INTRODUCTION

A fundamental problem in neuroscience is explaining the neurobiological mechanisms involved in learning. One way this issue has been studied is by investigating the effects of drugs on neurobiological systems that are thought to be involved in memory and learning. The present thesis addresses the issue of the effects on spatial learning of drugs from three classes (N-methyl-D-aspartate receptor antagonists, gamma-aminobutyric acid agonists, and muscarinic cholinergic antagonists) and examines the way that these drugs interact when compounds from each class are administered in combination.

Glutamate and acetylcholine (excitatory neurotransmitters) and GABA (inhibitory neurotransmitter) are three neurotransmitter systems that have been linked to learning and memory. These neurotransmitters differ in their general effects on postsynaptic membranes. Excitatory neurotransmitters, like glutamate and acetylcholine, depolarize the postsynaptic membrane. Conversely, GABA hyperpolarizes the postsynaptic membrane, producing an inhibitory postsynaptic potential. The two simple amino acids, glutamate and GABA, are the most abundant neurotransmitters in the CNS (Feldman, Meyer and Quenzer, 1997).

Neuropharmacology of Glutamate

Four types of glutamate receptors have been discovered. Three are ionotropic (the NMDA receptor, the AMPA receptor, and the kainate receptor) and the fourth receptor is metabotropic. Ionotropic receptors are receptors that contain a binding site for a neurotransmitter (the location on a receptor protein to which a ligand binds) and an ion channel, which opens or closes when a

molecule of the transmitter attaches to the binding site. Metabotropic receptors activate an enzyme that begins a series of chemical events that affect ion channels elsewhere in the membrane of the postsynaptic cell when a molecule of neurotransmitter attaches to the binding site. Although the AMPA (alpha-amino-3-hydroxy-5-methylisoxazole propionate) receptor appears to be the most widely distributed glutamate receptor, the N-methyl-D-aspartate receptor has attracted much attention because of several unique properties of this receptor. It has six different binding sites (one of which is for glutamate), and when it is open, the ion channel controlled by the NMDAR allows sodium and calcium ions to enter the postsynaptic cell, which causes a depolarization (Feldman et al., 1997). According to numerous reports, excessive release of glutamate and prolonged stimulation of NMDA receptors are produced by ischemia and seizure. NMDAR antagonists have been shown to decrease neuronal damage and to have anticonvulsive properties in the experimental models of such neuropathologies (Ylinen, Pitkanen, Sirvio, Hartikainen, Sivenius, Koivisto, and Riekkinen, 1995).

There are two principal concepts in neuropharmacology of neurotransmitters. Drugs that affect synaptic transmission are classified into two general categories. Antagonists are compounds that oppose or inhibit an effect of particular neurotransmitter on the postsynaptic membrane. Agonists are the drugs that facilitate the effects of transmitter (Feldman et al., 1997). Obviously, N-methyl-D-aspartate (NMDA) serves as direct agonist of NMDA receptors. A hallucinogenic synthetic drug phencyclidine (PCP) binds to one of the six binding sites. PCP blocks the calcium channel and, thus, serves as an indirect

antagonist (indirect means that drug does not interfere with the binding site for the principal ligand when it attaches to its receptor). There are several other drugs that affect glutamatergic synapses: competitive NMDAR antagonists (AP5, CPP, CGS 19755) bind to NMDA receptors, and the non-competitive antagonist MK-801 (dizocilpine) binds to PCP receptor-site. Competitive binding of agonists or antagonists acts directly on the neurotransmitter binding site whereas non-competitive binding of indirect agonists or antagonists acts on alternative binding site and modifies the effects of the transmitter on the ion channel controlled by the neurotransmitter (Feldman et al., 1997). Because non-competitive agents MK-801 and PCP are effective after systemic administration, they have been studied intensively by behavioral pharmacologists who are interested in exploring the role of NMDA receptors in memory (Wozniak, Olney, Kettinger III, Price, and Miller, 1990).

Neuropharmacology of GABA

Two GABA (gamma-aminobutyric acid) receptors have been identified: $GABA_A$ and $GABA_B$. The $GABA_A$ receptor is ionotropic and controls a chloride channel; the $GABA_B$ receptor is metabotropic and controls a potassium channel (Feldman et al., 1997). The GABA system has a wide-spread distribution throughout the brain and spinal cord.

Like the NMDA receptor, the $GABA_A$ receptor is complex and consists of the multiple binding sites. GABA binds to the main site and this binding can be antagonized by bicuculline. A second site binds with benzodiazepines.

Barbiturates bind to yet a third binding site. All of the mentioned compounds serve as modulators of the $GABA_A$ receptor-complex (Feldman et al., 1997).

Neuropharmacology of Acetylcholine

Like glutamate and GABA, acetylcholine is a neurotransmitter that is widely distributed throughout the brain. Released ACh generally produces excitatory effects. The acetylcholine neurons located in the basal forebrain and medial septum are involved in facilitating learning and formation of memories (Feldman et al., 1997). There are two different types of ACh receptors - ionotropic and metabotropic with different drugs activating each type of receptors. The ionotropic receptors are stimulated by nicotine and metabotropic are stimulated by muscarine, a substance naturally found in poison mushrooms. Thus, the two types of receptors are referred to as nicotinic and muscarinic receptors. Although both types are present in the central nervous system, muscarinic receptors predominate in CNS. Nicotinic receptors are contained in muscle fibers. Because metabotropic receptors control ion channels by the chain of secondary messengers, action of muscarinic receptors are slower and more prolonged than those of nicotinic ones (Feldman et al., 1997).

Drugs known as antimuscarinic or muscarinic cholinergic blocking agents antagonize the muscarinic actions of ACh and produce their effects at nicotinic receptor sites. The best-known member of this class of drugs is atropine, a naturally occurring alkaloid from the plant Atropa belladonna. Another well-known antimuscarinic drug is an alkaloid scopolamine, which is naturally found in the shrub *Hyoscyamus niger* and *Scopolia carniolica*.

Drugs that block central acetylcholine (ACh) muscarinic receptors have long been known to impair higher cognitive functions and induce amnestic states (Coyle, Price & DeLong, 1983). Behavioral effects of these antimuscarinic compounds will be of particular interest with respect to the experiments described in this thesis.

NMDAR Antagonist Effects on Spatial Learning

Rats are skillful at learning locations. Morris (1981) demonstrated that rats can rapidly learn to locate an object that they can never see, smell or hear provided it remains in a fixed spatial location relative to distal room cues. In Morris's classic study (1981) rats were placed in the circular pool of water and had to swim to an escape platform, which was submerged below the surface of murky water. Morris referred to this experimental condition as the place condition. In another experimental condition the top of the platform was several centimeters above the surface and rats could see it, which Morris termed as the cue condition. In the experiment the following conditions were contrasted: 1) *cue+place* - the escape platform was always visible and always in the same location; 2) *place* - the platform was submerged but always in the same location; 3) *cue only* - the platform was always visible but in the different locations on different trials; 4) *place random* - the platform was submerged and in different locations. The rats performed well in all conditions but the fourth one, in which rats took much longer to find the escape platform. During a probe test in which the escape platform was removed from the pool, rats trained in the *place* and *cue only* conditions preferentially searched in the region of the pool where the

platform was located during training. The fact that rats rapidly learned to swim directly to the hidden platform in the *place* condition and searched for the missing platform in the place where it was located during training both indicated that rats can use extrapool stimuli to navigate to the escape platform (Morris, 1981).

To investigate whether NMDA receptors were necessary for place learning, Morris, Anderson, Lynch and Baudry (1986) carried out an experiment in the water maze after chronically infusing rats implanted with minipumps with the drug aminophosphonovaleric acid (AP5, a competitive NMDAR antagonist), with saline infused and unoperated animals serving as controls. As in the Morris (1981) study described above, rats were trained to find a hidden platform in the pool. The first phase of training involved 15 trials; the inter-trial interval (ITI) was 4 hours and escape latencies were measured. Originally, AP5-infused animals learned the first location more slowly, but their performance was not statistically different from that of controls. AP5-infused rats also exhibited an obvious non-specific impairment (swim off the escape platform). However, AP5-treated rats stabilized their escape latencies at the end of the training. Then a transfer test was performed, the purpose of which was to find out how much had been learned about the spatial location of the platform. Analysis of the paths during the transfer test showed that AP5-treated rats learned little about the location of the platform because they did not demonstrate spatial bias to the training quadrant. Then animals were given 8 training trials with the platform back at the original location with 30-sec ITI, and, finally, on reversal trials rats were trained to swim to a new location. During the reversal-training phase escape latencies of

rats both in the AP5 and control groups initially increased from 20 sec on the last trial block of original training to 60 sec on the first block of reversal training trials. As training proceeded, performances of rats in both groups improved but controls appeared to learn the new position at a faster rate than the AP5 treated rats did, again suggesting that AP5 impaired place learning. Additionally, visual-discrimination task was performed using two visible platforms, one with vertical and the other with horizontal stripes. One of the platforms provided escape from the water, and black curtains that surrounded the perimeter of the pool to eliminate extra-maze cues. This test showed that rats in all groups were similar in terms of their abilities to learn a visual discrimination task. Morris et al. (1986) concluded that secondary sensorimotor and motivational impairment would unlikely be a cause of the place-learning deficits observed in AP5-infused rats.

As with central administration of the competitive NMDAR antagonist AP5, systemic administration of non-competitive and competitive NMDAR antagonists have been found to impair performance of rats on numerous behavioral tasks designed to assess learning and memory (Danysz, Wroblewski, and Costa, 1988; Heale and Harley, 1990; McLamb, Williams, Nanry, Wilson, and Tilson, 1990; Parada-Turska and Turski, 1990; Wozniak et al., 1990, Caramanos and Shapiro, 1994). One important issue that has been raised recently, however, is that NMDAR antagonists are known to produce numerous behavioral, non-cognitive effects. In particular, high doses produce such grossly observable changes in behavior such as peculiar rolling and circling, head weaving, increasing in locomotion, stereotypes, or ataxia, impairments in sensory

processing (Ahlander, Misane, Schott, and Ogren, 1999; Cory-Slechta, 1994). Therefore, it is crucial that researches apply the most sophisticated methods for dissociating learning and memory deficits caused by NMDAR antagonists from nonspecific behavioral effects.

$GABA_A$ Agonist Effects on Spatial Learning

Though their pharmacological mechanisms are fundamentally different, benzodiazepine drugs produce effects on spatial learning tasks that are similar to those produced by NMDAR antagonists. The first attempt to study spatial learning in the water maze under a benzodiazepine (chlordiazepoxide) in rats was reported by McNaughton and Morris (1987). Rats were placed in the pool, one trial per day, and were required to find a hidden platform and then, after thirteen trials of such training, a probe trial with the platform removed was given to test the extent to which changes in performance depended on knowledge of the spatial location of the platform. Path length data were used, since latency to escape could be contaminated by changes in swimming speed (due to muscle relaxant properties of CDZ). In contrast to previous results with the radial arm maze, data in this study indicated that 5 mg/kg CDZ can produce an impairment in spatial acquisition of the platform location in the swimming pool. In contrast to the effects of hippocampal lesions (Riedel et al., 1999; Brooks, Cory-Slechta, Murg, and Federoff, 2000; Morris, Garrud, Rawlins, and O'Keefe, 1982), drug-treated rats showed clear improvements in spatial navigation throughout the acquisition. Additionally, performance on the probe trial detected no bias in choosing the quadrant and tendency to swim directly towards a particular distal

stimulus in CDZ-treated rats. This observation led the authors to idea that deficits are related to pure impairment of spatial localization (McNaughton and Morris, 1987).

Researchers employing various behavioral paradigms have studied effects of benzodiazepines on spatial behavior and, in general, the hypothesis that BZDs impair learning has been supported (Broekkamp, Pichon, and Lloyd, 1984; Ferguson and Paule, 1993; Cole and Michaleski, 1986; McNaughton, 1985; McNamara and Skeleton, 1991).

A strong challenge to the view that NMDAR antagonists and GABA agonists impair learning and memory while sparing general performance abilities was brought by Cain's (1997) and of Saucier, Hargreaves, Boon, Vanderwolf, and Cain (1996) findings. Cain (1997) rejected the idea that the impairment of spatial navigation observed after diazepam administration can only be due to learning disturbances. In Cain's (1997) experiment, prior non-spatial pretraining eliminated impairments in water maze learning caused by diazepam.

Basically, Cain (1997) had two groups of animals tested on hidden-platform-task and given the same range of diazepam doses with the only difference that one of them had received non-spatial pretraining and the other one had not. Pretraining sessions involved letting rats swim to an escape platform that is moved to a new location on each trial. Black curtains around the pool eliminated any cues that rats could have being using to navigate to the platform. The procedure was therefore considered "non-spatial" pretraining because the position of the platform was never correlated with spatial cues in the

surrounding. Two non-drugged groups (naïve and pretrained) were also studied. The critical finding of Cain's study was that diazepam did not disrupt navigation to the hidden platform in pretrained rats whereas behaviorally naïve rats were severely impaired by diazepam. Furthermore, Cain reported that diazepam caused behaviorally naïve rats to spend more time swimming in the periphery, to swim off, or over, the hidden platform, and produced ataxia on a beam-task. Such sensorimotor disturbances were not apparent in non-spatially pretrained rats. Interestingly, behaviorally naïve rats that were tested on the visible-platform task rather than the hidden-platform task were not impaired relative to placebo treated controls or pretrained rats.

The findings cited above were paralleled by those of Saucier et al. (1996) with NMDA and muscarinic cholinergic antagonists suggesting that visible platform task can serve as a control for groups that exhibit pronounced sensorimotor disturbances but the absence of a deficit on this task does not guarantee that a naïve drug group is free of subtle sensorimotor dysfunctions that can affect acquisition during the hidden-platform task.

In summary, doses of both NMDAR antagonist and GABA agonist that produced marked non-specific disturbances and acquisition impairments in naïve rats did not produce these in subjects that received non-spatial pretraining. Thus, according to Cain, occupation of receptors by these drugs does not interfere with spatial learning in the Morris water maze and glutamate and GABA neurotransmitter systems may not be involved in spatial learning (Cain, 1997). The story of whether $GABA_A$ agonists and NMDAR antagonists can affect

learning and memory at doses that do not affect general behavioral performance was incomplete, however, because a narrow range of doses was studied by Cain and his colleagues. Thorough dose-effect curves should provide a more complete picture of how drugs in these classes affect learning.

Repeated Acquisitions Procedures in Behavioral Pharmacology

One procedure that allows researchers to compare drug effects on acquisition and performance is the multiple-component, repeated-acquisition procedure (RAP). The original RAP procedure introduced by Thompson and Moerschbaecher (1979) was situated in a traditional operant arrangement and involved teaching subjects patterns of reinforced keypeck or leverpress responses. In the presence of one stimulus (e.g., a tone), a fixed response sequence, or pattern, is always effective (performance component). In the presence of another stimulus (e.g., a light), the reinforced response pattern changes each session (acquisition component). In this procedure, within a single experimental session, researchers can study drug effects on a well-learned sequence and compare them directly with the acquisition of a new sequence.

