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Blockade of Muscarinic M1 Receptors Disrupts Performance on an Attention-Demanding Visual Discrimination Task

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ABSTRACT PAGE

Previous studies have shown that muscarinic receptor blockade decreases signal detection in a sustained attention task that requires discrimination of visual signals from trials with no signal presentation. However, the exact role of specific muscarinic receptor subtypes in attentional performance remains unclear. The present experiments examined the effects of blocking M1 receptors on attentional performance in rats. Rats were trained in a two-lever sustained attention task that required discrimination of visual signals (500, 100, 25 ms) from "blank" trials when no signal was presented. In Experiment 1, rats received the M1 receptor antagonist, dicyclomine (0, 0.625, 1.25, 2.5, and 5.0 mg/kg; ip), prior to performance in this task and prior to performing the task with a houselight flashing throughout the session or with a shorter intertrial interval. Dicyclomine (5.0 mg/kg) decreased the detection of signals in the standard task when compared to vehicle. Similarly, accuracy was decreased following 5.0 mg/kg dicyclomine compared to vehicle with a shorter intertrial interval, however, the deficit was only observed following the 100 ms signal. In the distracter task, when vehicle was compared to drug treatment, animals exhibited significant signal detection deficits at lower doses compared to the standard task. In Experiment 2, a guide cannula was surgically implanted into either the right or left ventricle of rats after reaching stable performance levels. All animals received infusions of the M1 receptor antagonist, pirenzepine (0, 10, 30, and 60 µg) prior to performing the standard attention task. Pirenzepine decreased accuracy of detecting the 500 ms signal following the highest dose (60 µg) when compared to vehicle. The lack of effects of dicyclomine and pirenzepine on trials with no signal presentation or on omissions suggests that M1 receptor blockade does not disrupt motoric functioning, motivation or the ability to respond based upon the rules of the task. The present results suggest that M1 receptors critically contribute to attentional processing mediated by the central cholinergic system.

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Blockade of muscarinic M1 receptors disrupts performance in an attention-demanding visual discrimination task

Attention has been described as the ability to detect and select stimuli for further processing (Knudsen, 2007). Attention is carried out by a network of anatomical areas, as opposed to a single brain region or a general function of the brain operating as a whole (Posner & Peterson, 1990). Neurological models of attention have differentiated separate subcomponents of attention, such as sustained attention and selective attention, which can be defined functionally and to some extent, anatomically. Vigilance or sustained attention concerns the ability of observers to maintain attention and remain alert to stimuli over prolonged periods of time (Parasuraman, Warm, & Dember, 1987). Being able to sustain attention is a critical behavioral adaptation because it is a basic requirement for the acquisition and recall of information (Sarter, Bruno, & Givens, 2003). The attentional system warrants further investigation because disruptions in this system could impair learning and memory, possibly contributing to deficits in some neuropsychiatric diseases including Alzheimer's disease.

Attentional deficits in Alzheimer's disease

Deficits in attention have been associated with the early stages of Alzheimer's disease (AD) (Perry & Hodges, 1999). Cholinergic neuron loss is thought to underlie the cognitive impairments seen in AD and provides the rationale for cholinergic replacement pharmacotherapies (Bartus, Dean, Beer, & Lippa, 1982). Based on the cholinergic hypothesis, acetylcholinesterase inhibitors

(AChE-Is) have emerged as the main therapeutic agents in treating AD. The AChE-Is act on the acetylcholine (ACh) pathway by inhibiting the enzyme acetylcholinesterase, which is normally responsible for hydrolysis of ACh. AChE-Is have been found to improve, or at least slow deterioration for some aspects of cognitive performance in patients with AD (Rogers, Farlow, Doody, Mohs, & Friedhoff, 1998). Systemic drug administration of an ACh-I, donepezil, has been found to enhance cognitive function (Rogers et al., 1998) including attentional performance (Salloway et al., 2004) in patients with mild to moderate AD. Several long-term clinical trials with AChE-Is have reported that these drugs actually decrease the rate of cognitive decline, and may delay the disease progression by 1 to 2 years (Farlow, 2002) but the benefits are modest (Courtney et al., 2004). AChE-Is are unable to slow progression from mild cognitive impairment to dementia (Raschetti, Albanese, Vanacore, & Maggini, 2007). While AChE-Is are the best current option for the treatment of AD, a drug that is more selective to the particular cholinergic receptors affected by the disease may be better at treating the symptoms and slowing the progression of the disease. AChE-Is act to increase the level and duration of ACh in the synapse so ACh can bind to all cholinergic receptors. A better understanding of the cholinergic receptors involved in attention may allow the development of more targeted drugs to provide maximal attention-related benefits via the cholinergic system.

There is evidence to support the hypothesis that cognitive disturbances associated with AD are due to disruptions in the cholinergic system (Auld,

Kornecook, Bastianetto, & Quirion, 2002). Neurons of the basal forebrain undergo a profound and selective degeneration in AD patients which leads to a cholinergic deficiency in their brains (Bartus et al., 1982; Whitehouse et al., 1982). While the behavioral effects of ACh on the brain have been extensively characterized by administering cholinergic drugs to human subjects, animal experiments have provided further insight into the function of the cholinergic system through controlled experiments. However, the assessment of attention in animals needs to be carefully considered before applying manipulations of brain function.

Measuring attention in the rat

A taxonomy for sustained attention has been developed to understand the different task parameters that can affect performance (Parasuraman et al., 1987). According to this taxonomy, one factor that contributes to sustained attention-demanding tasks is the event rate. Events that are presented quickly or at a variable rate have been shown to disrupt performance in tests of sustained attention (Parasuraman et al., 1987). Another important dimension that places demands on sustained attention is whether signals and nonsignals are presented successively (successive discrimination) from a single location. Successive discrimination places additional demands on the animal because signals have to be distinguished from a nonsignal reference represented in recent memory (Parasuraman et al., 1987). The use of dynamic stimuli, such as signals with variable duration also increases attentional load because they are more difficult to

discriminate (McGaughy & Sarter, 1995). Taking these factors into consideration, McGaughy and Sarter (1995) modified a task by Bushnell, Kelly, and Crofton (1994) to develop an attention-demanding two-lever task that required rats to discriminate between successively presented signals and nonsignals. The task includes several features (competing response rules, variable signal duration, and variable intertrial interval (ITI)) that impose a cognitive load, ensuring that even the basic version of the task cannot be successfully completed on the basis of side biases or simple response timing. The task instead requires directed attention to the presence or absence of brief visual stimuli on each trial. Distraction (in the form of a flashing houselight) (Gill, Sarter, & Givens, 2000; Himmelheber, Sarter, & Bruno, 2000) and decreasing the time between signal and nonsignal events (short ITI) (McGaughy & Sarter, 1995) can be introduced to challenge performance and increase attentional demands. The development of this task has allowed for more in depth research of attention because it improves upon previous tasks that did not provide a valid measure of the subjects' ability to discriminate between signal and nonsignal events. In previous tasks, baseline lever pressing rates could confound the false alarm rate (McGaughy & Sarter, 1995). Specifically, previous tasks had a lever extended throughout the session and animals were trained to press the lever after a stimulus (e.g., a brief light) was presented. Lever presses in the absence of the light were considered false alarms. However, any manipulation (e.g, drug treatment) that influences baseline lever pressing rate would be expected to impact the false alarm rate. Thus, it was

difficult to establish whether changes in the false alarm rate reflected changes in signal processing or the baseline lever pressing rate. The modified task prevents spontaneous lever pressing because the levers are only extended after a signal or nonsignal event and are retracted during the ITI.

Basal forebrain cholinergic system

The basal forebrain is a collection of structures that lie near the bottom of the anterior portion of the brain, ventrally to the striatum that includes the nucleus basalis of Meynert, diagonal band of Broca, and medial septal nuclei (Mesulam, Mufson, Wainer, & Levey, 1983). Collectively, from these structures within the basal forebrain, cholinergic neurons send projections widely throughout the brain, including to the amygdala, thalamus, cortical mantle, hippocampal formation, and the olfactory bulb (Mesulam et al., 1983).

Experiments that have selectively damaged the basal forebrain have been used for elucidating the function of this structure, particularly with regard to cognitive processing. Excitotoxic lesions of the basal forebrain have been shown to decrease accuracy on a five-choice serial reaction time task which is designed to assess multiple aspects of cognition, such as sustained attention and impulsivity (Muir, Everit, & Robbins, 1994). Tasks that place explicit demands on attentional processes rather than learning and memory appear particularly sensitive to lesions of the basal forebrain (Voykto et al., 1994). The development of a selective cholinergic immunotoxin, 192 IgG-saporin, has provided further support for the role of the basal forebrain corticopetal cholinergic neurons in attention

(McGaughy, Everitt, Robbins, & Sarter, 2000). 192 IgG is an antibody to the rat p75 nerve growth receptor. This antibody can be coupled to saporin, a ribosome-inactivating protein that causes cell death (Wiley, Oeltmann, & Lappi, 1991). 192 IgG-saporin, specifically targets and destroys cortically-projecting cholinergic neurons of the basal forebrain, the only neurons in the basal forebrain that express the p75 nerve growth factor receptor (Wiley et al., 1991). McGaughy, Kaiser, & Sarter (1996) found that intrabasalis infusions of 192 IgG-saporin decrease signal detection in a sustained attention task. In a separate experiment employing a five-choice serial reaction time task, intrabasalis infusions of high or low doses of 192 IgG-saporin produced different degrees of damage that correlated with the degree of accuracy deficit (McGaughy, Dalley, Morrison, Everitt, & Robbins, 2002). Furthermore, the accuracy deficit was significantly correlated with a reduction in cortical ACh efflux in rats with extensive lesions only.

Although cortical cholinergic inputs are critical for maintaining attentional performance they are of particular importance under conditions of increased attentional demands. Burk, Lowder, and Altemose (2008) found that loss of basal forebrain corticopetal cholinergic neurons can decrease signal detection in a sustained attention task, but only under conditions when multiple aspects of the task are attention-demanding. Animals were initially trained with minimal attentional demands and then following loss of cortical cholinergic inputs the task parameters were altered to increase attentional demands. No single task parameter disrupted signal detection following loss of basal forebrain corticopetal

cholinergic neurons, but signal detection was disrupted under conditions when multiple aspects of the task were attention-demanding. Thus, cortical ACh is critical for attentional effort or the recruitment of attentional mechanisms in response to challenges (Sarter, Gehring, & Kozak, 2006).

