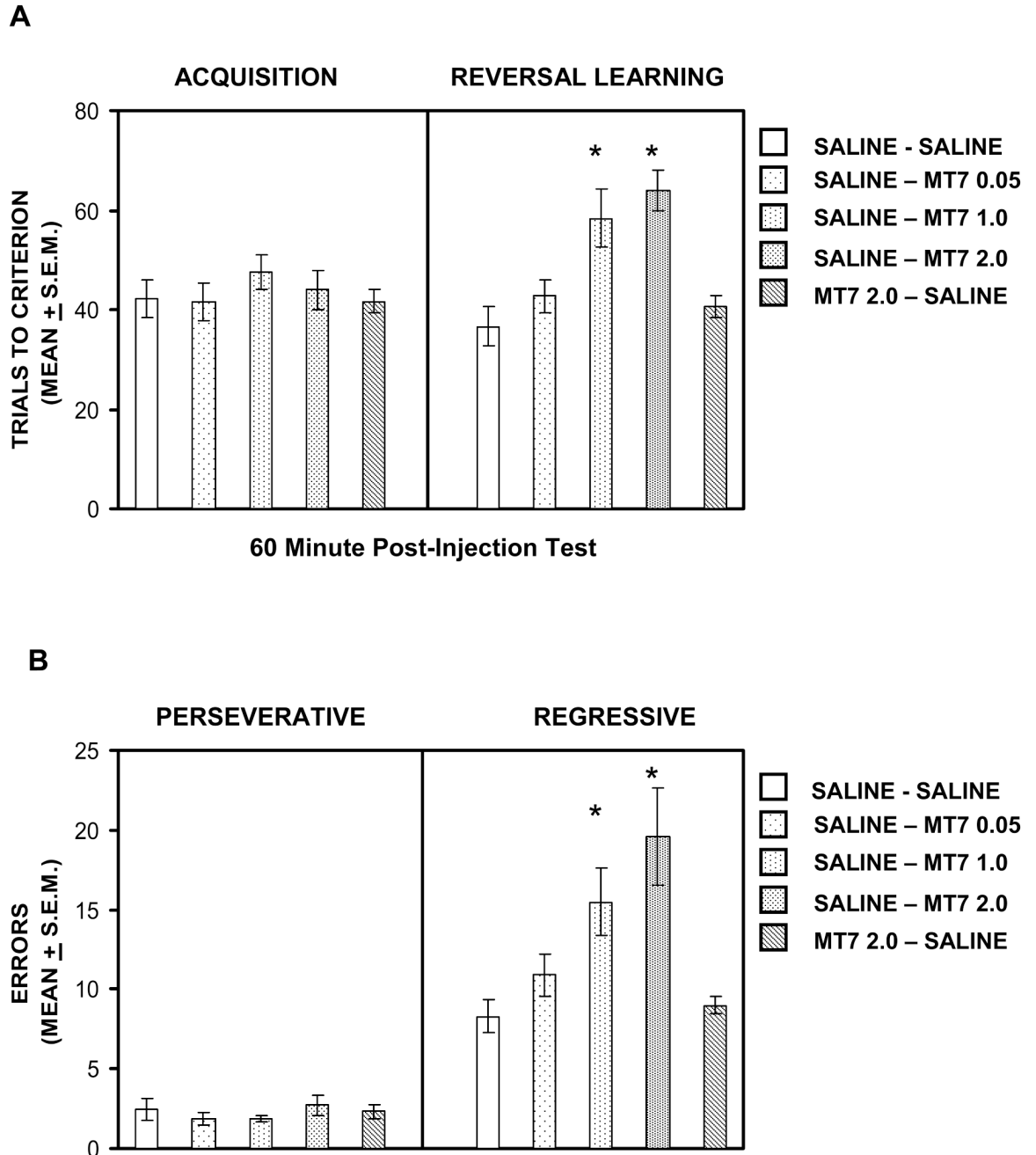


Figure 2.