The effects of phencyclidine (PCP) and pentobarbital (PB) on the acquisition and performance of the conditional discriminations in monkeys were studied by Moerschbaecher and Thompson (1980). In each of two components of a multiple schedule, monkeys were required to respond on a right or left lever depending upon the stimulus combination (a color and geometric form). Reinforcement of a response in the presence of one stimulus (the form) was conditional upon the other stimulus (the color). In the performance component of

the multiple schedule the discriminative stimulus (a combination of color and form stimuli) for lever presses was the same from session to session. In the acquisition component, unlike the performance component, the discriminative stimuli changed every session. The completion of a two-member chain of discriminations produced a food pellet. The task components alternated after 50 reinforcers were obtained or 25 min had elapsed, whichever occurred first. The drugs (PCP and PB) were administered intramuscularly (*im*) 5 min prior the session. Drug sessions were separated by five days, during which baseline and control sessions were conducted. The data were analyzed in terms of (a) the overall response rate (total responses/min, excluding timeouts) and (b) the overall accuracy or percent errors ((errors/total responses) x 100). The data for each individual subject were analyzed by comparing a given drug session with the control range of variability (saline sessions). Under this schedule both drugs produced a dose-dependent decrease in the overall rate of responding in comparison to saline controls. At the highest doses studied, both drugs disrupted accuracy under both the acquisition and performance components. At lower doses, however, each drug produced selective effects on accuracy. PCP selectively impaired accuracy (percent errors) at 0.056 - 0.1 mg/kg and PB selectively impaired accuracy at the dose of 5.6 mg/kg. Generally, errors increased in the acquisition component at lower doses than those required to disrupt behavior in the performance component (Moerschbaecher and Thompson, 1980). These results suggest that, at least under the conditions studied by Moerschbaecher and Thompson (1980), some PCP and PB doses are

both capable of disrupting mechanisms that enable learning and memory without interfering with processes that support general performance.

The RAP procedure also has been adapted to procedures that involve spatial navigation learning. Specifically, Keith and Galizio (1997) used a multiple-component, repeated-acquisition procedure in the Morris water maze. In the original procedure, developed by Morris (1981) the issue of distinguishing drug effects on learning versus performance was represented by carrying out hidden platform task versus visible platform task. The visible platform task served as a control for effects of drugs and brain lesions on general performance variables. Baring impairment on the visible platform tasks, researches have interpreted rodents' performances on a hidden platform task as a relatively pure measure of spatial learning (Morris, 1981; Morris et al., 1986). As mentioned above, NMDAR antagonists and $GABA_A$ agonists impaired spatial learning (behavior on the hidden task) at doses that did not impair visible platform performance. However, after Cain's findings, these interpretations have been questioned in terms of their ability to distinguish effects of drugs on learning versus performance.

To distinguish drug effects on performance factors from effects on mechanisms specific to learning, Keith and Galizio (1997) trained rats to swim to a hidden escape platform in two different pools. In one pool the platform always remained in the same location (performance). In the other pool, the platform was moved to a new location for each session (acquisition). The pools were painted different colors (back versus white) and surrounded by distinctively

different extra-pool cues to make them discriminable to the rats. After stable baselines for both components were achieved, the effects of DZP and CDZ were studied. DZP doses tested were 0.05, 0.075, 0.1, 0.2, and 0.3 mg/kg. CDZ doses studied were 0, 10, and 30 mg/kg. Drug or saline injections were given *ip* twice per week.

Keith and Galizio found that dizocilpine increased latencies in the acquisition component in a dose-dependent manner with comparable impairments in the performance component. Therefore, the selectivity of DZP on learning versus performance components was not found. The dose-response functions for CDZ were quite different from those seen with DZP. There was evidence of selective effect of this drug on acquisition. A 10 mg/kg CDZ dose increased latencies in the acquisition component but not in performance. Thus, Keith and Galizio concluded that their results contradicted the hypothesis that NMDA antagonists selectively disrupt processes involved in acquisition but were in agreement with common finding that BZDs impair acquisition of new behaviors but not performance of established ones (Keith and Galizio, 1997).

A more recent study by Keith, Pitts, Pezzuti and Galizio (2003) further demonstrated the utility of the RAP procedure in the swimming pool as a way for differentiating drug actions on acquisition and performance components. In this study the effects of three GABA modulators were evaluated: midazolam, chlordiazepoxide (both benzodiazepines), and pentobarbital (a barbiturate). Procedures similar to those used by Keith and Galizio (1997) were employed. However, only one pool was used for both performance and acquisition training.

The curtains that encompassed the perimeter of the pool were configured differently for the two task components. The dependent variables of swim path ratio, escape latency, and average swim speed were analyzed (for details see Keith et al., 2003). The researchers found that MDZ, CDZ, and PB all affected these variables in dose-dependent fashion. The important difference between two subtypes of $GABA_A$ agonists emerged: both benzodiazepines produced evidence of selective disruption of acquisition at doses that did not disrupt performance. The barbiturate, pentobarbital, however, produced acquisition impairment only at doses that also affected performance dependent variables (Keith et al., 2003).

In summary, studies that used repeated acquisitions procedures provided direct comparison of drug effects on acquisition and performance. Operant studies suggested that both NMDAR antagonists and GABA agonists produce a selective impairment of the acquisition component (Moerschbaecher and Thompson, 1980). On the other hand, studies that used modified version of RAP procedure in the spatial navigation task suggest that $GABA_A$ agonists but not NMDAR antagonists can disrupt the behavior in the acquisition component at the doses that do not affect general performance (Keith and Galizio, 1997).

Interactions between Pharmacological Systems that Influence Spatial Learning

Evidence is now emerging that some diseases of the central nervous system that cause learning and memory impairment interfere with the normal functioning of multiple neurotransmitter systems, including glutamate, GABA, and acetylcholine (Cain, Ighanian and Boon, 2000; Rush, 1988; Wozniak et al.,

1990). One way to investigate functional relationships between multiple neurotransmitter systems and behavior is to characterize differences in behavioral effects produced by drugs from two different classes given individually and in combination with one another.

A recent study by Cain et al. (2000) investigated the interactions between drugs that act on glutamate, GABA, and muscarinic cholinergic receptor systems. Cain et al. (2000) studied spatial learning in the Morris water maze by giving injections of the following agents: NPC17742 (a competitive NMDAR antagonist), scopolamine (muscarinic cholinergic receptor antagonist), and diazepam (GABA agonist) alone and in NPC-scopolamine and diazepam-scopolamine combinations.

In the Cain et al. (2000) study, one group of rats was pretrained (as described on page 9) and the others were behaviorally naïve prior to drug testing. The effects of 3.0 mg/kg NPC dose, 3.0 mg/kg diazepam dose, and 0.5 mg/kg scopolamine dose were studied alone and in combination. The experiment included the following groups: naïve scopolamine, pretrained scopolamine, naïve NPC, pretrained NPC, naïve diazepam, pretrained diazepam, naïve scopolamine + NPC, pretrained scopolamine + NPC, naïve scopolamine + diazepam, pretrained scopolamine + diazepam, naïve and pretrained controls. After place navigation testing with the submerged platform the rats received ten trials with visible platform as a control measure of non-associative effects of drugs on performance.

The main findings reported by Cain et al. (2000) were that: 1) separate or combined administration of drugs to naïve rats substantially impaired acquisition of water maze task strategies and learning the location of the hidden platform, 2) pre-trained rats given a single drug used appropriate behavior and learned the hidden platform location as quickly as controls; 3) pre-trained rats given the NPC-scopolamine and diazepam-scopolamine combinations used appropriate behavior to navigate but failed to learn the location of the hidden platform. The fact that pre-trained rats have used the appropriate behavioral strategy was apparent from the improvements in the search time on the hidden platform task. The conclusion that they learned the location of this platform after receiving a single drug was obvious after moving the submerged platform to a different location and observing rats swimming in tight loops centered on the former location.

The overall pattern of results was consistent with previous findings from Cain's group: poor performance of naïve rats was associated with an increased incidence of behavioral strategy impairments (Cain et al., 2000). Pretraining (experience with task-specific behaviors) eliminated drug-induced behavioral strategy impairments. Based on that, Cain and his co-workers concluded that for rats familiar with the required strategies, muscarinic cholinergic, NMDA, and $GABA_A$ receptor systems are, individually, not essential for robust place learning in the water maze (Cain et al., 2000).

Similar studies have been carried out using operant procedure. To directly compare drug actions alone and in combinations on learning versus

performance Thompson and Moerschbaecher (1981) used the RAP procedure in the operant chamber with patas monkeys. As their previous results showed, when administered alone phencyclidine and pentobarbital generally decreased the overall response rate in the dose-dependent manner. At higher doses, the percent errors increased and the performance component tended to be less sensitive in terms of accuracy than the learning component (Thompson and Moerschbaecher, 1980).

Thompson and Moerschbaecher, (1981) extended their previous study by investigating how these agents affected repeated acquisitions when the drugs were combined. Subjects were trained on a four-response chain by pressing the correct key in the presence of each of four geometric forms. During the performance component the four-response chain remained the same from session to session, whereas for the learning component it was changed every session. A steady state of repeated acquisition was established before initiating the drug study. The data were analyzed in terms of (a) the overall response rate and (b) the overall accuracy or percent correct. The order for giving drugs was as following: 1) the doses of PCP were tested in a mixed order with two determinations of each dose; 2) next, 3 mg/kg of PB was administered alone; 3) varying doses of PCP (in a mixed order again) were tested in combination with the 3 mg/kg dose of PB; 4) 3 mg/kg of PB was administered alone then. Then, using the same procedure, a higher dose of PB, either 7.5 or 10 mg/kg, was tested alone and in combination with varying doses of PCP. Finally, the dose-response curves for PCP alone were re-determined.

The higher doses of NMDAR antagonist and GABA agonist, when administered alone, again decreased the overall response rate and increased the percent errors in the both components of multiple schedule. The acquisition component was disrupted in that monkeys began making errors at smaller doses than were required to disrupt the performance component. When PCP was administered in combination with PB, the NMDAR antagonist dose-response curves for both accuracy and rate of responding were shifted progressively to the left as the dose of GABA agonist was increased. Since the original effect of PCP alone was replicated after PCP- PB combinations, it was clear that the shift couldn't be attributed to the development of sensitization to NMDAR antagonist. The authors concluded that the most reasonable explanation for this shift would be that pentobarbital "potentiated" the effects of phencyclidine, that is, combinations of NMDAR antagonist with GABA agonist consistently produced greater rate-decreasing and error-increasing effects than expected from simple addition of the effects of each drug given alone (Thompson and Moerschbaecher, 1981).

These results were consistent with a subsequent study reported by Thompson and Moerschbaecher (1982) in which pigeons were subjects. The task was that three response keys were illuminated at the same time by one of four colors and the animal was to acquire a four-response chain by pecking the correct key in the presence of each color. The order of injections was the same as in the previous study. The general results of this study were in agreement with study on monkeys described above. It appeared that phencyclidine-

pentobarbital combinations produced supra-additive effects on operant behavior in pigeons and patas monkeys (Thompson and Moerschbaecher, 1982).

Somewhat different results emerged from the study of effects of NMDAR antagonist - GABA agonist (PCP and PB respectively) combinations on schedule-controlled behavior in the squirrel monkeys by Chait and Balster (1978). Animals leverpressed under variable schedule (starting with FR 1, then moving to VI 15 and, later, gradually increasing the interval to VI 100). No evidence was found for the hypothesis that NMDAR antagonist enhances the disruption of responding produced by GABA agonist. In fact, most combinations in this study yielded rates of responding higher than expected based on additive effects. Chait and Balster (1978) concluded that less disruption of responding occurred than would be expected based on simple addition of the effects of each drug given alone, i.e. the two compounds antagonized one another.

In summary, experiments that investigated the effects of NMDAR antagonist-ACh antagonist and $GABA_A$ agonist-ACh antagonist on spatial learning and memory (Cain 2000) suggest that simultaneous blockage of the systems can cause failure of learning. The studies based on chain-response are inconsistent in terms of their findings. Some experiments indicate that combined administration of NMDAR antagonist-$GABA_B$ agonist produces "potentiated" disruptions (Thompson and Moerschbaecher, 1981; Thompson and Moerschbaecher 1982). The others argue that PCP-PB combination would cause an impairment even less than expected from adding the effects of two agents administered alone (Chait and Balster, 1978).

ACh Antagonist Effects on Spatial Learning

Changes in acetylcholine-releasing neurons have been documented in patients with Alzheimer's disease (Coyle et al., 1983). Thus, researchers have focused much attention on exploring the possibility that neuronal processes that depend upon acetylcholine are necessary for learning and memory. In particular, the behavioral effects of centrally active muscarinic receptor blockers have been intensively investigated. The important role played by cholinergic neurons in memory has been strengthened by the findings that ACh central agonists enhance recent memories and reverse learning deficits caused by anticholinergic drugs (Coyle et al., 1983). Furthermore, the ACh blocker scopolamine has been shown to produce impairments in recent memories but spared immediate registration and long-term memory in young adult humans (Coyle et al., 1983).

Similar to that, Savage, Faust, Lambert, and Moerschbaecher (1996) obtained evidence that scopolamine probably does not affect long-term memory storage but rather disrupts short-term memory processes. Savage and his colleagues evaluated effects of scopolamine in monkeys responding under operant procedures designed to evaluate drug effects on learning and memory. In their procedure, responding was maintained by food presentation under a multiple-component schedule. One component of the schedule was a repeated-acquisition task in which the discriminative stimuli for left- and right-key responses changed each session (learning). In the other component, the discriminative stimuli for responses were the same each session (performance). Doses of scopolamine ranged from 0.0032 to 0.032 mg/kg. In both components

of the multiple schedule, scopolamine produced dose-related decreases in responding; there was little evidence of differential rate-decreasing effects between components. Errors in learning increased in a dose-related manner, whereas percent errors in performance were generally unaffected except at high doses, which also produced substantial decreases in response rate. These results suggested that acquisition is more sensitive to the disruptive effects of scopolamine than is performance.

The second procedure utilized repeated acquisition and delayed performance as a technique to study the effects of scopolamine on memory. In this procedure, each session was divided into three phases: acquisition, delay, and performance. Two different delays were studied. After a 24-h delay, scopolamine had little or no effect on retention, accuracy or rate of responding. In contrast, after a 60-min delay, scopolamine decreased retention in a dose-related manner. These data suggest that scopolamine produces a greater disruptive effect when short (60-min) delays intervene between training and testing than when long (24-h) delays intervene (Savage et al., 1996).

Consistent with the previous finding that scopolamine produces selective deficits in short-term memory, Higgins, Woodward and Henningfield (1989) provided evidence that another anticholinergic drug, atropine, when administered to humans under RAP baselines, affected behavior in the acquisition component at lower doses than required to disrupt performance (Higgins et al., 1998). These results along with Savage et al. findings suggest selective nature of anticholinergic actions.

As mentioned above, Cain and his colleagues (2000) found that after the administration of the 0.5 mg/kg dose of scopolamine to the rats all subjects performed as well as rats that were injected with saline. Analysis of swim path patterns and qualitative observations of rats led authors to argue that rats learned the spatial location of the platform and retained the behavioral strategies required to perform the task. However, when given the scopolamine-diazepam or scopolamine-NPC combination, the platform locations were no longer learned, although general performance was still spared (Cain et al., 2000).