Cortical ACh and attention

Cortical ACh release is more highly elevated in rats performing a sustained attention task compared with control tasks (Arnold, Burk, Hodgson, Sarter, & Bruno, 2002; Passetti, Dalley, O'Connell, Everitt, & Robbins, 2000). Studies assessing cortical ACh release in attentional task-performing rats have generated preliminary evidence suggesting that increased demands on sustained attention performance are associated with increases in cortical ACh efflux (Himmelheber et al., 2000). ACh efflux in the frontoparietal cortex was studied with *in vivo* microdialysis while rats performed in an operant task designed to assess sustained attention (Himmelheber et al., 2000). A visual distracter was used during task performance to increase attentional demands. When animals performed under increased attentional demands there was an increase in cortical ACh efflux. The authors speculate that the effects of the distracter on performance and cortical ACh efflux were due to its ability to increase background noise and disrupt active attentional processing. This finding extends hypotheses regarding the crucial role of cortical cholinergic transmission for attentional functions. The effects of the distracter stimulus provide evidence for a direct relationship between attentional effort and cortical ACh release.

Recent evidence from studies employing choline-sensitive biosensors suggest that ACh regulates attentional processing on multiple time scales, involving a tonic component that relates more to arousal and a phasic component that appears to contribute more to cue detection (Parikh, Kozak, Martinez, & Sarter, 2007). These studies support the general hypothesis that cortical cholinergic inputs, while not exclusively mediating attentional processes, are likely to be activated by behavioral situations taxing attentional capabilities.

Muscarinic receptors

ACh binds to both muscarinic and nicotinic cholinergic receptors (Cooper, Bloom, & Roth, 2003). The blockade of muscarinic receptors, but not nicotinic receptors, disrupts attentional performance in monkeys (Herrero et al., 2008) and in rats (Johnson & Burk, 2006; McQuail & Burk, 2006). Muscarinic receptors belong to a class of metabotropic receptors which use G proteins as their signaling mechanism (Cooper, et al., 2003). Muscarinic acetylcholine receptors are involved in memory and attention (Broks et al., 1988), and it is hypothesized that a muscarinic agonist could provide a replacement therapy in AD. However, the exact role of specific muscarinic receptor subtypes in attentional performance remains unclear. Five different muscarinic receptor subtypes (M1-M5) have been identified. In terms of a role in cognitive processing, most research has assessed the contributions of M1 and M2 receptors. M1 receptors are primarily post-synaptic receptors and M2 receptors are pre-synaptic autoreceptors, which act to negatively modulate acetylcholine release (Clader & Wang, 2005). Muscarinic

receptor blockade produces cognitive impairments (McQuail & Burk, 2006), and it is thought that these effects most likely involve M1 receptors because M2 receptor blockade would be expected to increase acetylcholine release. M2 receptors have also been shown to decline in AD brains while M1 receptor levels remained unchanged (Flynn, Ferrari-DiLeo, Levey, & Marsh, 1995). With decreasing levels of M2 receptors there may not be adequate neural substrate for M2 receptor antagonists to act upon for treating age- and dementia-related cognitive decline. Because M1 receptors are retained it makes the M1 receptor an attractive target for symptomatic treatment of AD. This approach is further supported by muscarinic M1 receptor agonists that have improved performance on cognitive tests in Alzheimer's patients (Bodick, et al., 1997) and cognition in animal models of the disease (Fisher, Brandeis, Chapman, Pittel, & Michaelson, 1998; Genis, Fisher, & Michaelson, 1999). Furthermore, impairment of M1 mediated signaling may underlie the cognitive decline of AD via effects on protein kinase C activity and NMDA receptor density (Tsang et al., 2007).

Muscarinic M1 receptor agonists have been shown to enhance learning and memory in animals. The muscarinic M1 receptor agonists, arecholine, pilocarpine, and McN-A-343, facilitated learning acquisition in an active-avoidance paradigm in rats which records an anticipatory conditioned avoidance apart from the classical conditioned avoidance response (Sen & Bhatacharya, 1991). This learning enhancement was later attenuated by the selective M1 antagonist, pirenzepine. In addition, M1 receptor agonists have been found to

significantly reduce age-related cognitive impairments (Brandeis, Dachir, Sapir, Levy, & Fisher, 1990). Memory functions were impaired in the Morris Water Maze and 8-arm radial arm maze in aged rats compared to young animals. The administration of AF102B, an M1 receptor agonist, significantly reduced the age-related cognitive impairments observed in both tasks. The ability of muscarinic M1 receptor agonists to enhance performance in these memory tasks may result from improved attention capacity that can aid in the acquisition of spatial navigation strategies (Aura, Sirvio, & Riekkinen Jr., 1997). These data support the hypothesis that enhancement of cholinergic function may reverse geriatric cognitive deficits.

Not surprisingly, M1 receptor antagonists have been found to disrupt cognitive performance. Pirenzepine, an M1 receptor antagonist, disrupted spatial memory (Bymaster, Heath, Hendrix & Shannon, 1993), as well as performance accuracy on a delayed non-matching to position task (Aura et al., 1997). Mice with a mutation of the gene coding for the M1 receptor showed impaired working memory on the radial arm maze as well as impaired acquisition and consolidation of contextual fear conditioning (Anagnostaras et al., 2003). This finding suggests that the M1 receptor is specifically involved in memory processes for which the cortex and hippocampus interact. Collectively, these data support the idea that the M1 receptor is critical in cognitive processing.

Despite the evidence that muscarinic M1 receptors are involved in memory, the role of these receptors in attentional functioning has not been

thoroughly investigated. Although it has been suggested that M1 receptors are critical for attentional performance there are currently no studies that examine the role of the M1 receptor in an attention demanding task. The present experiments were designed to investigate the hypothesis that muscarinic M1 activation is necessary for performance in an attentional task. Experiment 1 examined the effects of systemic administration of dicyclomine, an M1 receptor antagonist, on attentional performance in rats. A previously validated attention task designed to place high demands on attentional processing (e.g., brief and variable signal durations and ITIs) and that required the discrimination of a brief visual signal from trials with no signal presentation was used. Furthermore, Experiment 1 investigated the role of the M1 receptor when attentional demands were increased. Accuracy on the task has been previously shown to decrease when background noise is increased by flashing a houselight as well as when the ITI is decreased (McGaughy & Sarter, 1995). M1 receptor blockade is hypothesized to differentially disrupt performance when attentional demands are increased. In Experiment 2 the effects of intracerebroventricular (icv) infusions of pirenzepine (M1 receptor antagonist) were investigated in the same sustained attention task used in Experiment 1. Different M1 receptor antagonists were used in Experiment 1 and Experiment 2 in order to test the generalizability of any effects of M1 receptor blockade on attentional performance. ICV administration was used to create widespread receptor blockade, while avoiding any peripheral effects of systemic administration. Widespread receptor blockade was important

because only extensive loss of cortical cholinergic inputs have been found to disrupt performance in the standard task (McGaughy et al., 1996; Newman & McGaughy, 2008). Administration of pirenzepine was expected to disrupt detection of visual signals, but not to affect accuracy on nonsignal trials. The lack of effect of dicyclomine and pirenzepine on trials with no signal presentation suggests that M1 receptor blockade does not disrupt the ability to respond based upon the rules of the task, motoric functioning or motivation. This selective pattern of results has also been observed after lesions of basal forebrain corticopetal cholinergic neurons (McGaughy et al., 1996), so a similar pattern of results would suggest that M1 receptors critically contribute to attentional processing mediated by the central cholinergic system.

Method

Experiment 1

Subjects

Subjects were nine male Long-Evans rats; weighing 151-175 g at the beginning of the experiment (Charles River Laboratories Inc., Wilmington, MA). Animals were housed individually in hanging wire cages in a vivarium with a 14/10 h light/dark cycle (lights on 0600-2000). All behavioral testing occurred during the light cycle between 0900 and 1200, for five or six days a week. Rats were permitted to feed freely, but were water restricted on testing days, receiving water during task performance and for 30 min following testing sessions. On days the rats were not tested they were allowed free access to water overnight or

for 1 h during the day. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the College of William and Mary.

Apparatus

Rats were trained in one of 12 chambers (Med Associates, Inc., Georgia, VT) each enclosed within a sound-attenuating box. One side of the chamber contained two retractable levers, a water port with a dipper to deliver water (0.01 ml) situated between the two levers, and a centrally located panel light located above the water port. A houselight was located in the back of the chamber. The behavioral testing programs and data collection were managed by a personal computer utilizing Med-PC version IV software.

Behavioral training

The houselight remained illuminated throughout all testing sessions. In the first stage of training, the levers were extended throughout the session and the dipper was raised following each lever press. However, following five consecutive presses on a single lever, the other lever had to be pressed to receive water access. The rule was included to attempt to prevent a lever bias. After reaching a criterion of 120 lever presses per session for three sessions, rats were trained to discriminate between signals (1 s illumination of the panel light) and nonsignals (no illumination of the panel light). After a signal or nonsignal, the levers were extended into the chamber. The rules for a correct response were counterbalanced across animals. For half the animals, following a signal, a press on the left lever was considered a "hit" and the rat received water access. A

response on the right lever was considered incorrect, scored as a "miss" and the rat received no water. After a nonsignal, a press on the right lever was considered correct, scored as a "correct rejection" and water was given, while pressing the left lever was considered a "false alarm". After a lever press, levers were retracted. Failure to press either lever within 3 s was considered an "omission". Half of the animals were trained based on these rules and the other half trained with the reverse rules (e.g., right lever press correct after a signal and left lever press correct after a nonsignal). The ITI varied (12±3 s) during training to prevent the rats from anticipating the onset of the next trial. Incorrect responses were followed by a correction trial that was identical to the previous trial. Three consecutive incorrect responses triggered a forced trial where only the correct lever was extended into the chamber for 90 s. When the three consecutive errors occurred on signal trials, the panel light remained illuminated for the duration of the forced trial. Animals were trained in this task until they reached criterion of 70% hits and 70% correct rejections for three consecutive sessions. In the next level of training the signal duration was reduced and varied within each session (500, 100, or 25 ms). A session consisted of 162 trials with an ITI of 9 ± 3 sec. The signal duration and ITI were decreased to place higher demands on attentional processing (McGaughy & Sarter, 1995). Animals were trained in this version of the task to a criterion of 70% hits at the 500 ms and 70% correct rejections for three consecutive sessions in order to move to the drug administration phase of the experiment.