A) Mean (\pm s.e.m.) trials to criterion in acquisition and reversal learning of a place discrimination following bilateral infusions of saline or MT-7 into the dorsomedial striatum 60 minutes post-injection. Treatment with MT-7 2.0 μ g into the dorsomedial striatum did not affect acquisition. MT-7 at 1.0 and 2.0 μ g significantly increased trials to criterion compared to that of saline controls. In the legend, the numbers juxtaposed to the MT-7 represent the microgram doses of MT-7 used for intracranial infusions. * = $p < 0.05$ vs. saline controls. B) Mean (\pm s.e.m.) number of perseverative and regressive errors in reversal learning after bilateral infusions of saline or MT-7 into the dorsomedial striatum. There was no difference among the groups in perseverative errors. MT-7 at 1.0 and 2.0 μ g significantly increased regressive errors

compared to that of the saline-saline group. The numbers juxtaposed to the MT-7 represent the microgram doses of MT-7 used for intracranial infusions * = $p < 0.05$ vs. saline-saline group.

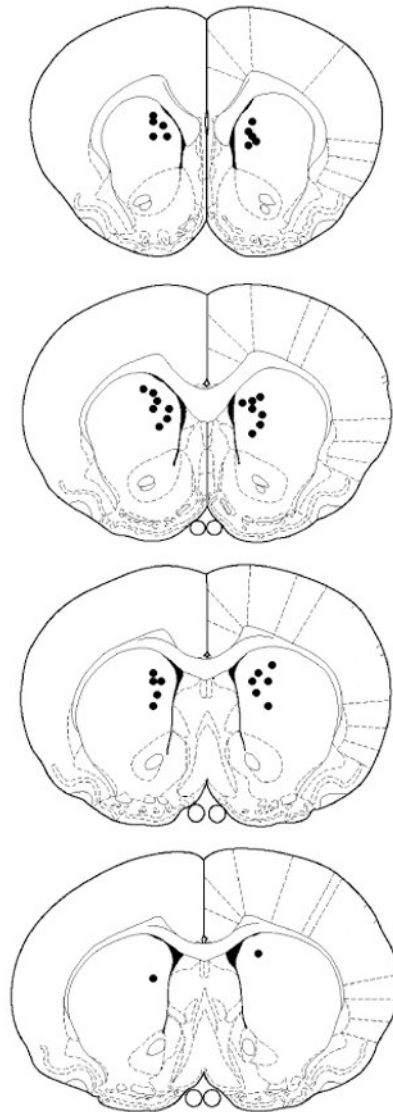


Figure 3.

Placement of cannula tips in the dorsomedial striatum for rats included in the behavioral analyses for Experiment 2. The location of the cannula tip placements ranged from 0.48 - 1.7 mm anterior to bregma. The rat brain sections were modified from the atlas of Paxinos and Watson (1996). The number of circles does not match the total number of cannula tips for rats included in the behavioral analyses because some cannula placements overlapped to such a large extent that a single circle represents more than one cannula tip placement.