In contrast, other evidence suggests that the core action of scopolamine appears to be related to disrupting appropriate behavioral strategies. Whishaw (1989) provided clues that it is likely that the main function of cholinergic system is to facilitate the selection of appropriately guided movements necessary to perform navigation tasks and that learning disruptions per se may be secondary to performance deficits. In the same manner as Cain suggests in his study that muscarinic cholinergic activity may not be essential for robust place learning, Whishaw's results indicate that disruptions in the use of motoric skills contribute remarkably to the deficits produced by cholinergic blockade in spatial navigation task. A number of aspects of rats' performance were examined in order to dissociate putative performance and learning deficits.

In the initial studies in the water maze, Whishaw (1989) found that while cue response (strategies involved in swimming to the visible platform), position response (turning body left or right and then swim away of the wall), and place navigation retention (evaluating performance of previously learned tasks) were

unimpaired in rats subject to anticholinergic blockade, place navigation acquisition was impaired. Rats were given various doses of atropine sulfate (0, 10, 50, 100 mg/kg). Subjects demonstrated impairments at doses as low as 10 mg/kg, although reaching the control levels of performance after substantial training and showing that they did learn the platform location. These results paralleled Cain's findings and raised a question of whether muscarinic cholinergic system is essential for the place response acquisition.

To address this issue, Whishaw sought to determine whether the deficit was related to the place learning per se or it was related to the systems that are only indirectly involved in effective learning. To do so, Whishaw carried out three experiments: one swimming place task and two dry-land tasks. The swimming task required rats to locate a submerged platform in the pool. The first dry-land task required rats to find a hole on the large circular platform from which they could escape to their cage; there were also several dark disks left on the apparatus to imitate the hole. The second dry-land task employed the same circular apparatus, which was now covered by 2 cm of sawdust. Rats were required to search for a food pellet, which was placed at specific location hidden under the layer of dust (rats were food deprived). Thus, in all tasks the goal was to find a target that remained at the consistent location relative to distal room cues.

Four trials per day were given for five days, then rats were given a probe trial on each of the tasks with the targets moved to a different location. All three tasks were compared to the performance on the swimming cue task (visible-

platform task). Atropine-treated rats performed significantly worse than controls in all three tests in terms of escape latencies. The observation of rats' behavior revealed that atropine-treated rats adopted different behavioral strategies than did controls. These strategies employed either thigmotactic movements in the pool of water or, again, movements against the wall on the circular apparatus with rats' backs arched and limbs extended. These results indicated that impairment found previously in the water maze task was not specific but rather can be demonstrated in a variety of navigation tasks. Whishaw's analysis revealed that atropine caused rats to adopt maladaptive motoric action patterns in each of the different tasks. Interestingly, when these three tasks were studied in well-trained rats, their performances were not disrupted.

The results of Whishaw's study demonstrated that naïve, atropine-treated rats were impaired on place acquisition tasks and that the impairments were likely due to subtle effects on motor patterns that can only be detected, Whishaw argued, by carefully observing the movements of the rats while they are performing the behavioral tasks used to measure learning (Whishaw, 1989).

The picture of anticholinergic actions described in this section is ambiguous. On one hand, some studies suggest that anticholinergic drugs produce selective learning/memory impairments in the chain-response RAP settings (Higgins et al., 1989; Savage et al., 1996). Other studies, however, imply that anticholinergic drugs cause motor impairments that interfere with the acquisition of new, but not previously learned, behaviors (Cain et al., 2000; Whishaw, 1989). One of the experiments presented in this thesis was designed

to further investigate the effects of anticholinergis agent scopolamine on learning and memory.

To summarize the literature reviewed above, data from the studies that employed serial chains suggest that compounds from all three classes produce selective action on non-spatial learning (Moerschbaecher and Thompson, 1980, Savage et al., 1996). Studies based on spatial learning tasks by Cain et al. (2000), Keith and Galizio (1997), Whishaw (1989), and Keith et al. (2003), however, suggest that NMDAR and ACh antagonists and $GABA_A$ receptor modulators all can produce impairments on spatial learning tasks but that they produce their effects by disrupting different behavioral processes. The Keith and Galizio (1997) study demonstrates evidence that $GABA_A$ agonists but not NMDAR antagonists can selectively impair spatial learning and memory. Findings from Cain et al. (2000) and Whishaw (1989) indicate that all three systems ($GABA_A$, NMDA, and ACh) targeted alone may not be essential for spatial learning/memory processes and that manipulations with agents mostly affect motor functions necessary to perform the tasks.

The experiments reported in this thesis were designed to reevaluate the effects of single compounds (dizocilpine, chlordiazepoxide, and scopolamine) on spatial learning and memory using the modified version of spatial navigation task. Additionally, no study has evaluated the simultaneous effect of NMDAR antagonist-GABA agonist and achetylcholinergic antagonist-GABA agonist on spatial learning in a water maze using multiple-component repeated-acquisition procedure. To investigate whether these neurotransmitter systems are

interdependent, the present study used RAP procedure in a Morris water maze given neurochemical agents that target NMDA, GABA, and ACh systems alone and in combination.

METHODS

Subjects

Subjects were four experimentally naïve, male Holtzman Sprague-Dawley rats (K3, K6, K8, and L4). Each animal was individually housed under 12:12 hr dark-light conditions. Food and water were available ad lib.

Apparatus

The apparatus was a circular white fiberglass pool (1.5 m diameter, 45.7 cm deep) filled with 22.5 cm of water and a small white platform (10 cm diameter, 20 cm high) that was submerged 2.5 cm below the surface of the water. White non-toxic paint powder was used to make the water opaque. The water temperature was maintained at 30° C (±2° C). The room that housed the pool was 3m x 3m with white painted cinderblock walls. The swimming pool was illuminated with two indirect halogen lights (500 Wt). A digital camera was mounted to the ceiling directly above the center of the pool to view only the area of the pool. Data from the camera were collected on a computer by running data acquisition software (Polytrack, San Diego Instruments) and included escape latency, total distance traversed, and pool quadrant entries. The animals were observed during the trials on a monitor that displayed the camera's view.

Experimental Variables

The experiment in this thesis used a within-subject design with multiple

dependent and independent variables. The dependent measures were escape latency, swim path ratio and swim speed. Escape latency was measured in seconds from the time the subject is placed in the pool until it reaches the platform. This dependent variable measures how much time it took rats to swim to the hidden platform. The swimming speed was measured in centimeters per second. This dependent variable captures the motoric aspects of the behavior and reflects how fast the rats' swimming was. Both escape latency and swimming speed are conventional measured often used in the studies investigation the effects of drugs on the spatial learning. The swim path ratio, measured from release to escape, was the ratio of the difference between the actual swimming distance and the minimum possible distance from the release location to the escape platform. This variable measures how accurate the path that the rat took was. Swim path ratio was computed using the following formula: (AD-MD)/MD, where MD is the minimal distance and AD is the actual distance swam on the given trial. A swim path ratio of zero indicated that the rat took the most direct route to the submerged platform. In the present study, decreased latencies and swim path ratio within a training session were used to define learning.

The independent variables included task component (performance or acquisition) and drug dose. The task involved two components - performance and acquisition. The performance component was designed to measure performance on a well-learned navigation problem. The acquisition component was designed to measure the rate of learning about new platform locations.

There were several doses of the compound in each of the experimental study. Saline injections were also administered to control for possible effects of the injection procedure on performance.

Preliminary Training

Initially, animals had six trials per day (Monday through Friday) during which they were trained to swim to a submerged platform that was always located at the same place throughout training. The curtain configuration that was used during training on the performance component is shown on the top panel of Figure 1. Four release points that corresponded with the points where the curtains met were used. The order of release location was determined prior to the beginning of the session in a pseudorandom manner. Each release point was used at least once per session but never twice in a row. To begin a trial, a rat was gently placed into the water facing the wall of the pool at one of four starting points and a stopwatch was started. Once an animal stepped onto the platform the trial ended. The subject was left on the escape platform for 15 s. If a subject failed to find the platform by 60 s, it was led to the platform by experimenter. Then a rat was returned to its home cage where it remained for 2-min intertribal interval (ITI). The sequence of start points was the same for each rat in the session but different from day to day.

Preliminary training lasted until subjects met a criterion of three consecutive sessions with escape latencies for each session averaging less than 10 sec per trial.

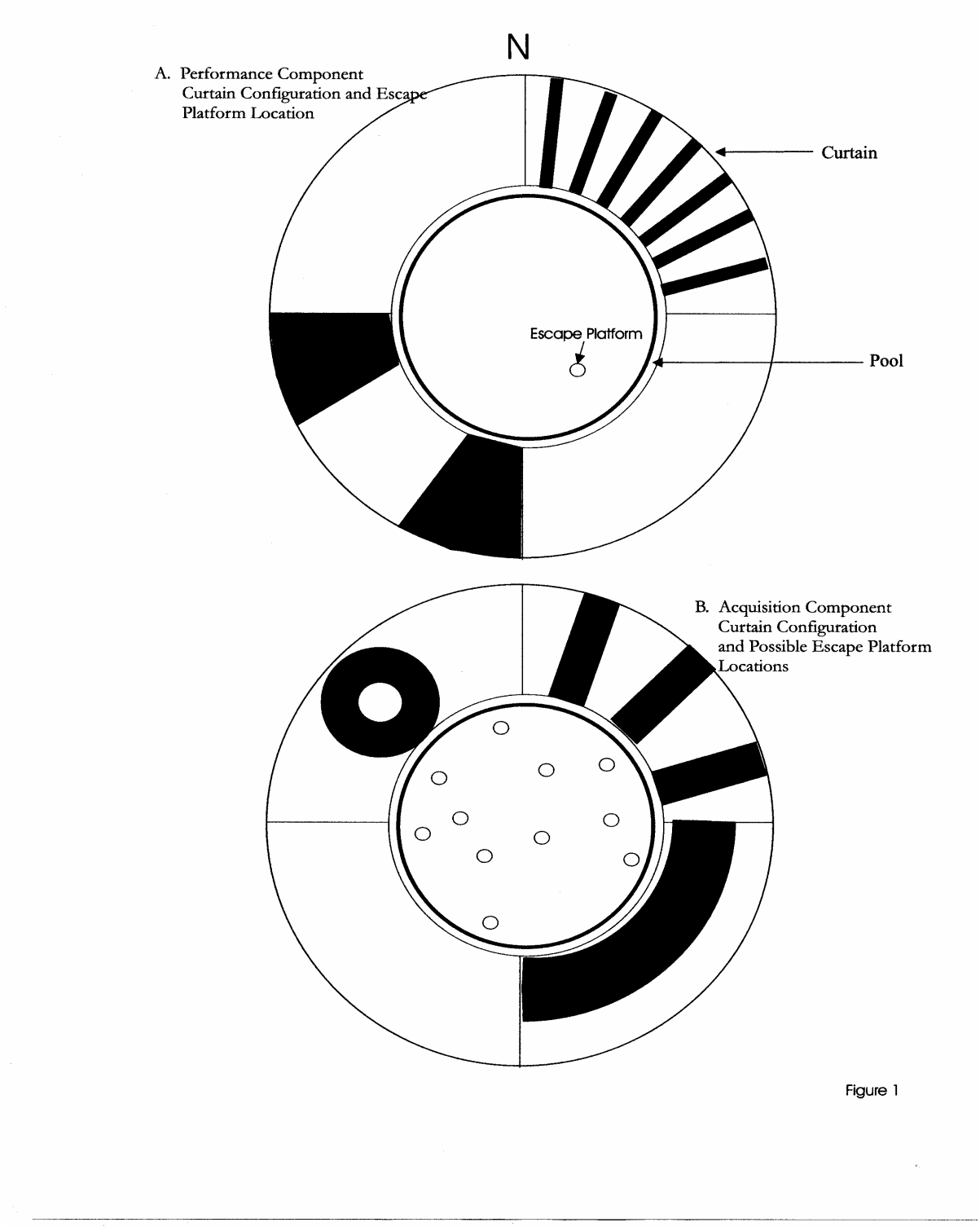

Figure 1. Escape platform positions and stimulus arrangement used during multiple-component training. Top panel presents a platform position and stimulus arrangement during the performance component. Bottom panel presents platform positions and stimulus arrangements during the acquisition component.

Repeated Acquisitions Training

Once animals met a criterion for preliminary training, they were moved to the repeated acquisitions training. During this stage, subjects received six daily trials on each of two spatial navigation tasks. During the performance component the submerged platform remained at the same location throughout training and testing. The performance component was the same as during preliminary training. During the acquisition component the submerged platform was moved to a different location on each day of training and testing but remained at the same place throughout a daily session.

The arrangement of curtains that surrounded the pool during the acquisition component is shown on the bottom panel of Figure 1. The sequence of platform positions was randomly determined with the constraint that the same position could not be used on consecutive days. The eleven locations of the escape platform for acquisition component are shown on the bottom panel of Figure 1.

For each rat, training trials were alternated between the performance and acquisition components. Each training and testing session began with the performance component, and was followed by 2 min ITI and then a trial in the acquisition component.

In order to meet a criterion for repeated acquisition training, rats' escape latencies had to be less than 10 sec per trial in the performance component for all six trials and less than 10 sec per trial in the acquisition task for trial 2-6 during

the final 10 sessions. After reaching the criterion subjects entered the drug study.

Throughout the drug study, injections were administered twice a week (Tuesday and Friday). Average number of determinations for each dose and each animal was 3 (range 1-5 determinations). The order at which doses were tested was semirandom. The time period allowed to wash the tested compound out of the animal's system after a particular experiment was conducted and before the next study began was one week.

Experiment 1: Dizocilpine

All four subjects received following doses of dizocilpine: 0.01, 0.03, 0.1, 0.18, and 0.3 mg/kg. The daily protocol for drug testing was the same as during baseline training. Testing was conducted five days a week with Thursdays serving as control baseline sessions and the drug was administered on Tuesdays and Fridays. An *ip* injection of DZP or vehicle was administered to the subjects 30 min prior to the session.

Experiment 2: Chlordiazepoxide

All four subjects received following doses of chlordiazepoxide: 1, 3, 5.6, 10, and 17 mg/kg. The daily protocol for drug testing was the same as during the experiment 1. An *ip* injection of CDZ or saline was administered to the subjects 15 min prior to the session.

Experiment 3: Dizocilpine-Chlordiazepoxide

All four subjects received the following combinations: 0 DZP-0 CDZ, 0.03 DZP-1.0 CDZ, 0.03 DZP-3.0 CDZ, 0.1 DZP-1.0 CDZ, and 0.1 DZP-3.0 CDZ

mg/kg. These doses of individual drugs were used in combination on the basis of pilot observation of the data obtained after administering dizocilpine and chlordiazepoxide alone. Doses of DZP and CDZ that after administering alone produced either no effect or selective effect were chosen to test the combined action of drugs. An *ip* injection was performed twice: the first injection contained dizocilpine and was given 30 min prior to the session, the second injection contained chlordiazepoxide and was given 15 min prior to the session (or 15 min after the first injection). Double injections of saline served as a control and was given in the same manner (15 min apart, 30 and 15 min prior to the control session).