Drug administration and behavioral testing

Dicyclomine was dissolved in saline and injected into the intraperitoneal cavity in a volume on 1.0 ml/kg. The doses used were 0.625, 1.25, 2.50, 5.0 mg/kg of dicyclomine. The dicyclomine solution was heated for approximately 5 min until the solution was visibly dissolved.

Rats each received five sessions with three different task manipulations: standard task, short ITI, and distracter task. In the short ITI version of the task, the ITI was decreased to 4.5 $\pm$ 3 sec. In the distracter task, the houselight flashed on and off at 1 s intervals for the entire session. The order of the task manipulations and drug administration was randomly assigned to each rat. Rats received each drug dose for all three task manipulations, for a total of 15 injections. After an injection, animals were placed in the testing chamber and the program was started. There was a 15 min delay between drug administration and testing to allow the drug to take effect (Fornari, Moreira, & Oliveira, 2000). Between drug administration days, rats returned to the standard task and were required to meet a criterion of 70% hits at the 500 ms signal and 70% correct rejections before proceeding to the next drug administration day.

Behavioral measures

The number of hits, misses, correct rejections, false alarms, and omissions were recorded for each testing session. Omissions were analyzed separately from measures of response accuracy.

Statistical analysis

The effects of the drug were analyzed separately for each task manipulation. The relative number of hits (percentage of hits divided by 100) was analyzed using a repeated-measures analysis of variances (ANOVA) with the factors of signal duration and drug dose. The relative number of correct rejections (percentage of correct rejections divided by 100) was also analyzed using a repeated-measures ANOVA. A repeated-measures ANOVA was also used to analyze the number of omissions. Data analyses were conducted with SPSS 15.0 for Windows (SPSS, Chicago, IL). *P* values were corrected with the Huynh-Feldt procedure. An α level of 0.05 was used to determine statistical significance.

Experiment 2

Subjects

Subjects were ten male Long-Evans rats (Charles River Laboratories Inc., Wilmington, MA). Animals were housed identically to those in Experiment 1. Rats were also water restricted for the duration of the experiment, receiving water during task performance and for 30 min following testing sessions. The experimental protocol was approved by the Institutional Animal Care and Use Committee at the College of William and Mary.

Apparatus and behavioral training

Animals were trained in the same chambers using the same procedure prior to drug administration as in Experiment 1.

Surgical procedures

Once animals met a criterion of 70% hits at the 500 ms and 70% correct rejections for three consecutive sessions they moved to the surgery phase of the experiment. Before surgery animals were anesthetized with an intraperitoneal injection of 90.0 mg/kg ketamine and 9.0 mg/kg xylazine. The head of the rat was shaved and then placed in a Kopf stereotaxic instrument. An incision was made through the skin down the center of the head to expose the skull. A hole was drilled and the cannula (8 mm, 22 gauge) was placed into either the right (50% of the rats) or left lateral ventricle at the following co-ordinates: AP = -0.8 mm posterior, L = 1.6 mm lateral to bregma and V = -2.5 mm relative to bregma. Three additional holes were drilled for supporting screws, one anterior to the guide cannula and two posterior to the guide cannula. The guide cannula and three supporting screws were covered in dental acrylic cement. Animals were given acetaminophen (2.7 mg/ml) in their water bottle 24 h before surgery and 3 days following surgery for pain relief.

Post-surgical drug administration and behavioral training

The animals were allowed one week of free water access after surgery before returning to training. After returning to a water-deprivation schedule, rats were required to maintain 70% hits and 70% correct rejections for 3 sessions before drugs could be administered. All rats received infusions of: 0, 10, 30, and 60 μg pirenzepine, dissolved into saline. Drug administration occurred via an internal cannula that extended 1.0 mm below the guide cannula. Polyethylene

tubing connected the cannula to a 10 µl Hamilton syringe. The syringe was loaded into a microinfusion pump (Pump II, Harvard Apparatus). Solutions were infused at a rate of 1.0 µl/min and the total volume injected was 2.5 µl. The infused solution was allowed to diffuse for 1 min before replacing the infusion cannula with dummy cannula to prevent clogging. There was a 10 min delay between drug administration and testing to allow the drug to take effect (Aura et al., 1997).

Histology

Following completion of the drug schedule, rats were anesthetized and intracardially perfused at 300 mm Hg with 10% sucrose followed by 10% formalin. Perfused brains were left overnight in formalin prior to being placed in 30% sucrose phosphate buffer for approximately three days. The brains were frozen and sectioned in the coronal plane with a microtome (50 µm). The brain sections were mounted on gelatin-coated slides and dried overnight before being stained with cresyl violet and cover-slipped. The sections were then examined to determine the placement of the cannula tips.

Behavioral measures and statistical analysis

The same behavioral measures and statistical analysis used in Experiment 1 was used in Experiment 2.

Results

Experiment 1

The data reported are from the nine rats that maintained stable performance levels between drug administration sessions. The effects of dicyclomine were analyzed separately for each task manipulation.

Standard task

A repeated-measures dose x signal duration ANOVA on the relative number of hits for the three signal durations on the standard task found a significant main effect for signal duration ($F(2, 16) = 142.74, p < .001$). Rats exhibited signal duration-dependent accuracy, with the hit rate higher following longer signal durations. A main effect of dose was also observed with a decrease in hits associated with an increase in the drug dose, ($F(4,32) = 3.49, p = .018$) (Figure 1). A paired samples t test revealed a significant difference between vehicle and the highest drug dose (5.0 mg/kg dicyclomine) ($t(8) = 2.52, p = .036$). A repeated-measures ANOVA for the relative number of correct rejections (Figure 2) or for omissions (Figure 3) found no significant main effects for dose.

Distracter task

A repeated-measures ANOVA found a significant main effect for signal duration on the relative number of hits ($F(2,16) = 130.45, p < .001$), with a higher degree of accuracy following the longest signal duration (Figure 4). No main effect of drug dose or signal duration x drug dose interaction was observed. However, when the drug doses were averaged together and compared to vehicle a

main effect of drug was observed ($F(1,8) = 7.51, p = .025$) as well as a dose x signal duration interaction ($F(2,16) = 146.08, p = .034$). A t test was performed at each signal duration comparing vehicle to the average of the drug doses. Of those, the 500 ms signal was the only signal duration to show a significant difference ($t(8) = 6.96, p < .001$). Further t tests at the 500 ms signal duration revealed significant differences in hits when vehicle was compared to 0.625 mg/kg dicyclomine ($t(8) = 4.01, p = .004$), 2.5 mg/kg dicyclomine ($t(8) = 5.93, p < .001$), and 5.0 mg/kg dicylomine ($t(8) = 2.94, p = .019$), but not 1.25 mg/kg dicylomine ($t(8) = 2.159, p > .05$). A repeated-measures ANOVA found no significant main effects for dose for the relative number of correct rejections (Figure 5) or for omissions (Figure 6).

Short ITI

A repeated-measures ANOVA found a significant main effect for signal duration on the relative number of hits for the three signal durations ($F(2,16) = 147.42, p < .001$), with the hit rate higher following the longest signal duration. For hits a significant interaction was found between drug dose and signal duration, ($F(8,64) = 3.05, p = .006$) (Figure 7). A one-way ANOVA was performed at each signal duration comparing drug dose. Of those, the 100 ms signal was the only signal duration to show significant differences between the drug doses ($F(4,32) = 3.83, p = .016$). A paired-samples t test revealed that hits were significantly reduced following the 5.0 mg/kg dose when compared to vehicle ($t(8) = 2.929, p$

= .019). No significant main effects for dose were found for the relative number of correct rejections (Figure 8) or for omissions (Figure 9).

Experiment 2

The data reported here are from the ten rats that maintained stable performance levels between drug administration sessions and had correct cannula placement. A repeated-measures dose x signal duration ANOVA on the relative number of hits for the three signal durations on the standard task found a significant main effect for signal duration ($F(2,18) = 99.9, p = .001$). Rats exhibited signal duration-dependent accuracy, with the hit rate higher following longer signal durations. For hits, a significant interaction was found for signal duration and dose ($F(6,54) = 4.766, p = .001$) (Figure 10). A one-way ANOVA was performed at each signal duration comparing drug doses. The 500 ms signal was the only signal duration to show significant differences between the drug doses ($F(3,27) = 5.485, p = .006$). A paired samples t-test compared the vehicle dose with each drug dose at the 500 ms signal. The highest drug dose (60 μg pirenzepine) significantly impaired performance when compared to vehicle ($t(9) = 2.944$, $p = .016$). No other significant effects were found for the hits. A repeated-measures ANOVA for the relative number of correct rejections (Figure 11) or for omissions (Figure 12) found no significant main effects for dose as a factor.

Discussion

The current experiments investigated the effects of M1 receptor blockade on performance in an attention demanding visual discrimination task. Animals were given M1 receptor antagonists prior to performing a sustained attention task

that required the discrimination of a brief visual signal from a nonsignal. Experiment 1 examined the effects of systemic administration of dicyclomine on three task manipulations designed to vary the amount of attentional demands needed to complete the task. In the standard task, accuracy on the signal trials was decreased following the highest dose of dicyclomine. In the distracter task, at the 500 ms signal duration, signal detection was disrupted following multiple dicyclomine doses (0.625 mg/kg, 2.5 mg/kg, 5.0 mg/kg) compared to vehicle. Under these conditions, the effects of 1.25 mg/kg dose of dicyclomine approached statistical significance compared with vehicle. Finally, in the short ITI task decreased signal detection was observed at the 100 ms signal duration at the highest doses of dicyclomine. In Experiment 2, icv administration of the M1 receptor antagonist pirenzepine decreased signal detection at the longest signal duration (500 ms) at the highest dose (60 μg) when compared to vehicle.

In both experiments, no drug-induced deficits were found on accuracy during the nonsignal trials as the number of correct rejections did not change across dose. The ability to correctly reject nonsignals has been used previously in attention tasks as an indicator that the subjects displayed no lever bias and were continuing to respond based on the task rules (McGaughy et al., 1996). The decrease in the number of hits was not caused by a side bias because an increase in the number of relative correct rejections would have been expected if rats were only pressing the miss/correct rejection lever. No severe motor deficits or effects

on motivation for reward were caused by the drugs, as evidenced by the lack of any significant drug-induced change in omissions.