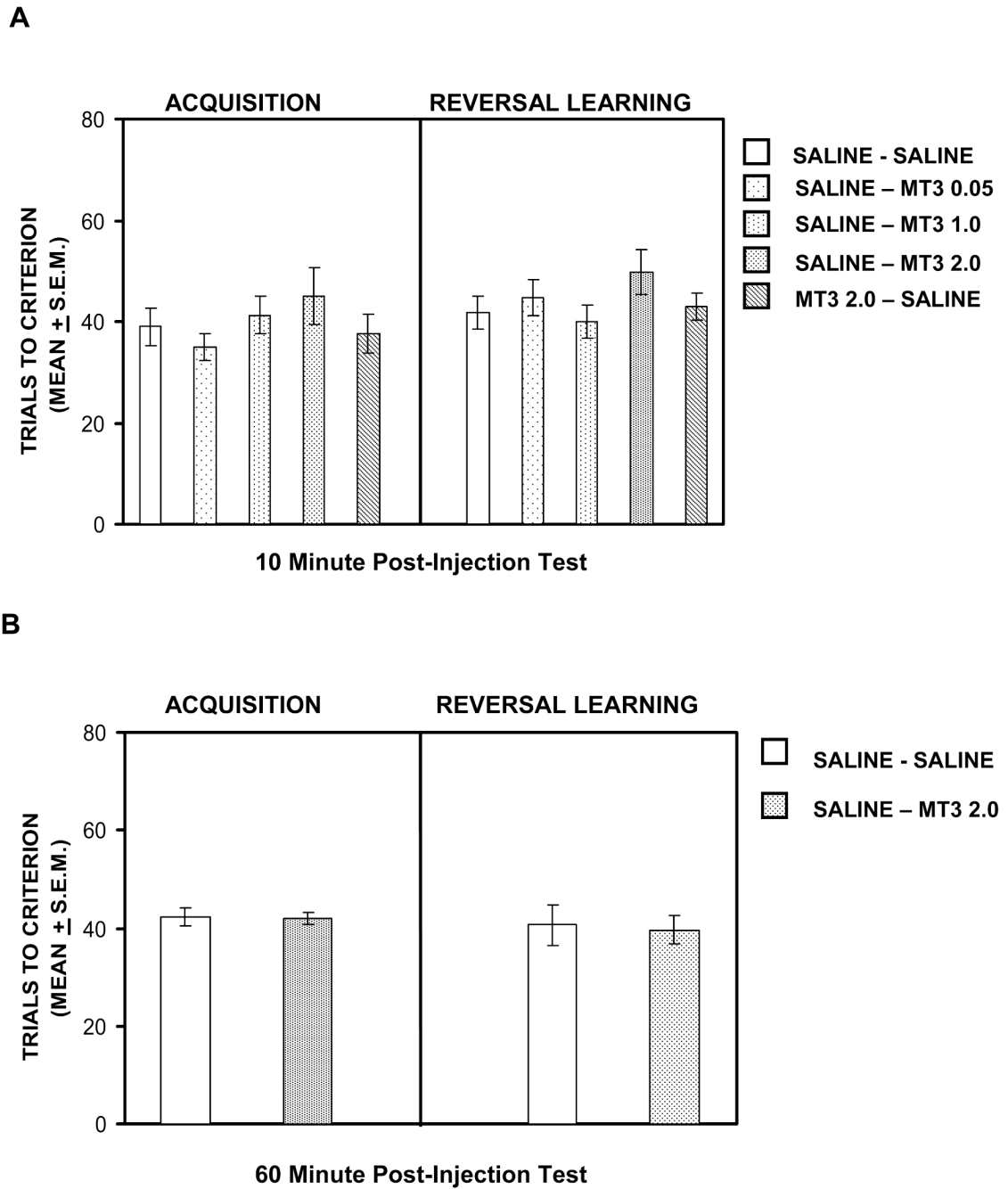


Figure 4.
 A) Mean (\pm s.e.m.) trials to criterion in acquisition and reversal learning of a place discrimination following bilateral infusions of saline or MT-3 into the dorsomedial striatum 10 minutes post-injection. Treatment with MT-3 2.0 μ g into the dorsomedial striatum did not affect acquisition. MT-3 at 0.05, 1.0 or 2.0 μ g did not affect reversal learning compared to that of saline controls. In the legend, the numbers juxtaposed to the MT-3 represent the microgram doses of MT-3 used for intracranial infusions. B) Mean (\pm s.e.m.) trials to criterion in acquisition and reversal learning of a place discrimination following bilateral infusions of saline or MT-3 into the dorsomedial striatum 60 minutes post-injection. Treatment with MT-3 2.0 μ g into the dorsomedial striatum did not affect acquisition. MT-3 at 0.05, 1.0 or 2.0 μ g did

not affect reversal learning compared to that of saline controls. In the legend, the numbers juxtaposed to the MT-3 represent the microgram doses of MT-3 used for intracranial infusions.