Experiment 4: Scopolamine

All four subjects received following doses of scopolamine: 0.03, 0.1, 0.17, 0.3, 1.0, 1.7 mg/kg. The daily protocol for drug testing was the same as during the previous experiments. An *ip* injection of SC or saline was administered to the subjects 20 min prior to the session.

Experiment 5: Scopolamine-Chlordiazepoxide

Three subjects received following combinations (subject L4 died prior to this experiment): 0 SC-0 CDZ, 0.3 SC-3.0 CDZ, 0.3 SC-5.6 CDZ, 1.0 SC-3.0 CDZ, and 1.0 SC-5.6 CDZ mg/kg. An *ip* injection was performed twice: the first injection contained scopolamine and was given 20 min prior to the session, the second injection contained chlordiazepoxide and was given 15 min prior to the session (or 5 min after the first injection). Double injection of saline served as a the control session).

Data Analysis

Statistical analyses were conducted using the Statview statistical software package (SAS Institute, Cary, NC). Repeated-measures ANOVAs were performed on each the dependent variable for each component. In cases where raw distributions were severely skewed, data for escape latency and swim path ratio were log-transformed to normalize the distributions. Unpaired t-tests were used as post-hoc tests to determine whether at specific doses differed significantly from those of saline sessions. Bonferroni corrections (α/n, where α is .05 and *n* is the number of unpaired tests performed) were conducted to adjust the α level for multiple comparisons. In addition to doing traditional analysis on the means of dependent variables on the different drug condition, the individual subject results were examined in detail. Data on the dependent measures were plotted as a function of drug doses or trial number. The hypothesis that chlordiazepoxide and scopolamine produce selective acquisition impairments would be supported in cases where a dose would produce reliably significant impairment of escape latency or swim path ratio in the acquisition, but not in performance, component. The hypothesis that dizocilpine does not produce selective acquisition impairments would be supported in case in which a dose would produce reliably significant impairment of escape latency or swim path ratio in each of the components. The hypothesis that dizocilpine-chlordiazepoxide and scopolamine-chlordiazepoxide combinations produce overadditive effects would be supported if a combination produced greater disruption than expected from adding the effects of two doses when given alone.

RESULTS

Preliminary Training

During preliminary training all four rats rapidly learned the first task component, the performance component. The criterion for this training (three consecutive sessions with escape latencies for each session averaging less than 10 sec per trial) was reached by all rats by the ninth session (range 3-9 sessions, M = 4.75). Each animal then received six trials per day in the multiple-component task (performance and acquisition). Multiple-component training required 11-36 sessions (M = 21.5) for rats to reach the criterion (escape latencies had to be less than 10 sec per trial in the performance component for all 6 trials and less than 10 sec per trial in the acquisition task for trial 2-6 during the final 10 sessions) and move into the drug study.

Figure 2 shows mean escape latencies presented as a function of trial averaged across the last ten days before the drug studies began. Open circles represent acquisition training latencies; closed squares represent performance training latencies. It is evident that all four subjects had mastered the performance component and showed clear evidence of learning during the acquisition component. Furthermore, the pattern obtained for swim path ratios during training was similar to the one presented for escape latencies.

Figure 3 shows each individual rat's escape latencies obtained for each animal during the last ten sessions before the drug treatment began. Each panel of this figure represents the subject's mean escape latencies for six trials for

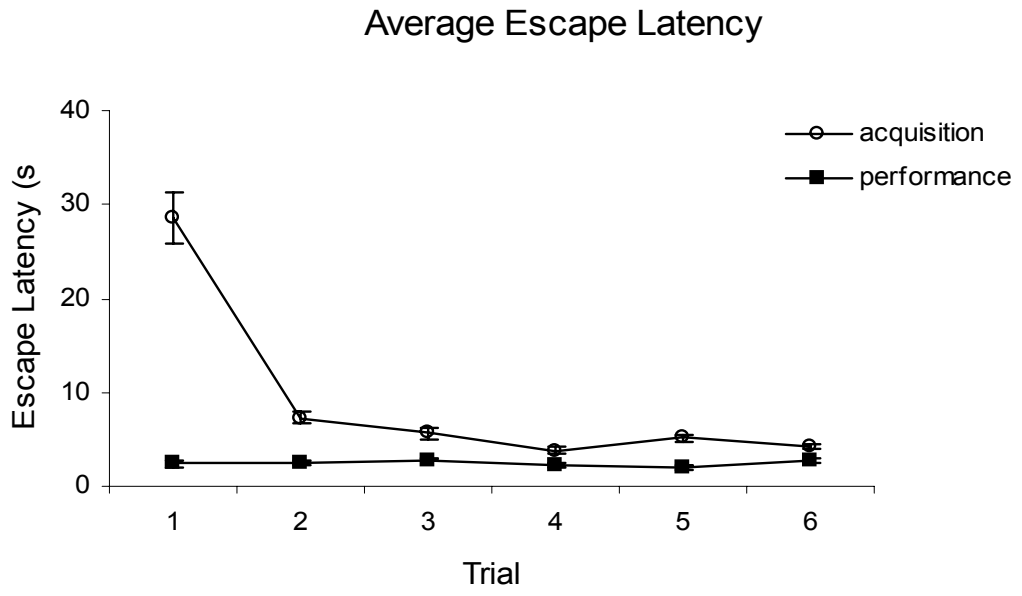

Figure 2. Mean escape latency during acquisition (open circles) and performance (closed squares) as a function of trials within last ten baseline sessions before drug study began.

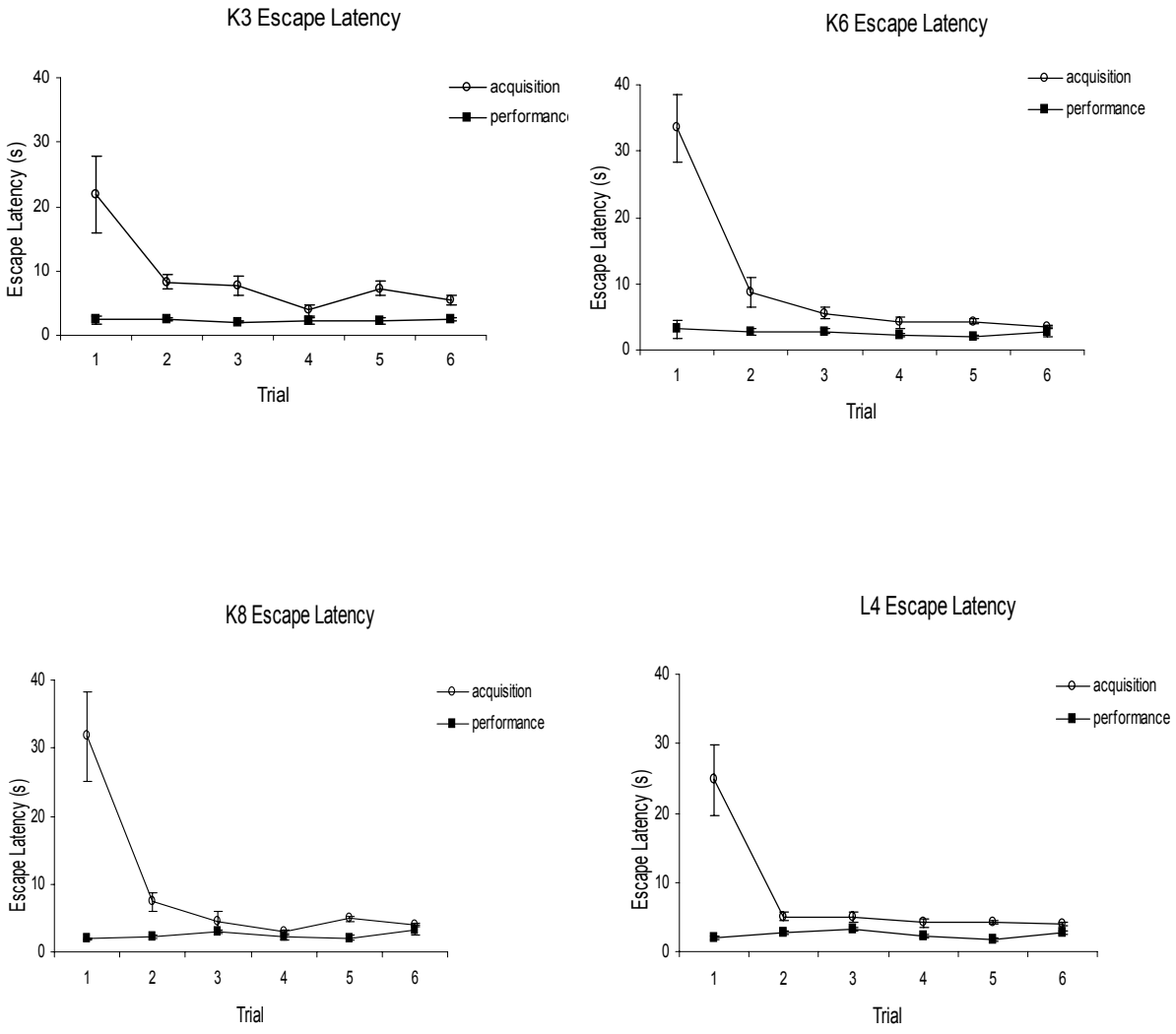

Figure 3. Individual mean subject escape latency during acquisition (open circles) and performance (closed squares) as a function of trials within last ten baseline sessions before drug study began.

both schedule components. It is apparent that all four subjects learned the new locations during training on acquisition component at approximately the same rate, although animal K3 tended to fluctuate a bit more than the rest of the animals. Nonetheless, K3's latencies on the last acquisition trial were consistently shorter than on trials 1-5 during acquisition component. Overall, the acquisition patterns were fairly consistent across all animals.

Baseline Behavior

After all subjects met the criterion required to enter the drug study, very stable behavioral patterns during both components were maintained throughout the study. Figure 4 shows mean swim path ratios for all four subjects during baseline sessions performed on Thursdays. Similarly, latency data are provided in Figure 5. Both figures present data from each of the drug studies. Both dependent variables under acquisition (open circles) and performance (closed squares) are reported as a function of a trial number. It is apparent from both figures that escape latencies and swim path ratios were low in the performance component and relatively high on the first trial of the acquisition component. Additionally, a within-session decline in acquisition component is apparent for both of the dependent variables.

Experiment 1: Dizocilpine

Figure 6 presents the effects of DZP on three dependent variables: escape latency (top panel), swim path ratio or SPR (middle panel), and swim speed (bottom panel). The closed squares represent group means on the performance component (all six trials of each session were analyzed) and

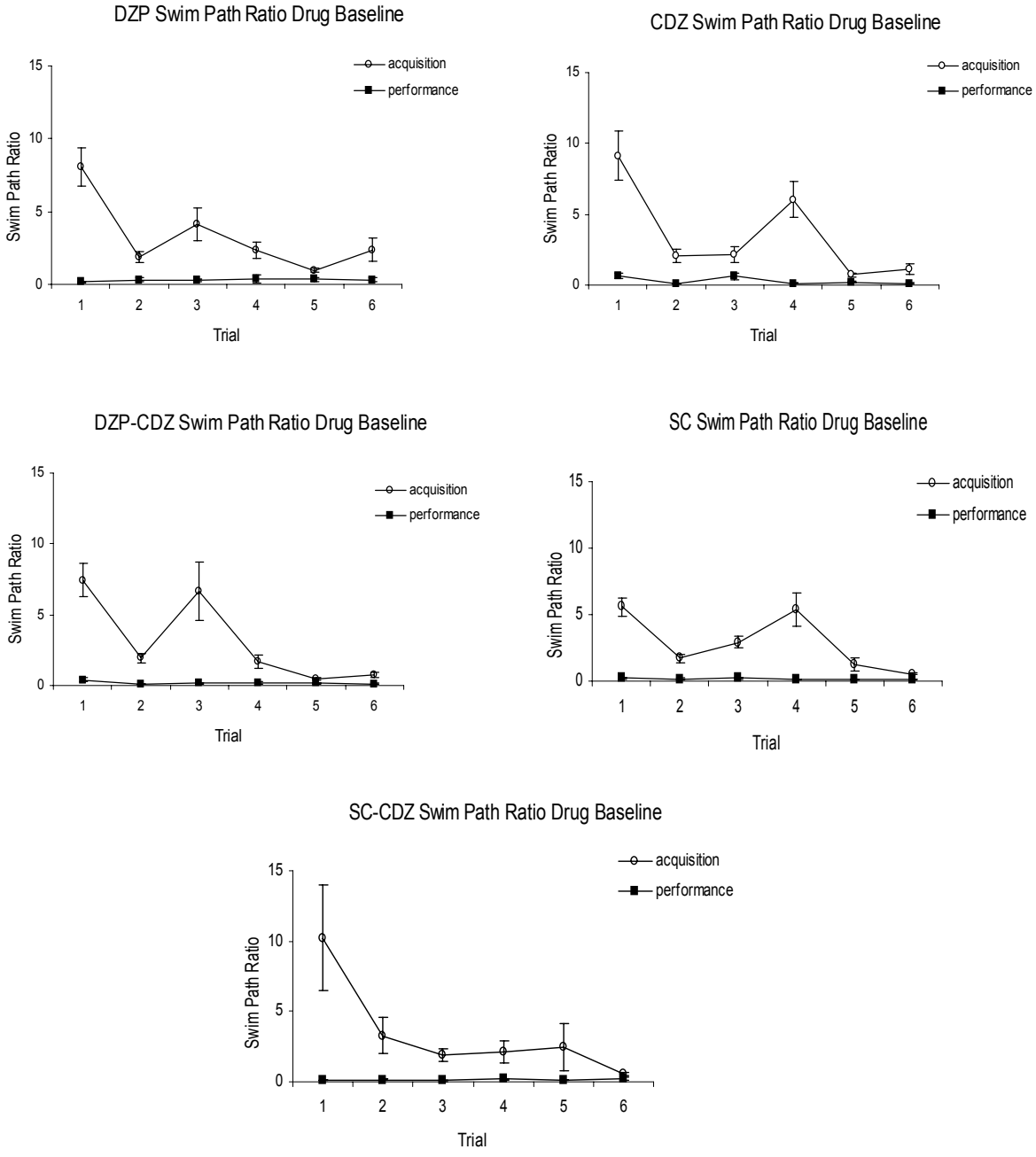

Figure 4. Mean swim path ratio during acquisition (open circles) and performance (closed squares) as a function of trials within drug baseline sessions (Thursdays). DBL sessions were followed by a drug testing session on the next day. Bars indicate standard error of the mean. Panels present: dizocilpine DBL (top left), chlordiazepoxide DBL (top right), dizocilpine-chlordiazepoxide DBL (middle left), scopolamine DBL (middle right), and scopolamine-chlordiazepoxide DBL (bottom center).