In the standard task, systemic injection of the highest dose of dicyclomine created a decrease in signal detection at all signal durations. Severe disruption of visual processing seems unlikely to explain these deficits in signal detection because accuracy remained signal duration dependent under all drug administration conditions. The impairment in signal detection at all signal durations following systemic M1 receptor blockade was expected and is consistent with the effects of systemic muscarinic receptor blockade (Johnson & Burk, 2006; McQuail & Burk, 2006). A separate group of animals received an M1 receptor antagonist into the lateral ventricle to test whether the effects of systemic M1 receptor blockade were mediated centrally. Intraventricular infusions of an M1 receptor antagonist produced similar, but not identical, disruptions in signal detection compared to systemic administration. Specifically, icv drug administration produced an accuracy that was only detected following the 500 ms signal. The disruption of performance at the 500 ms signal duration after icv administration of pirenzepine is similar to less extensive cortical cholinergic deafferentation created by intracortical infusions of 192 IgG-saporin, which produced loss of only 40 to 60 percent of cholinergic fibers in the anterior two-thirds of the cortex (McGaughy & Sarter, 1998). Similar to McGaughy and Sarter (1998) pirenzepine administration may have only produced a moderate impairment of the cholinergic system, resulting in the pattern of detection deficits

only at the longest signal duration. Icv administration of pirenzepine may have only produced a moderate impairment because the drug may not have diffused completely throughout the brain. An increase in the pirenzepine dose may have produced more substantial deficits in the detection of signals and provided a clearer idea of the effects of the drug manipulation.

In the short ITI task signal detection was disrupted at the 100 ms signal duration at the highest dose of pirenzepine. The lack of effect at the 25 ms signal duration may be due to a floor effect. Furthermore, the hit rate following the 25 ms signal was relatively low in both experiments indicating that rats most commonly responded as if no signal was presented on these trials. This relatively low hit rate may have contributed to difficulty observing further drug-induced declines in accuracy following the 25 ms signal.

In the distracter task, signal detection was disrupted at the 500 ms signal duration. Additionally, performance was disrupted at low doses of dicyclomine administration, while in the standard and short ITI tasks signal detection was only disrupted at the higher doses (see Figure 13 for a summary of the effects of pirenzepine on the standard and distracter task). The distracter task is more taxing on the attentional system because the flashing houselight disrupts the animal's ability to discriminate between signals and nonsignals. This increase in attentional demand may recruit different brain regions for performance which explains why performance was disrupted at lower doses of dicyclomine. Distinct subregions in the cortex are implicated depending on the attentional demands

(Dalley, Cardinal, Robbins, 2004). One subregion, the prefrontal cortex, is important for maintenance of attention in the presence of distraction (Newman & McGaughy, 2008). Cholinergic lesions of the prefrontal cortex increased susceptibility to irrelevant stimuli during attention task performance (Newman & McGaughy, 2008). Other studies have shown that prefrontal cholinergic inputs contribute to distracter-related processing in animals performing a sustained attention task (Gill et al., 2000). Gill et al. (2000) demonstrated that increasing attentional demand by presenting a visual distracter increased prefrontal cortical neuronal firing rates. Removal of cholinergic projections to the medial prefrontal cortex decreased the firing rate of prefrontal cortical neurons and attenuated the frequency and amplitude of increased neuronal firing rates that were associated with the response of a distracter. In other words, the correlation between prefrontal cortical neuron firing rates and attentional demand is dependent on the integrity of cholinergic inputs to the prefrontal cortex (Gill et al., 2000). Moreover, blockade of prefrontal cortical muscarinic receptors was found to disrupt performance in the five choice serial reaction time task (Chudasama et al., 2004). Infusions of scopolamine, into the prefrontal cortex produced deficits similar to those produced by basal forebrain cholinergic lesions. These findings, along with the present data, suggest that muscarinic receptors within the prefrontal cortex may be critical for mediating attentional performance, particularly when irrelevant stimuli are presented.

The above studies all illustrate the importance of ACh in the prefrontal cortex under increased attentional demands. The present data offer some indication that the M1 receptors in the prefrontal cortex may be sensitive to low doses of dicyclomine. If these receptors are inhibited at low doses of dicyclomine, higher doses will not be able to inhibit the receptors further and similar deficits will be found at both high and low doses of the drug. The available literature suggests that there are regional and layer-specific differences in M1 and M2 receptors in the cerebral cortex, although exactly how these differences may contribute to altered sensitivity to M1 receptor antagonists remains unclear (Lidow, Gallager, Rakic, & Goldman-Rakic, 1989). In the standard task, a higher drug dose was needed to disrupt performance which suggests that prefrontal cortical M1 receptors may not be as critical for performance of the standard version of this attention task. Previous research shows excitotoxic prefrontal cortical lesions, which destroy a variety of inputs to this region, disrupt decisional processes necessary to complete the task (Miner, Ostrander, & Sarter, 1997). However, prefrontal ACh is not needed for performance on the standard task (Newman & McGaughy, 2008). Thus, dicyclomine may be affecting M1 receptors in other brain regions in the standard task. Similarly, the short ITI task performance was only disrupted at high doses of dicyclomine. The short ITI task may not be as attentionally demanding as the distracter task, which is supported by McGaughy & Sarter (1995) who found a high event rate did not disrupt

performance as greatly as a flashing houselight. Prefrontal cortical acetylcholine is most likely not being recruited to perform the task with a shorter ITI.

It is thought that in the presence of a distracter, top-down processing is required to sustain attention and filter out background "noise" (Sarter, Givens, Bruno, 2001). Top-down processing refers to knowledge driven mechanisms designed to enhance the processing of relevant sensory input to facilitate discrimination between targets and distracters (Kastner & Ungerleider, 2000). The basal forebrain cholinergic system is proposed to contribute to the functionally top-down processes in sustained attention (Sarter et al., 2001). According to present hypotheses, when a visual distracter is introduced during task performance, the basal forebrain cholinergic system becomes activated in proportion to the level of attention required to maintain performance. When the basal forebrain is activated, under conditions of high attentional demand, a subset of neurons in the medial prefrontal cortex detect this change and elevate their activity and subsequently project this enhanced activity to the posterior cortical regions to modulate the processing of signals (Nelson, Sarter, & Bruno, 2005). This hypothesis is supported by lesion studies that have found bilateral infusions of 192 IgG-saporin into the medial prefrontal cortex resulted in selective impairments in sustained attention (Newman & McGaughy, 2008). Signal detection accuracy was disrupted during the presence of a visual distracter, but not under standard attentional conditions. The lack of effect of bilateral medial prefrontal cortex cholinergic lesions on performance under standard attentional

conditions suggests that medial prefrontal cortex cholinergic inputs may have less of a role in enhancing signal detection and more of a role in filtering out irrelevant stimuli. Loss of cholinergic inputs to the posterior parietal cortex increases response latencies, but does not affect accuracy, when the irrelevant flashing houselight is presented in the present attention task (Broussard, Karelina, Sarter, & Givens, 2009). Thus, it seems unlikely that the effects of M1 receptor antagonists in the present experiment, which decrease signal detection accuracy, are mediated within the posterior parietal cortex.

The present experiment is consistent with previous studies that have found cognitive deficits following M1 receptor blockade. Disruptions in memory performance have been reported after administration of an M1 receptor antagonist (Bymaster et al., 1993; Aura et al., 1997; Anagnostaras et al., 2003), but the effect on attention has not been studied. The present experiment found that blocking M1 receptors decreases accuracy in an attention demanding task and further disrupts performance when attentional demands are increased. The disruption in memory performance found in previous studies may be due to a disruption in attentional capacity caused by blocking the M1 receptor.

These results further support the role of the M1 receptor in AD. Improvements in cognition have been reported following the use of muscarinic M1 receptor agonists (Clader & Wang, 2005) while an impairment of M1 mediated signaling may underlie the cognitive decline associated with the disease (Tsang et al., 2007). However, Tsang et al. (2007) found that M1/G protein

decoupling was correlated with reductions in PKC activity and NMDA receptor density. They suggest postsynaptic cholinergic dysfunction may underlie the cognitive features of AD. In addition, these data suggest that M1 receptor agonists may be beneficial during the early stages of AD, while M1/G protein coupling is unaffected.

The current study was limited by the fact that dicyclomine was administered systemically, so the drug could have had peripheral effects. In addition, pirenzepine was administered into the lateral right or left ventricle so it affected all brain regions, not just regions specialized for attention. It may be beneficial to investigate the effects of M1 receptor blockade on specific areas in the brain, such as the prefrontal cortex. Future studies could also investigate if M1 receptor agonists could attenuate the attentional impairments caused by moderate impairment of the cholinergic system.

In summary, M1 receptor blockade decreased signal detection accuracy in an attention task. Deficits in attention were observed following systemic administration of dicyclomine. Additionally, when attentional demands were increased deficits in signal detection were observed at lower doses of pirenzepine that did not affect performance in the standard task. Signal detection was also disrupted following icv administration of pirenzepine. The effects of acetylcholine on attention seem to be mediated, in part, by the M1 receptor. The present data offer a starting point to understanding the effect of the M1 receptor on attention.

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Figure Captions

Figure 1. Relative hits across dicyclomine doses at each signal duration in the standard task. Relative hits were significantly decreased following the 5.0 mg/kg dicyclomine dose.

Figure 2. Relative correct rejections across dicyclomine doses in the standard task. Correct rejections were not significantly affected by dicyclomine administration at any dose.

Figure 3. The number of omissions across drug doses in the standard task. Omissions were not significantly affected by dicyclomine administration at any dose.

Figure 4. Relative hits across dicyclomine doses at each signal duration in the distracter task. Relative hits were significantly decreased at the 500 ms signal duration following the 0.625, 2.5, and 5.0 mg/kg dicyclomine doses and approaching significance at the 1.25 mg/kg dose. No other significant effects were observed at the 25 and 100 ms signal duration.

Figure 5. Relative correct rejections across dicyclomine doses in the distracter task. Correct rejections were not significantly affected by dicyclomine administration at any dose.

Figure 6. The number of omissions across drug doses in the distracter task. Omissions were not significantly affected by dicyclomine administration at any dose.