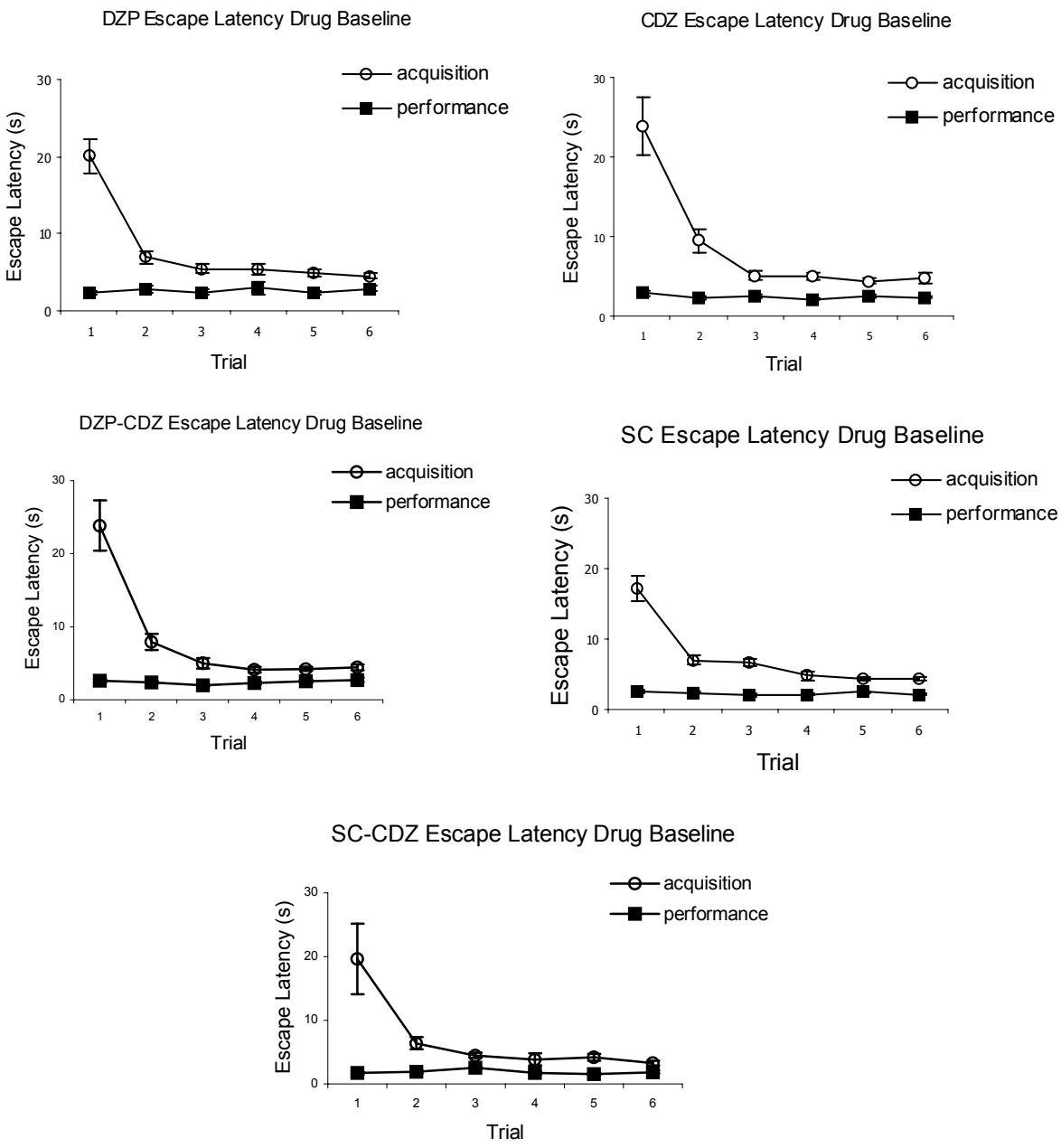

Figure 5. Mean escape latency during acquisition (open circles) and performance (closed squares) as a function of trials within drug baseline sessions (Thursdays). DBL sessions were followed by a drug testing session on the next day. Bars indicate standard error of the mean. Panels present: dizocilpine DBL (top left), chlordiazepoxide DBL (top right), dizocilpine-chlordiazepoxide DBL (middle left), scopolamine DBL (middle right), and scopolamine-chlordiazepoxide DBL (bottom center).

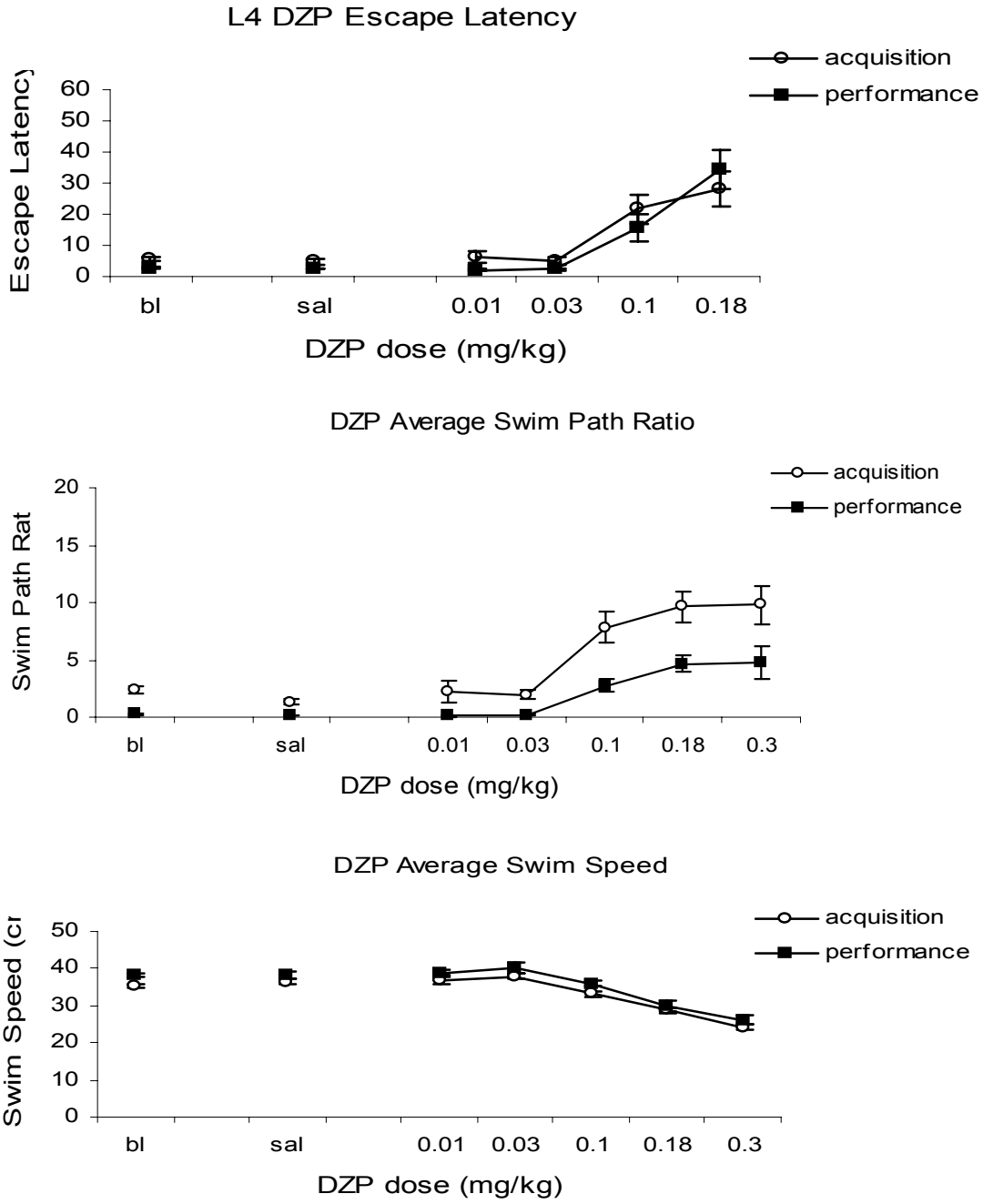

Figure 6. Mean escape latencies (top), swim path ratios (middle), and swim speeds (bottom) as a function of dizocilpine dose during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean.

the open circles represent the group means under the acquisition component (trials two through six of each session were used to compute acquisition means). Three out of four subjects were exposed to six doses of dizocilpine: Subject L4, however, did not receive the highest (0.3 mg/kg) dose of dizocilpine because he was severely impaired by the 0.18 mg/kg dose.

It is evident that administration of dizocilpine disrupted behavior on the task in both components in a dose-dependent fashion. No evidence of a selective acquisition impairment at a dose that spared performance was found; all doses that impaired acquisition also impaired performance. The raw data from escape latencies and swim path ratios were highly positively skewed and were therefore unsuitable for analysis using parametric statistics. The logarithmic transformation of the raw data from these two variables, however, produced normal distributions and the transformed data were analyzed using ANOVAs. A repeated measures ANOVAs revealed effects of dose on acquisition for SPR [$F(6,114)=11.049$, $p<.0001$], escape latency [$F(6,84)=12.632$, $p<.0001$] 0.1 mg/kg DZP dose was the lowest that produced consistent disturbances under both task components. The 0.1 mg/kg dizocilpine dose produced significantly longer escape latencies relative to saline under both acquisition and performance(p's$<.0001$), for SPR (p's$<.0001$). The effect of the dose 0.18 mg/kg was found to be significantly different from that of saline under acquisition and performance for the escape latencies (both p's$<.0001$), SPR (both p's$<.0001$) and swimming speed ($p<.0001$ and $p=.0003$, respectively). The effect of the 0.3 mg/kg dose was found also to be significantly different from that of saline under

acquisition and performance for the escape latencies (both p's<.0001), SPR (both p's<.0001) and swimming speed (p<.0001 and p=.0003, respectively). The effect of the 0.3 mg/kg dose was found also to be significantly different from that of saline administration under acquisition and performance for the escape latencies (both p's<.0001), SPR (both p's<.0001), and swimming speed (both p's<.0001). Individual subject raw escape latency data is available in the appendix E (raw data on all three dependent variables is available in the electronic version of the present thesis). The trial-by-trial data shown in Figure 7 revealed that the 0.1-0.3 mg/kg doses caused increases in both escape latencies and swim path ratios consistently on all six trials on the performance component. During acquisition component these doses caused a modest impairment that was reflected in both dependent variables. It is clear, however, that the data points of the last two trials on acquisition after administering 0.1-0.3 mg/kg were never as good as those achieved under the 0-0.03 mg/kg DZP doses.

Discussion

In agreement with previous findings (Keith and Galizio, 1997), dizocilpine, an NMDAR antagonist, impaired spatial navigation behavior in a dose-dependent manner. But the doses that disrupted acquisition latencies also produced evidence of impairment in the performance component. In fact, this effect was seen with all three dependent variables. The 0.1 mg/kg dose produced a consistent impairment in both components. It was also the smallest dose required to impair escape latencies (see Figure 8) and swim path ratios (see Figure 9) under both components in all four subjects. In the study by Keith and

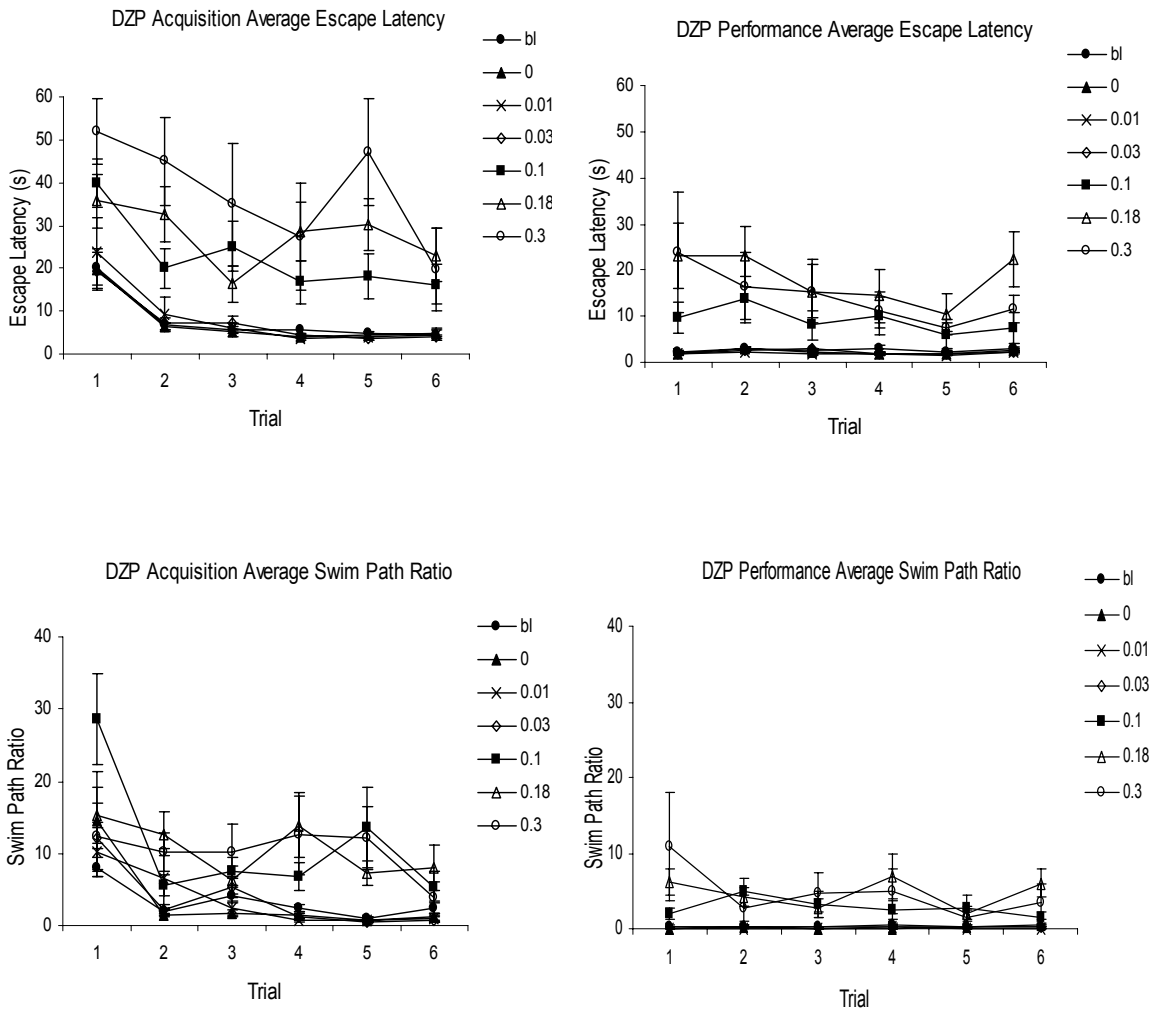

Figure 7. Mean escape latencies during acquisition (top left) and performance (top right), swim path ratios during acquisition (bottom left) and performance (bottom right) components as a function of trials for all dizocilpine doses tested. Bars indicate standard error of the mean.

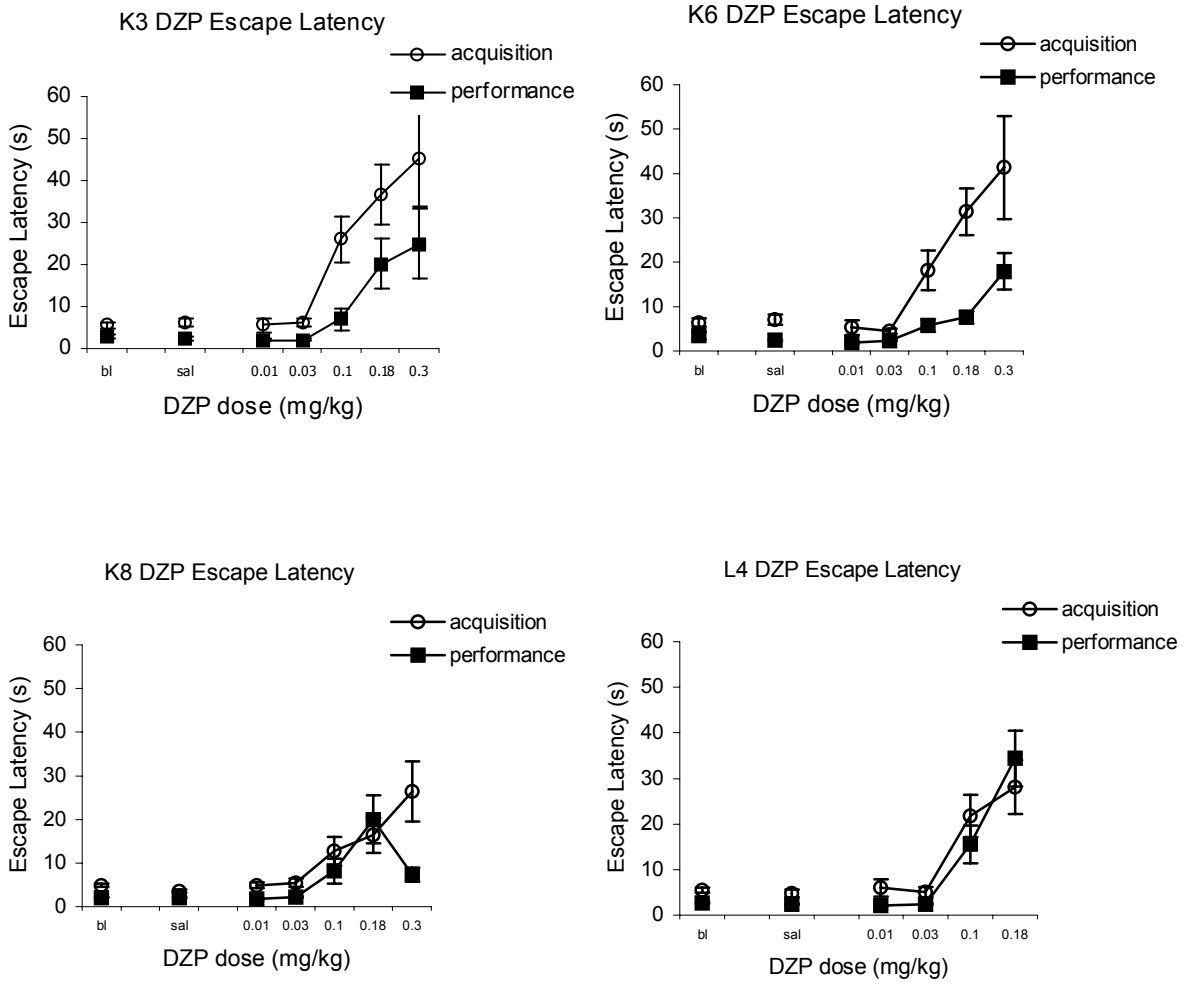

Figure 8. Individual mean subject escape latencies as a function of dizocilpine dose during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean of all determinations at a dose.

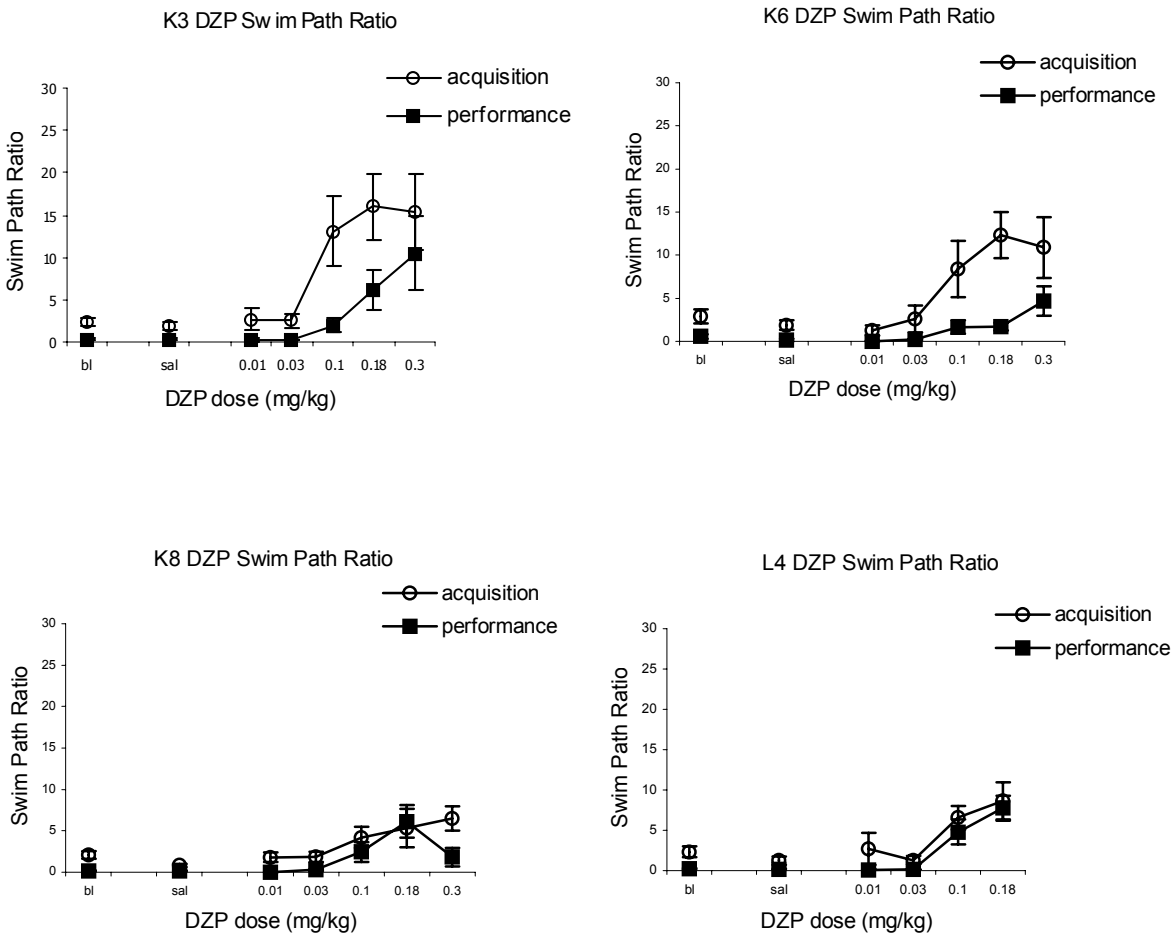

Figure 9. Individual mean subject swim path ratios as a function of dizocilpine dose during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean of all determinations at a dose.

Galizio, (1997), other doses of dizocilpine were tested using RAP protocol (0.1, 0.2, 0.3 mg/kg). In that study 0.2 mg/kg was the dose that, on average, produced impairment on both the performance and acquisition components of the spatial RAP task.

Consistent with Keith and Galizio's (1997) report, the present experiment demonstrates clearly that although dizocilpine produced general performance impairments, learning was nevertheless still apparent in the presence of relatively high DZP dose (0.1 mg/kg). This evidence is apparent from Figure 7 that show trial-by-trial plots in which some extent of learning of a new spatial location still occurred although the acquisition rate decreased (Morris et al., 1986: Danysz, Wroblewski, and Costa, 1988; Heale and Harley, 1990; McLamb, Williams, Nanry, Wilson, and Tilson, 1990).

Experiment 2: Chlordiazepoxide

Figure 10 shows the effects of chlordiazepoxide on escape latency, swim path ratio, and swim speed averaged across four subjects. The open circles represent the group means under the acquisition component (trials two through six) and the closed squares represent the group means under the performance component (all six trials). CDZ impaired behavior in both components in a dose-dependent manner. As it was the case for dizocilpine, latency and swim path ratio raw data were transformed to a log scale. Swim speeds, however, were not transformed. Repeated-measures ANOVAs revealed significant effects of dose on acquisition for SPR [$F(6,144)=5.466$, $p<.0001$], escape latency [$F(6,144)=20.624$, $p<.0001$] and swim speed [$F(6,144)=26.405$, $p<.0001$], and on

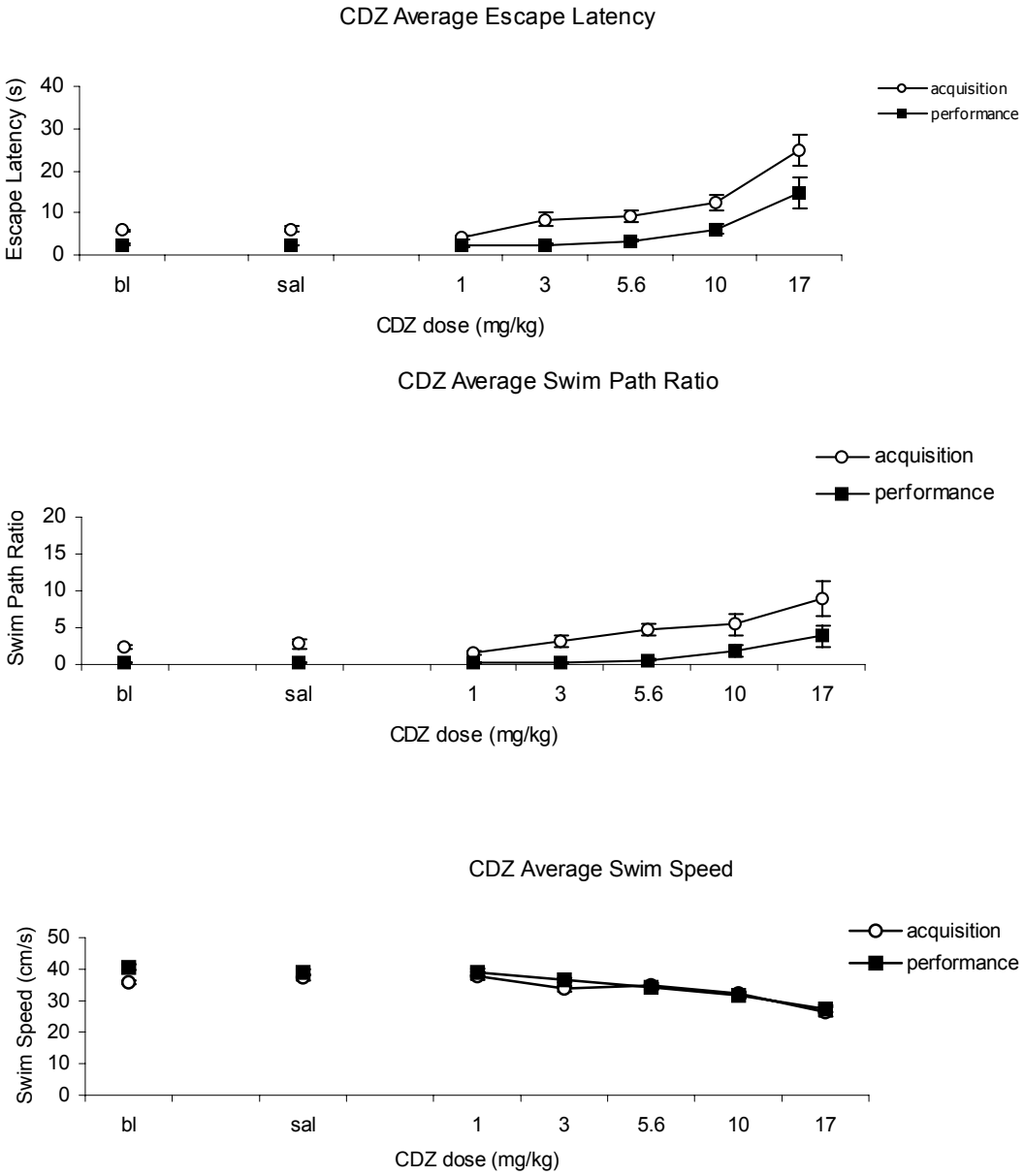

Figure 10. Mean escape latencies (top), swim path ratios (middle), and swim speeds (bottom) as a function of chlordiazepoxide dose during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean.

performance for SPR [F(6,174)=6.552, p<.0001], escape latency [F(6, 138)=23.171, p<.0001], and swim speed [F(6,174)=25.565, p<.0001]. Doses 0 and 1.0 mg/kg produced no disruption at either performance or acquisition component on any of the dependent measures. Figure 10 shows that the effect of the 3.0 mg/kg CDZ dose on escape latency did not reliably differ from those of saline (p=.2233 and p=.3973, respectively). However, the 3.0 mg/kg dose was selective for two out of four subjects (K3 and K8), causing increases in escape latencies and SPRs during the acquisition but not the performance component relative to saline baseline (see Figure 11). Figure 11 also indicates that although the dose 3.0 mg/kg selectively disrupted behavior of rats K3 and K8, this effect disappeared at the dose 5.6 mg/kg and then reoccurred in both subjects again at 10 mg/kg. On average, however, the effect of 10 mg/kg was found significantly different from that of saline administration under acquisition and performance on escape latencies (p=.0006 and p<.0001). The dose 5.6 mg/kg produced a selective effect on escape latencies in one out of four subjects (L4), increasing significantly its acquisition but not performance latency value. Nevertheless, on average this dose produced a significantly different effect from that of saline in the performance component (p<.0001) but not in the acquisition component (p=.0835). So, overall, three out of four subjects showed a selective impairment, albeit at different doses. The highest dose (17 mg/kg) further disrupted the behavior of all subjects under both components: the effect of 17 mg/kg was found statistically different from that of saline under both acquisition and performance on escape latencies (p's<.0001). Individual subject raw escape latency data is