Figure 7. Relative hits across dicyclomine doses at each signal duration in the short ITI task. Relative hits were significantly decreased at the 100 ms signal

duration following the 5.0 mg/kg dicyclomine dose. No other significant effects were observed at the 25 and 500 ms signal duration.

Figure 8. Relative correct rejections across dicyclomine doses in the short ITI task. Correct rejections were not significantly affected by dicyclomine administration at any dose.

Figure 9. The number of omissions across drug doses in the short ITI task. Omissions were not significantly affected by dicyclomine administration at any dose.

Figure 10. Relative number of hits across pirenzepine doses at each signal duration in the standard task. Relative hits were significantly decreased at the 500 ms signal duration following the 60 μg pirenzepine dose. No other significant effects were observed at the 25 and 100 ms signal duration.

Figure 11. Relative correct rejections across dicyclomine doses in the standard task. Correct rejections were not significantly affected by dicyclomine administration at any dose.

Figure 12. The number of omissions across drug doses in the standard task. Omissions were not significantly affected by pirenzepine administration at any dose.

Figure 13. Relative hits were averaged across the various signal durations for each dicyclomine dose in the standard and distracter task (Experiment 1). In the standard task, relative hits decreased at the highest dicyclomine dose (5.0 mg/kg)

while in the distracter task relative hits decreased across most doses (0.625, 2.5, 5.0 mg/kg).

Figure 1.

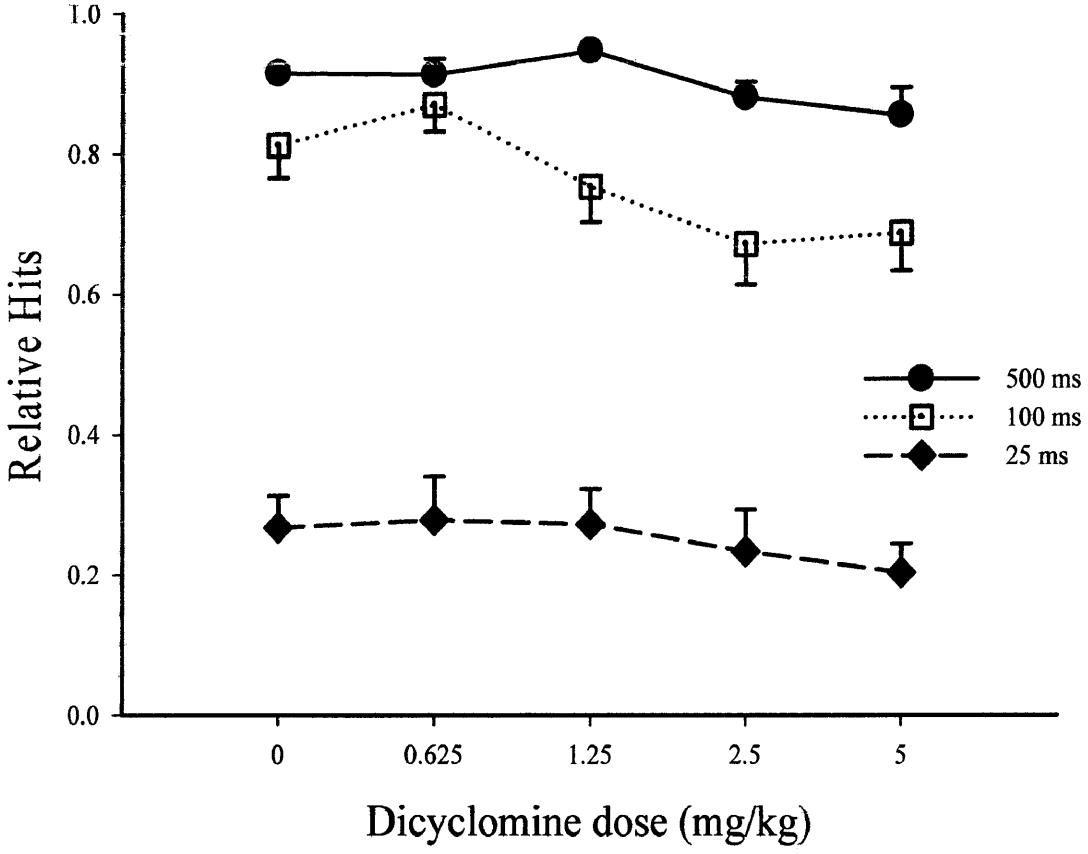

1.0
0.8
0.6
0.4
0.2
0.0
Relative Hits
0
0.625
1.25
2.5
5
Dicyclomine dose (mg/kg)
500 ms
100 ms
25 ms

Figure 2.

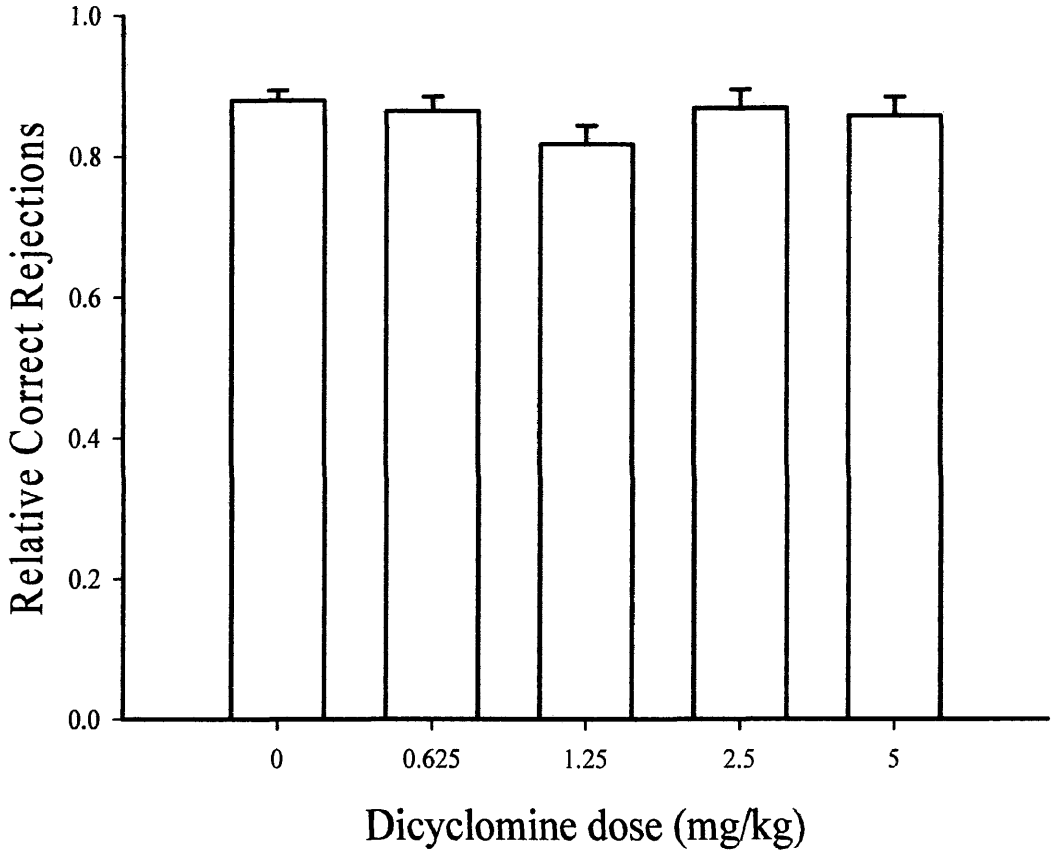

1.0
0.8
0.6
0.4
0.2
0.0
Relative Correct Rejections
0
0.625
1.25
2.5
5
Dicyclomine dose (mg/kg)

Figure 3.

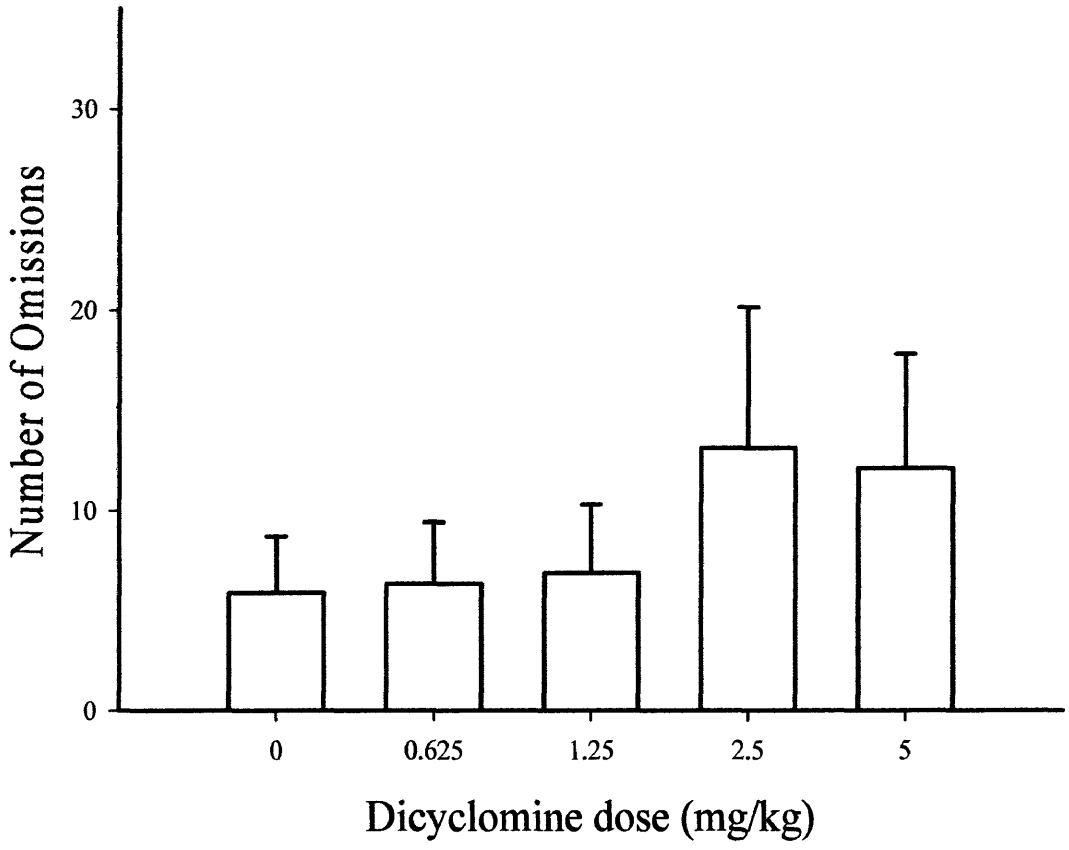

Number of Omissions
30
20
10
0
0
0.625
1.25
2.5
5
Dicyclomine dose (mg/kg)

Figure 4.

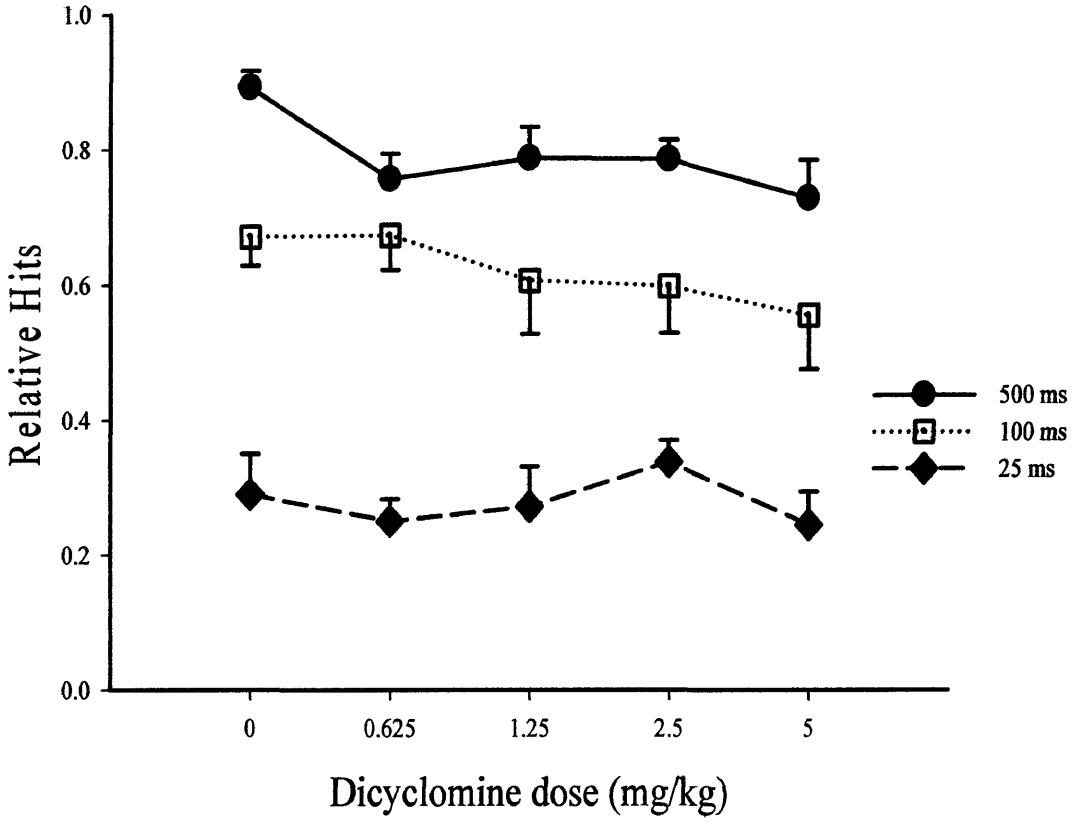

1.0
0.8
0.6
0.4
0.2
0.0
Relative Hits
0
0.625
1.25
2.5
5
Dicyclomine dose (mg/kg)
500 ms
100 ms
25 ms

Figure 5.

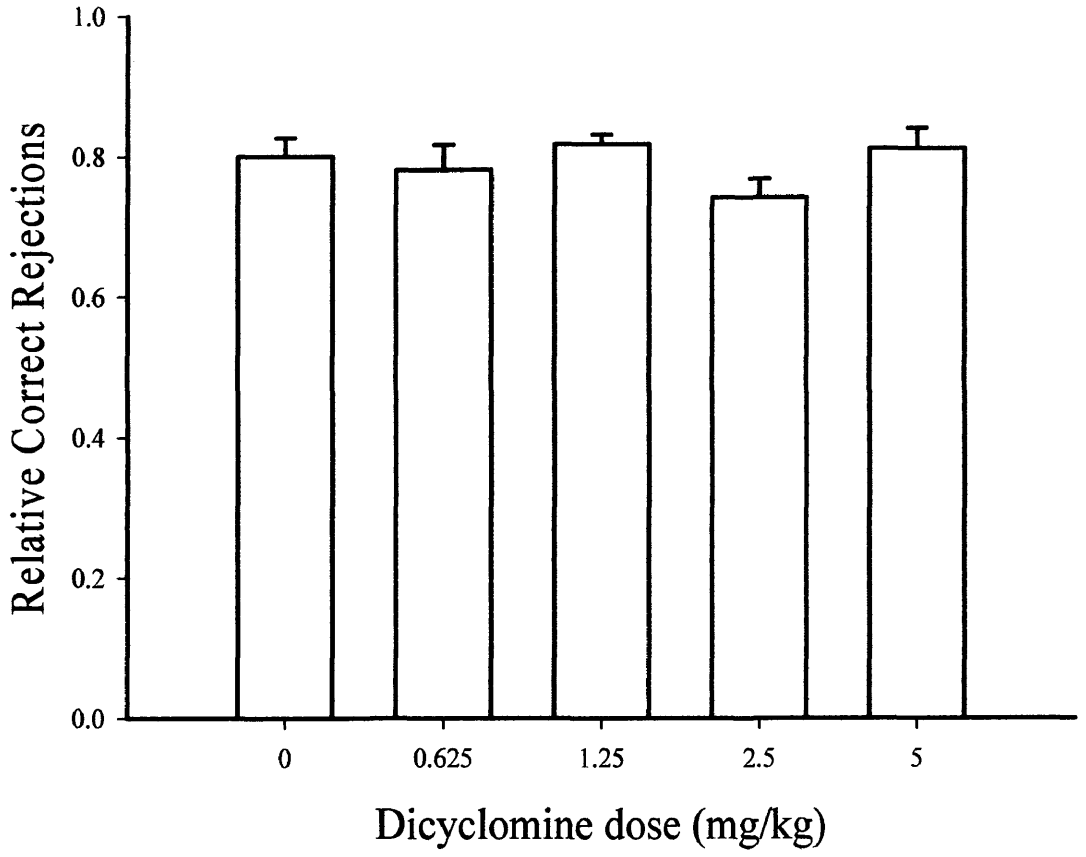

1.0
0.8
0.6
0.4
0.2
0.0
Relative Correct Rejections
0
0.625
1.25
2.5
5
Dicyclomine dose (mg/kg)

Figure 6.

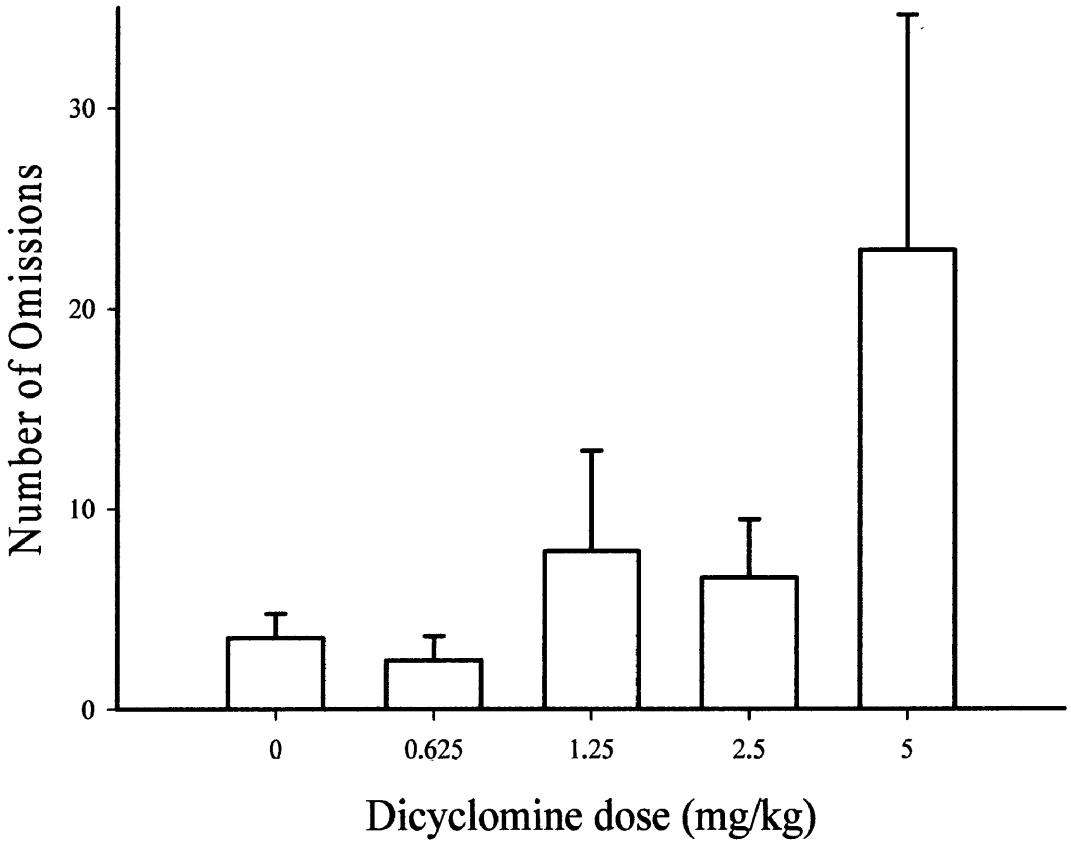

Number of Omissions
30
20
10
0
0
0.625
1.25
2.5
5
Dicyclomine dose (mg/kg)

Figure 7.