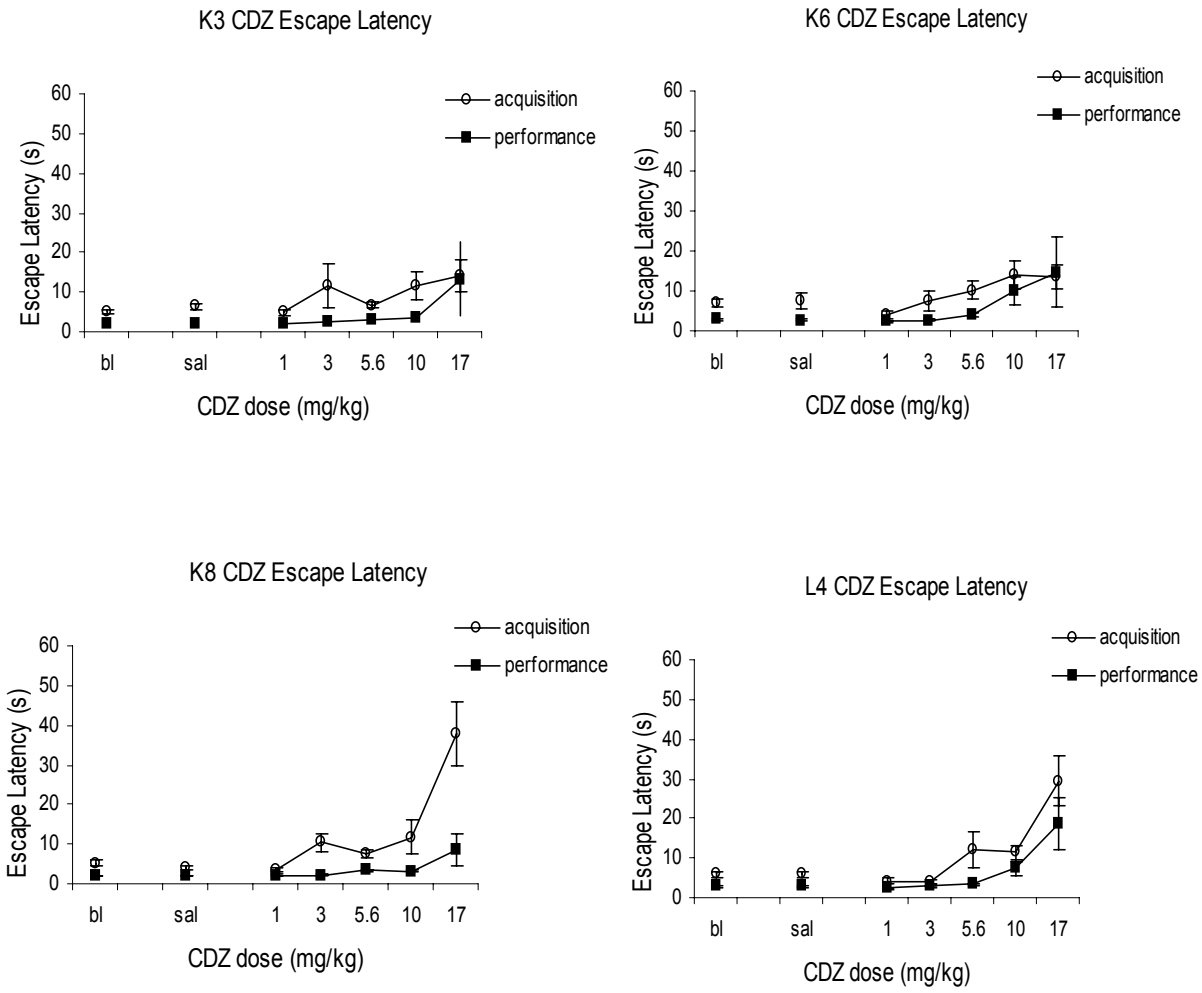

Figure 11. Individual mean subject escape latencies as a function of chlordiazepoxide dose during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean of all determinations at a dose.

available in the appendix F (raw data on all three dependent variables is available in the electronic version of the present thesis).

The 5.6 mg/kg dose of CDZ did not affect swim ratios whereas the 10 and 17 mg/kg doses increased averaged swim path ratio in both task components. The effect of 10 mg/kg of CDZ was significantly different from that of saline administration under acquisition and performance on SPR ($p=.0012$ and $p=.0066$, respectively). The effect of the highest dose, 17 mg/kg, was significantly different from that of saline administration under acquisition and performance on SPR ($p's<.0001$).

Individual rat swim path ratio plots (Figure 12) indicate the different extent of path accuracy disruption in the experimental animals. Swim accuracy of the subject L4 was selectively affected by the 5.6 mg/kg dose of CDZ; 3.0-5.6 mg/kg caused a selective impairment in the rat K8. Rats K3 and K6's accuracy, however, was never affected during acquisition component at the dose that did not disrupt their swim accuracy during the performance component.

The 5.6 mg/kg dose of CDZ was the lowest dose that affected swim speed: it significantly decreased speed in the performance component ($p=.0003$) but produced no effect in acquisition component. Doses of 10 and 17 mg/kg of chlordiazepoxide significantly decreased swim speed in both components (all $p's<.0001$) causing non-selective effect on this variable. Interestingly, data presented as a function of trial (Figure 13) revealed that doses of 10 and 17 mg/kg caused a remarkable increase in swim path ratio and escape latency on the first trial of the performance component. Such effect on latencies appeared

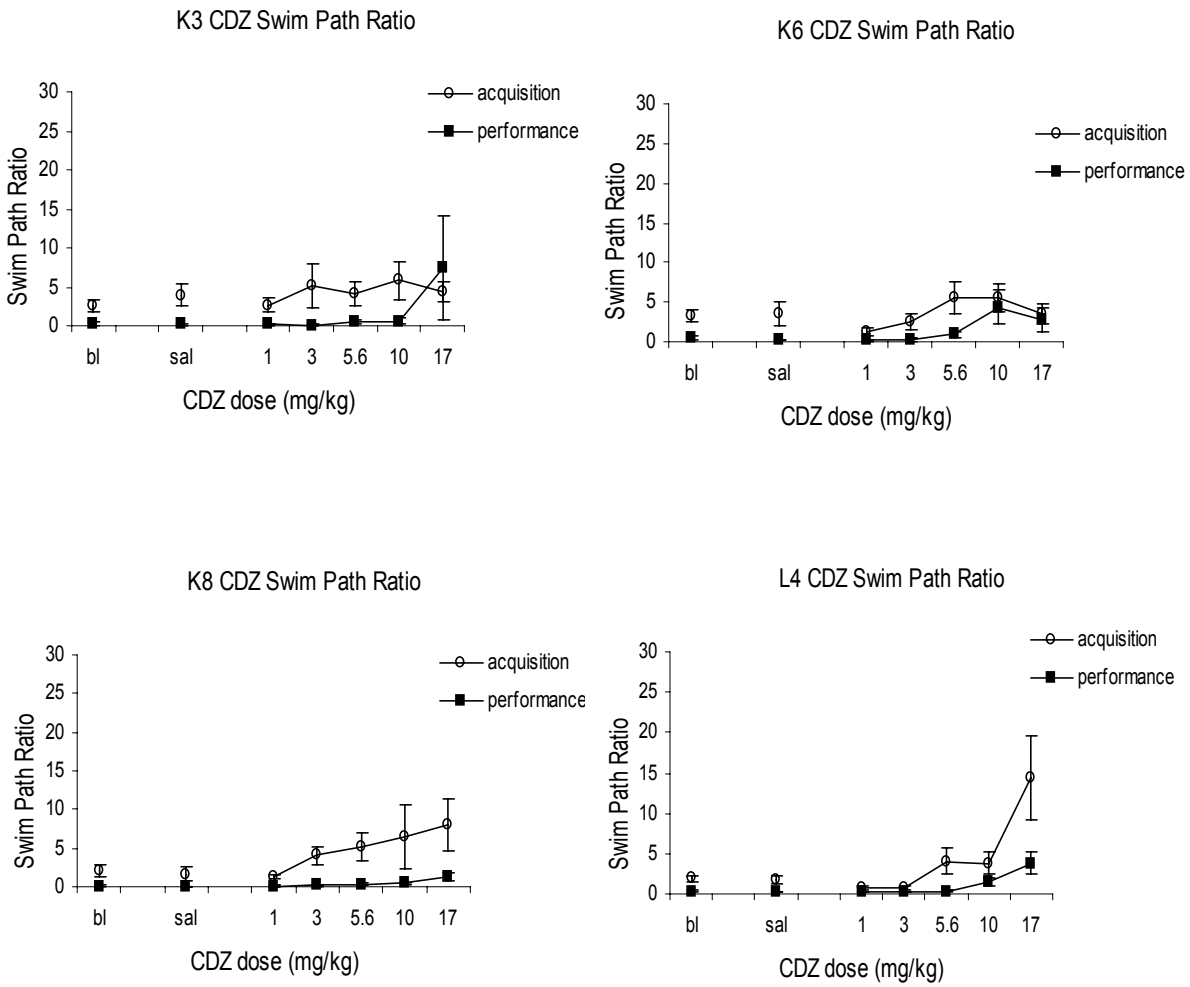

Figure 12. Individual mean subject swim path ratios as a function of chlordiazepoxide dose during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean of all determinations at a dose.

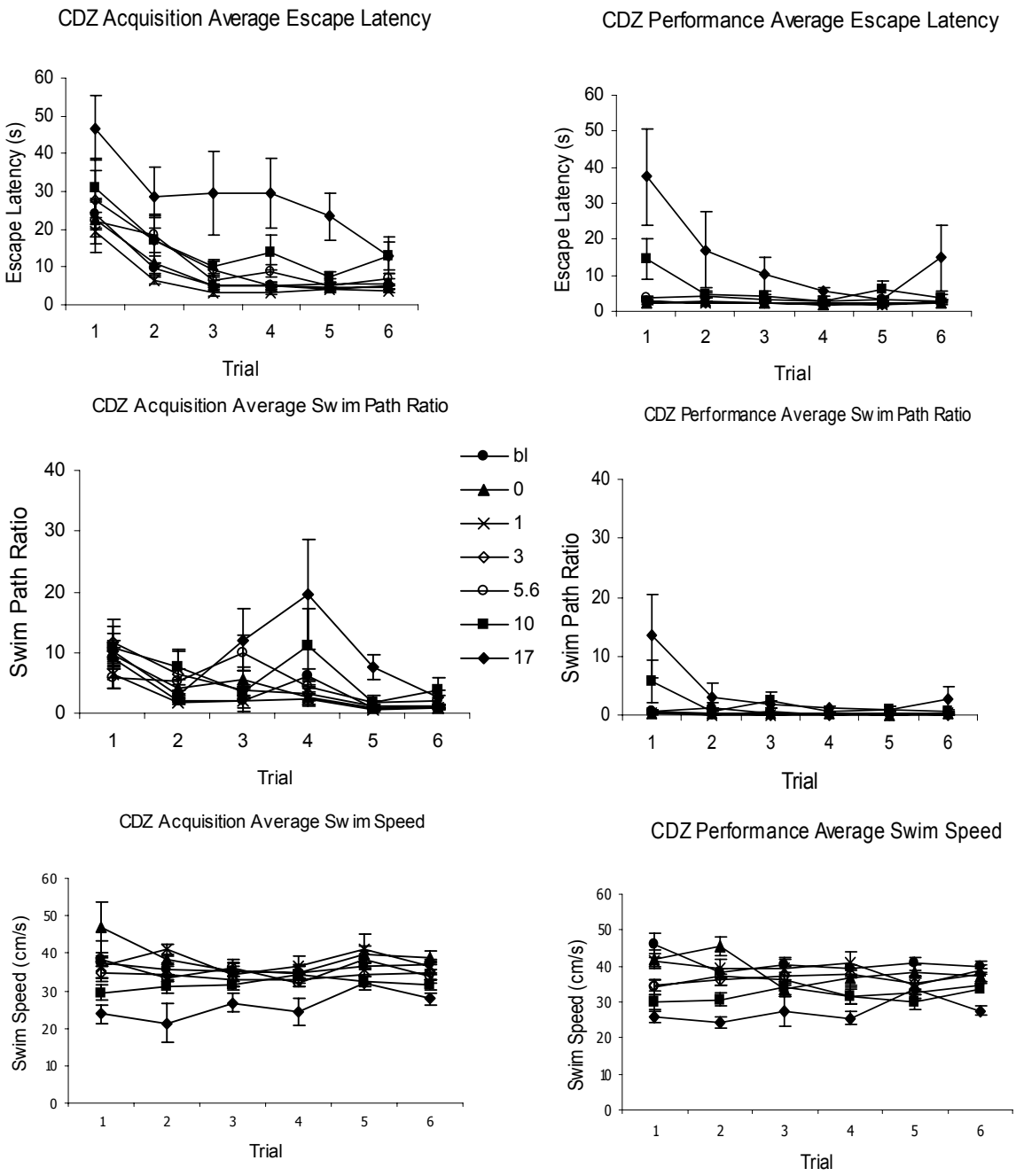

Figure 13. Mean escape latencies during acquisition (top left) and performance (top right), swim path ratios during acquisition (middle left) and performance (middle right), and swim speeds during acquisition (bottom left) and performance (bottom right) components as a function of trials for all chlordiazepoxide doses tested. Bars indicate standard error of the mean.

not to be due to changes in swim speed and state-dependency (see Figure 13), raising the possibility that the impairments caused by the high chlordiazepoxide doses during the performance component of the task may owe to retrograde amnesia.

Discussion

The main finding of this experiment replicates the results of Keith and Galizio (1997) and Keith et al. (2003) studies that used the same paradigm to study effects of chlordiazepoxide on spatial learning and memory. In mentioned above studies, it has been a consistent experimental outcome that this $GABA_A$ agonist causes selective impairments of acquisition swim path ratios and escape latencies at doses that do not produce impairments in the performance or affect swimming speed. In the study by Keith et al. (2003) six out of eight subjects showed this pattern but at the dose 5.6 mg/kg, although different degrees of drug sensitivity were noticed. In the present study, three out of four rats were impaired during the acquisition component at the doses lower than those required to produce performance impairments. Two rats were selectively impaired at the 3.0 mg/kg dose of CDZ, one rat was selectively impaired at the 5.6 mg/kg dose.

These results further confirm the previous findings in literature on benzodiazepines that drugs from this class impair learning of new behavior without disrupting performance of well-established behaviors (Broekkamp et al., 1984, McNamara and Skelton, 1991, McNaughton, 1995). Present experiment's findings contradict the hypothesis of Cain's (1997) study that benzodiazepines

suppress mechanisms involved in general performance of the task. His study suggested the possibility that when general strategies used to perform the task are well established, benzodiazepines do not impair spatial learning. In the present study all subjects received an extensive training on both components and stable baselines were obtained. The chlordiazepoxide effects observed in the present study are unlikely to be a reflection of the sedative properties of chlordiazepoxide because such properties would have affected escape latencies or swim speed in both task components to an equal extent.

Another interesting finding of this experiment is revealed by studying trial-by-trial plots that indicated that general performance-related processes are affected by the high doses of CDZ (10 and 17 mg/kg), but just on the very first trial. When the data for these doses were averaged across all of six performance component trials it appeared that performance was generally impaired. But a closer inspection of the data revealed that performance on trials 2-6 were unaffected, suggesting that the general performance processes that support behavior in this task were not disrupted. One interpretation is that these chlordiazepoxide doses produced retrograde amnesia but did not impair acquisition processes. Other laboratories have previously reported that benzodiazepine drugs can cause retrograde amnesia (Netter, 1988; Ott, Rohloff, Aufdembrinke, and Fichte, 1988).