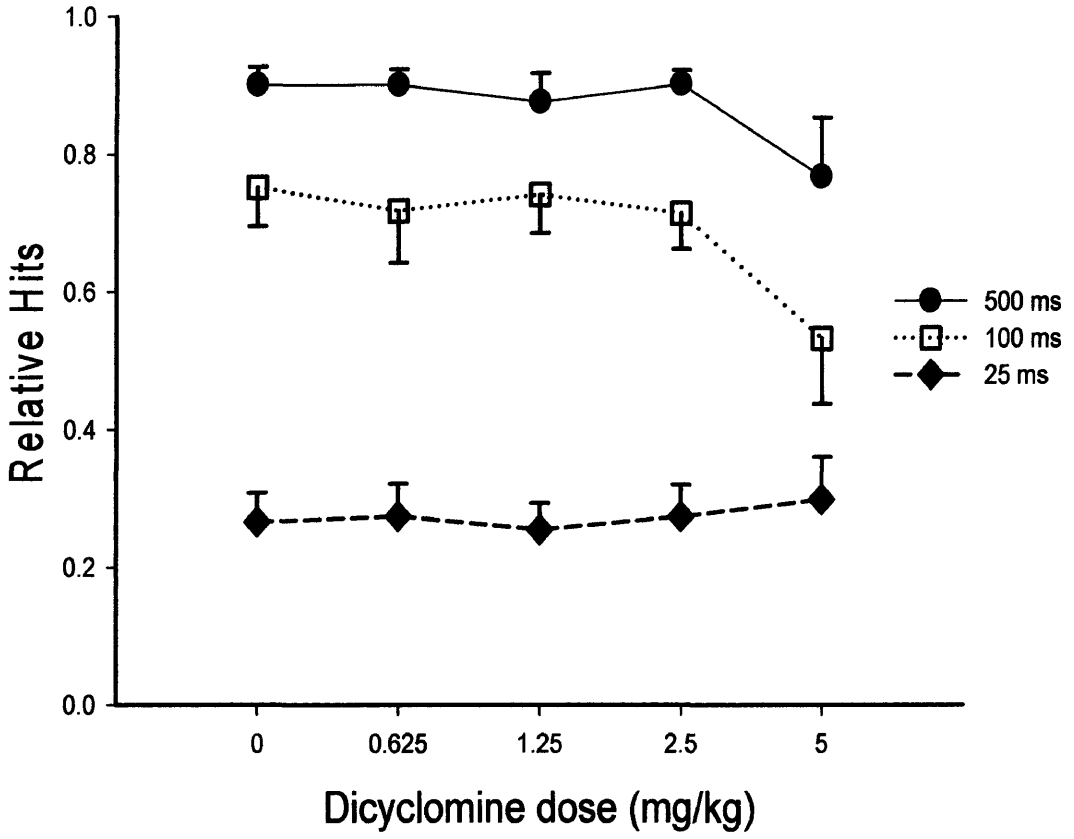

1.0
0.8
0.6
0.4
0.2
0.0
Relative Hits
0
0.625
1.25
2.5
5
Dicyclomine dose (mg/kg)
500 ms
100 ms
25 ms

Figure 8.

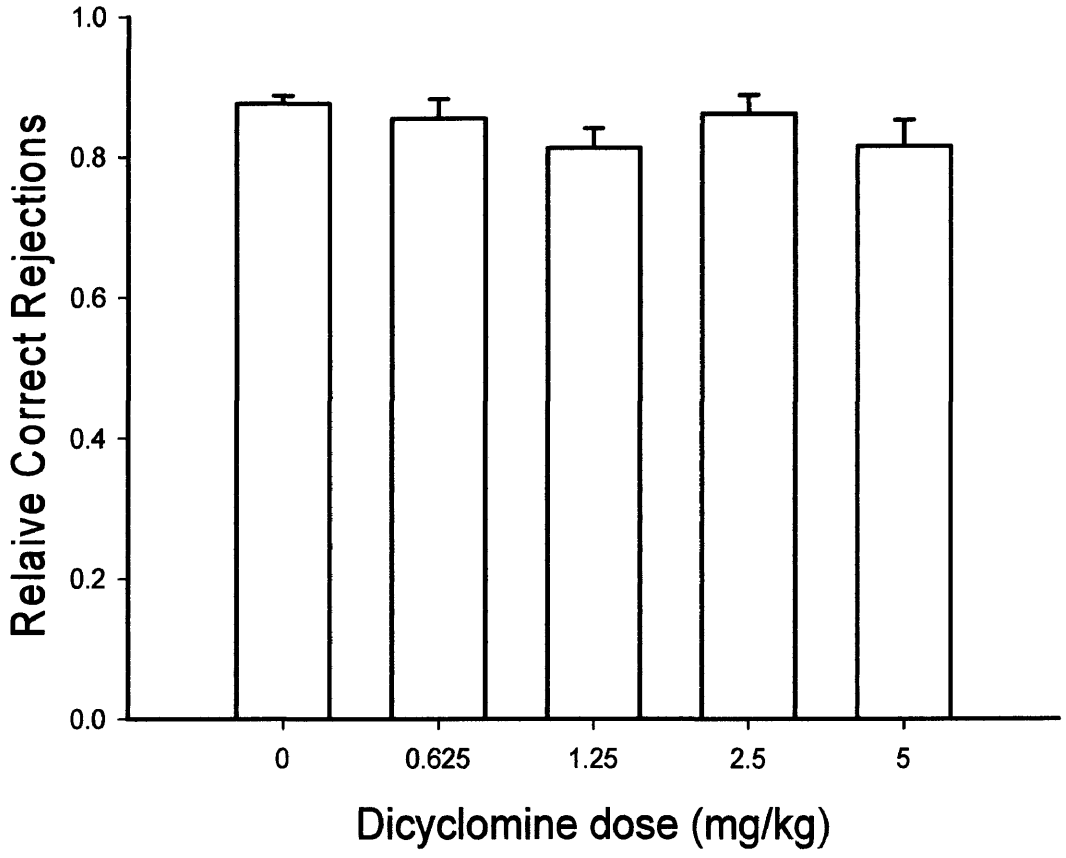

1.0
0.8
0.6
0.4
0.2
0.0
Relaive Correct Rejections
0
0.625
1.25
2.5
5
Dicyclomine dose (mg/kg)

Figure 9.

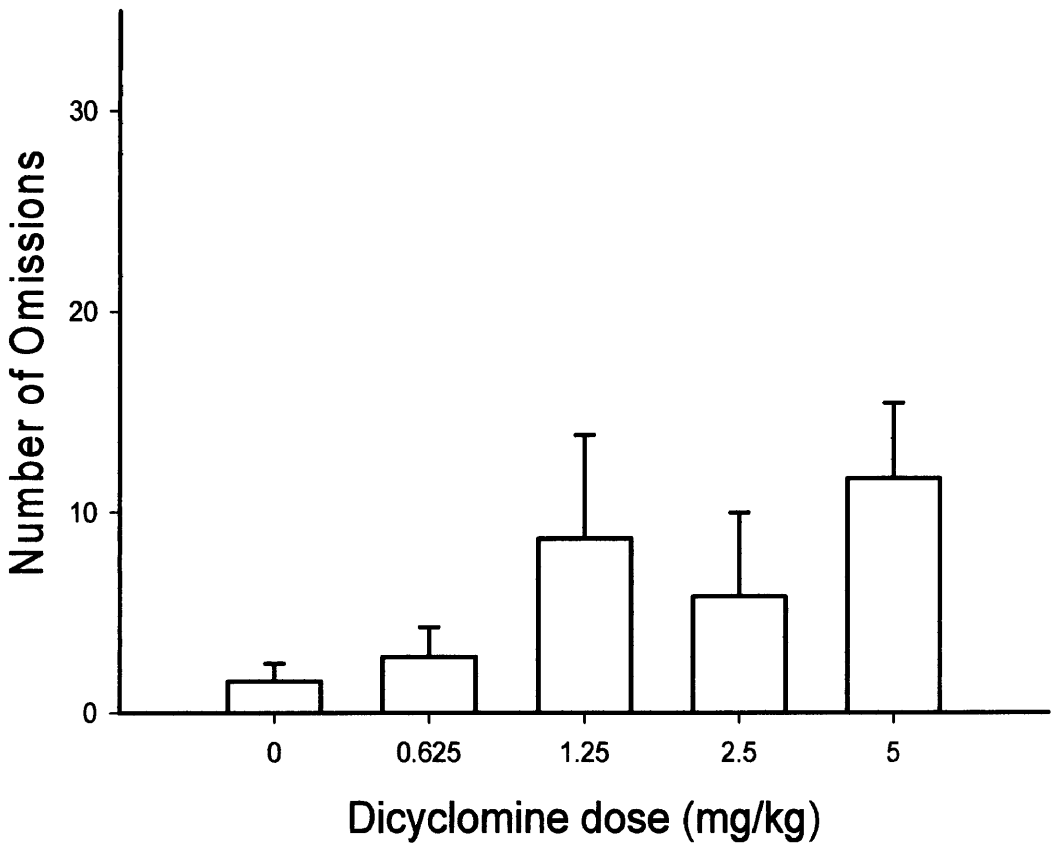

Number of Omissions
30
20
10
0
0
0.625
1.25
2.5
5
Dicyclomine dose (mg/kg)

Figure 10.

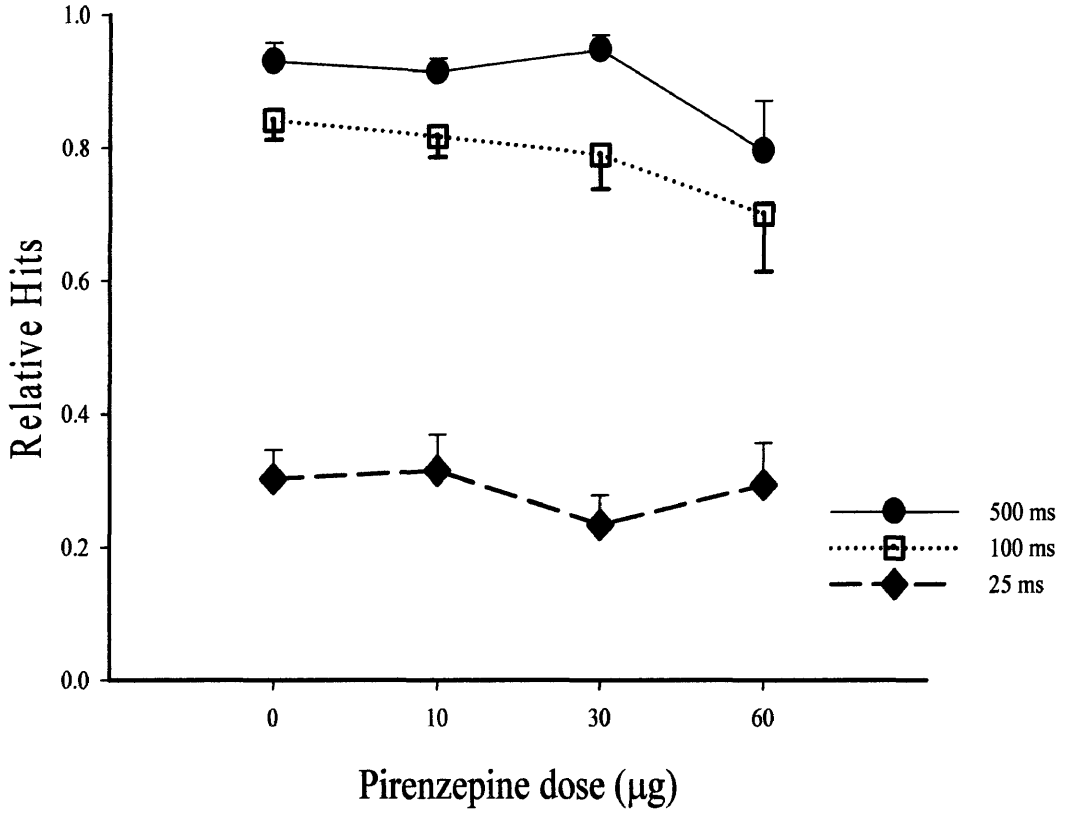

1.0
0.8
0.6
0.4
0.2
0.0
Relative Hits
0
10
30
60
Pirenzepine dose (µg)
500 ms
100 ms
25 ms

Figure 11.

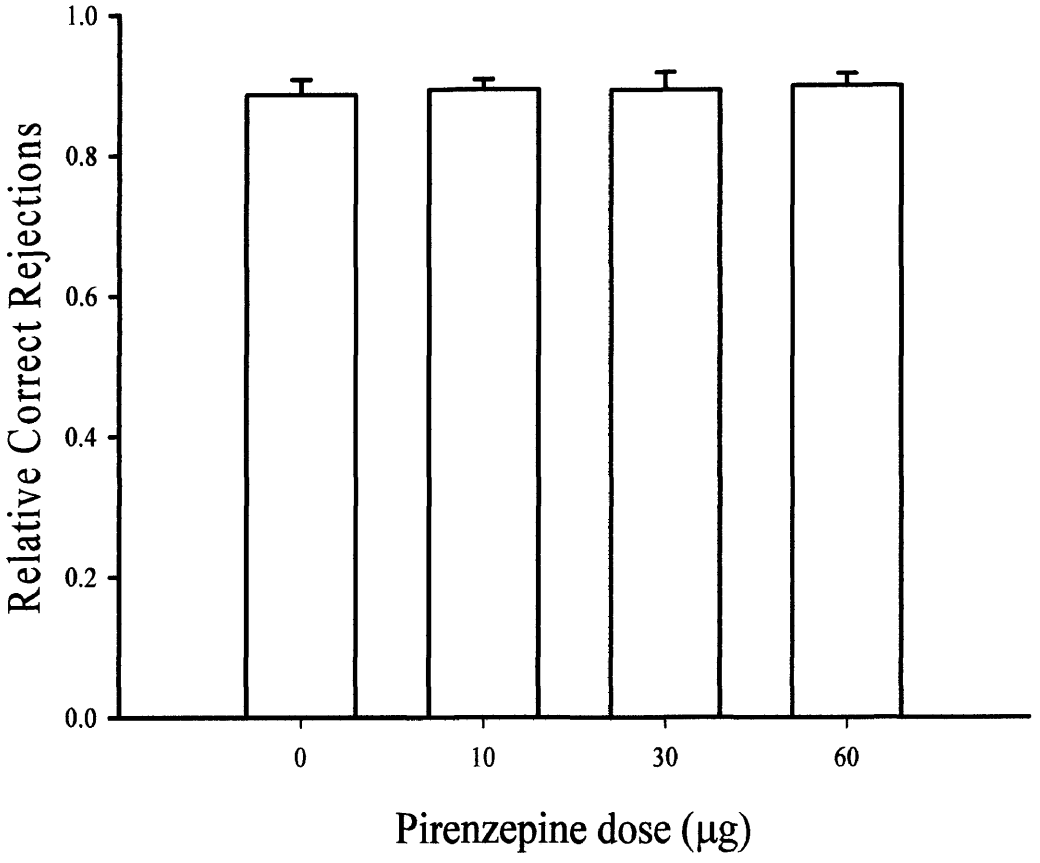

1.0
0.8
0.6
0.4
0.2
0.0
Relative Correct Rejections
0
10
30
60
Pirenzepine dose (μg)

Figure 12.

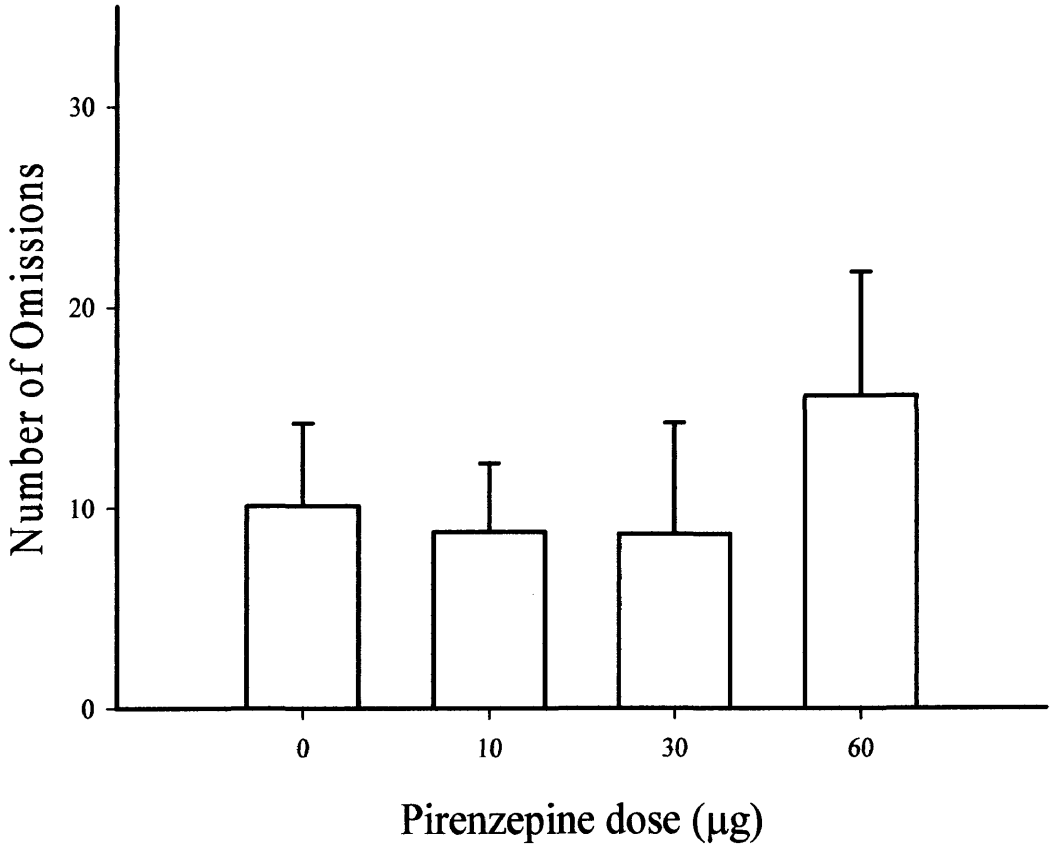
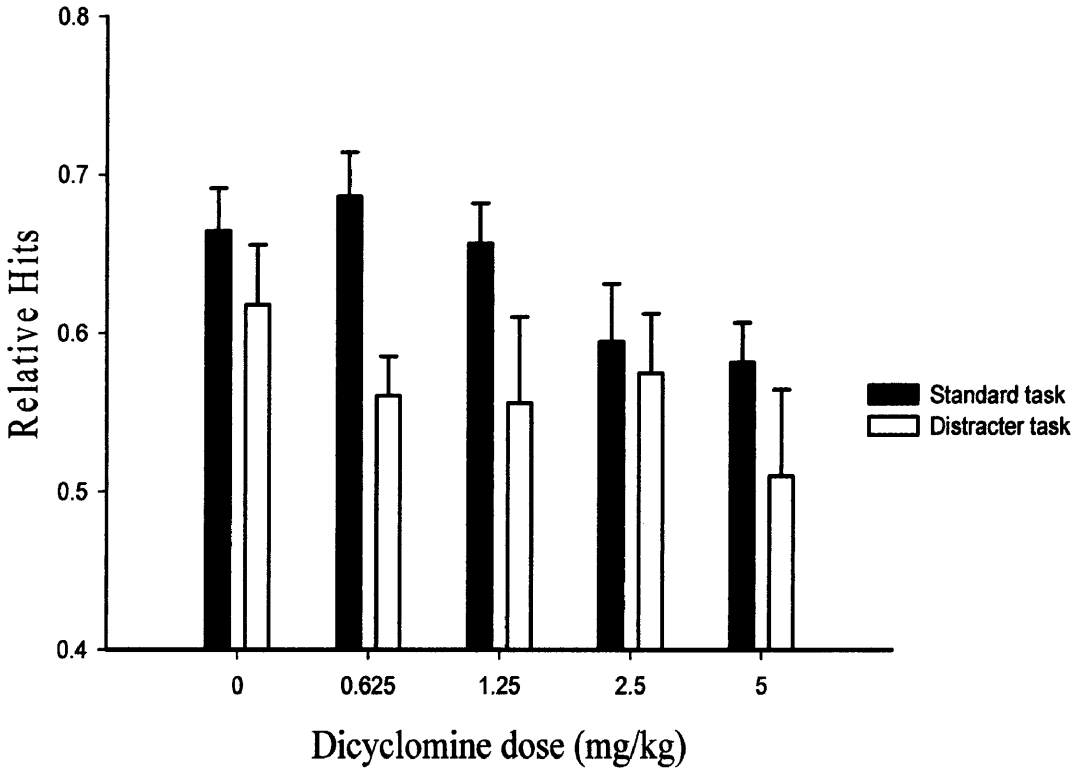


Figure 13.


0.8
0.7
0.6
0.5
0.4
Relative Hits
0
0.625
1.25
2.5
5
Dicyclomine dose (mg/kg)
Standard task
Distracter task