Experiment 3: Dizocilpine-Chlordiazepoxide

Figure 14 shows the effects of DZP-CDZ dose combinations on the three dependent variables. Swim speed data sets were not transformed, whereas

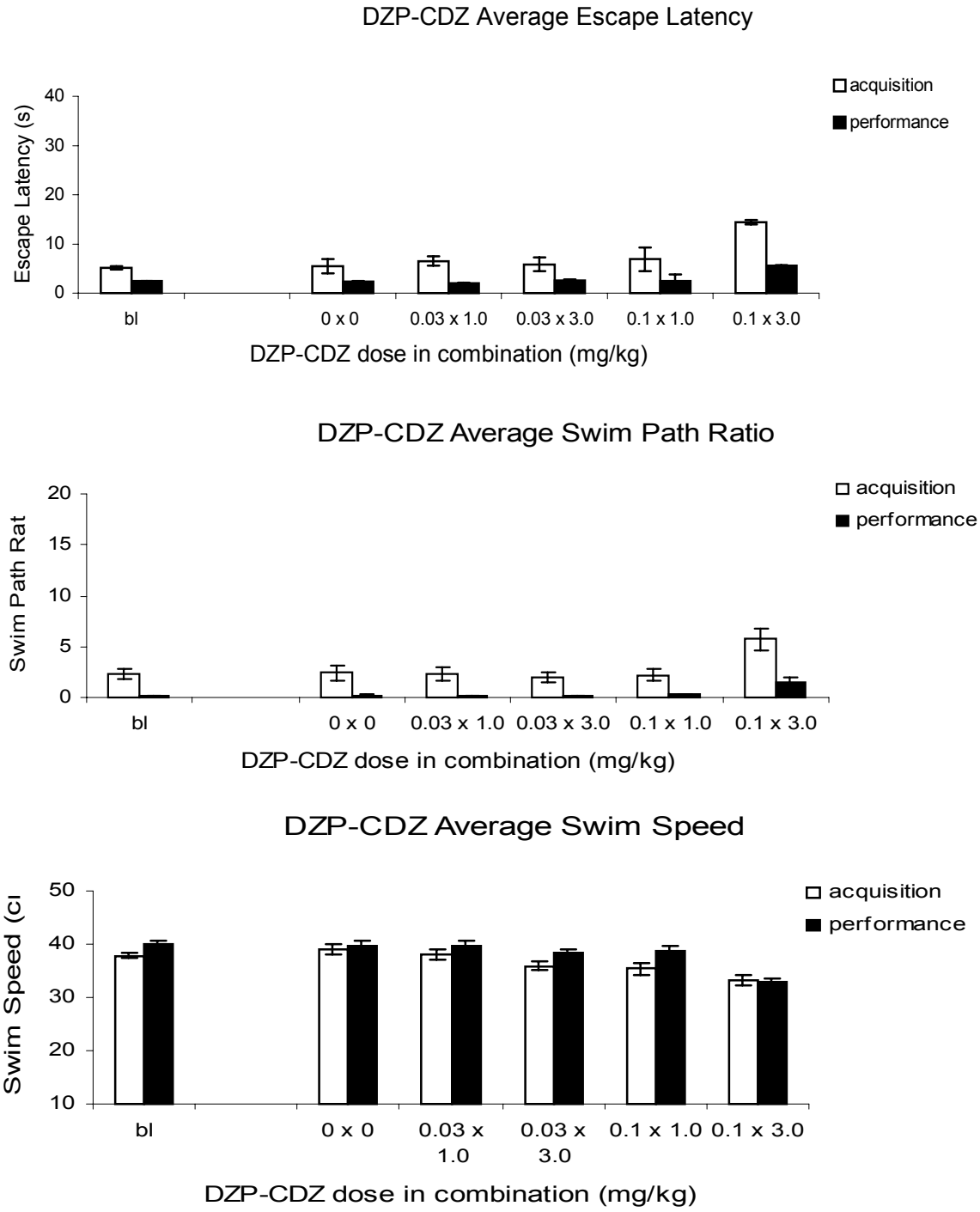

Figure 14. Mean escape latencies (top), swim path ratios (middle), and swim speeds (bottom) as a function of dizocilpine-chlordiazepoxide dose combination during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean.

escape latency and swim path ratio data sets were transformed logarithmically. Repeated-measures ANOVAs revealed significant effects of combination dose on acquisition for escape latency [$F(5, 195)=5.035$, $p=.0002$] and swim speed [$F(5,195)=4.627$, $p=.0005$], and on performance for escape latency [$F(5, 235)=9.397$, $p<.0001$], and swim speed [$F(5, 235)=9.313$, $p<.0001$] but no effect of combination dose for SPR on acquisition [$F(5,195)=1.971$, $p=.0851$] or on performance [$F(5,235)=.849$, $p=.5284$]. Unpaired t-tests revealed that the combination of the highest doses of two compounds (0.1 DZP-3.0 CDZ) had a significantly different effect from that of saline-saline administration in both acquisition and performance on escape latencies ($p=.0004$ and $p<.0001$, respectively), on swim speed ($p=.0004$ and $p<.0001$, respectively), and swim path ratio in the performance component only ($p=.0097$).

As can be seen from Figure 15, 0.1 mg/kg of DZP produced a substantial nonselective disruption of behavior when administered alone. When administered in combination with 1.0 mg/kg of CDZ, however, the detrimental effects of DZP were abolished and latencies during the performance component were virtually identical to those obtained in saline-saline condition. When the 0.1 mg/kg of DZP was administered in combination with 3.0 mg/kg of CDZ, again, dizocilpine produced less behavioral disruption than when it was administered alone.

As it is evident from trial-by-trial data shown in Figure 16, escape latencies and swim path ratios improved across the trials, demonstrating that substantial learning occurred under the acquisition component within individual sessions

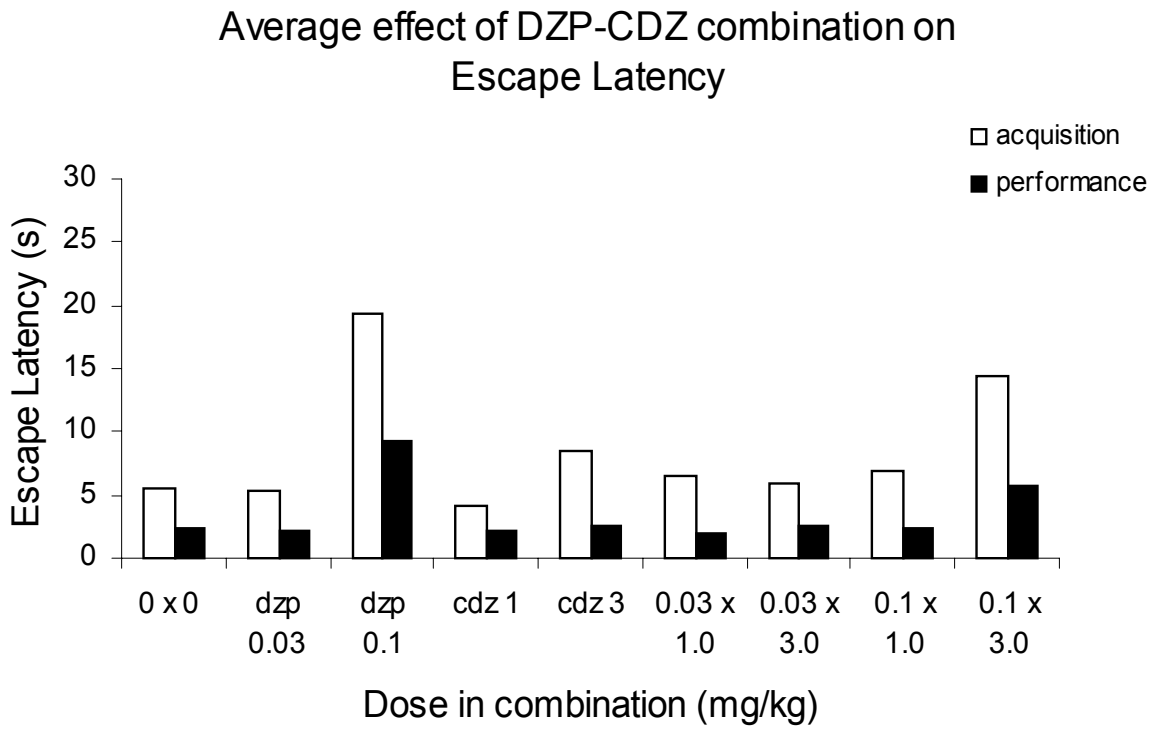

Figure 15. Mean escape latencies during acquisition (white bars) and performance (black bars) of selected dose of dizocilpine and chlordiazepoxide given alone and in combination.

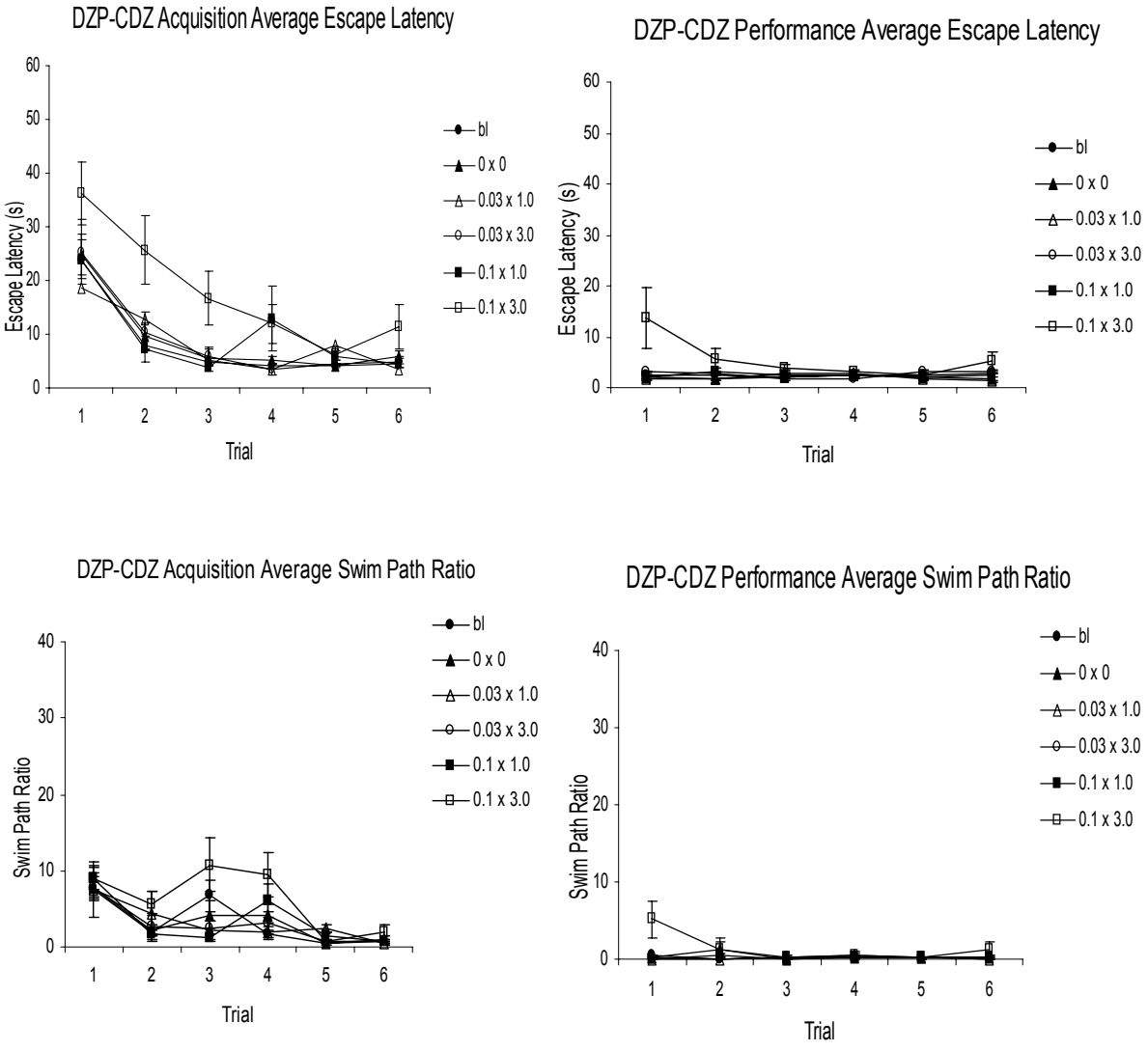

Figure 16. Mean escape latencies during acquisition (top left) and performance (top right), swim path ratios during acquisition (bottom left) and performance (bottom right) components as a function of trials for all dizocilpine-chlordiazepoxide dose combinations tested. Bars indicate standard error of the mean.

under each of the drug combinations. The performance component was generally undisturbed in terms of both latencies and SPR with the exception of under the highest combination (0.1 DZP-3.0 CDZ), which was slightly poorer than under the other drug combinations. The graphs on acquisition trial-by-trial data for both latencies and SPR revealed that the values of 0.1 DZP-3.0 CDZ administration never quite reached those of the other dose combinations but did, nevertheless, show a substantial degree of within session improvement. The individual mean subject escape latencies and swim path ratios as a function of DZP-CDZ dose combination are presented in the appendix of the present thesis (appendices A and B, respectively). Additionally, individual subject raw escape latency data on chlordiazepoxide administration is available in the appendix G (raw data on all three dependent variables is available in the electronic version of the present thesis).

Discussion

The results of this experiment were surprising. Based on previous studies by Thompson and Moerschbaecher (1981, 1982) we predicted that combined administrations of NMDAR antagonist and GABA agonist would interact in a synergistic manner where chlordiazepoxide would increase the behavioral impairment caused by dizocilpine. Thompson and Moerschbaecher, however, studied the effects of simultaneous administration of phencyclidine and pentobarbital on performances and acquisitions of serial response chains. They reported that phencyclidine dose-response curves shifted to the left (i.e., lower

doses of phencyclidine were required to produce behavioral disruptions) when the NMDAR antagonist was administered in combination with the barbiturate.

In contrast with Thompson and Moerschbaecher (1981 and 1982), Chait and Balster (1978) reported that combined administrations of PCP and pentobarbital disrupted behavior less than one would expect if the compounds interacted in an additive fashion. The between-study differences could be due to different compounds chosen for these and the present studies and procedural between-task differences (navigation versus non-navigation), the type of reinforcement used (appetitive versus non-appetitive motivation), and between-species difference (rats versus monkeys and pigeons).

The results of the present study were clear. When the 0.1 mg/kg DZP doses were given alone, behavior was consistently disrupted in all four rats on both the acquisition and performance components. When 0.1 DZP doses were given in combination with 1.0 CDZ doses, behavior was never disrupted in any of the subjects in either task component (see Appendix A).

Experiment 4: Scopolamine

Figure 17 shows the effects of scopolamine on escape latency, swim path ratio, and swim speed. Scopolamine impaired behavior in both components in a dose-dependent manner. Data on escape latency and swim path ratio were transformed to a logarithmic scale to obtain normal distributions. Repeated-measures ANOVAs revealed significant effects of dose on acquisition for SPR [$F(7, 273)=6.137$, $p<.0001$], escape latency [$F(7, 273)=9.304$, $p<.0001$] and swim speed [$F(7,273)=6.200$, $p<.0001$], and on performance for escape latency

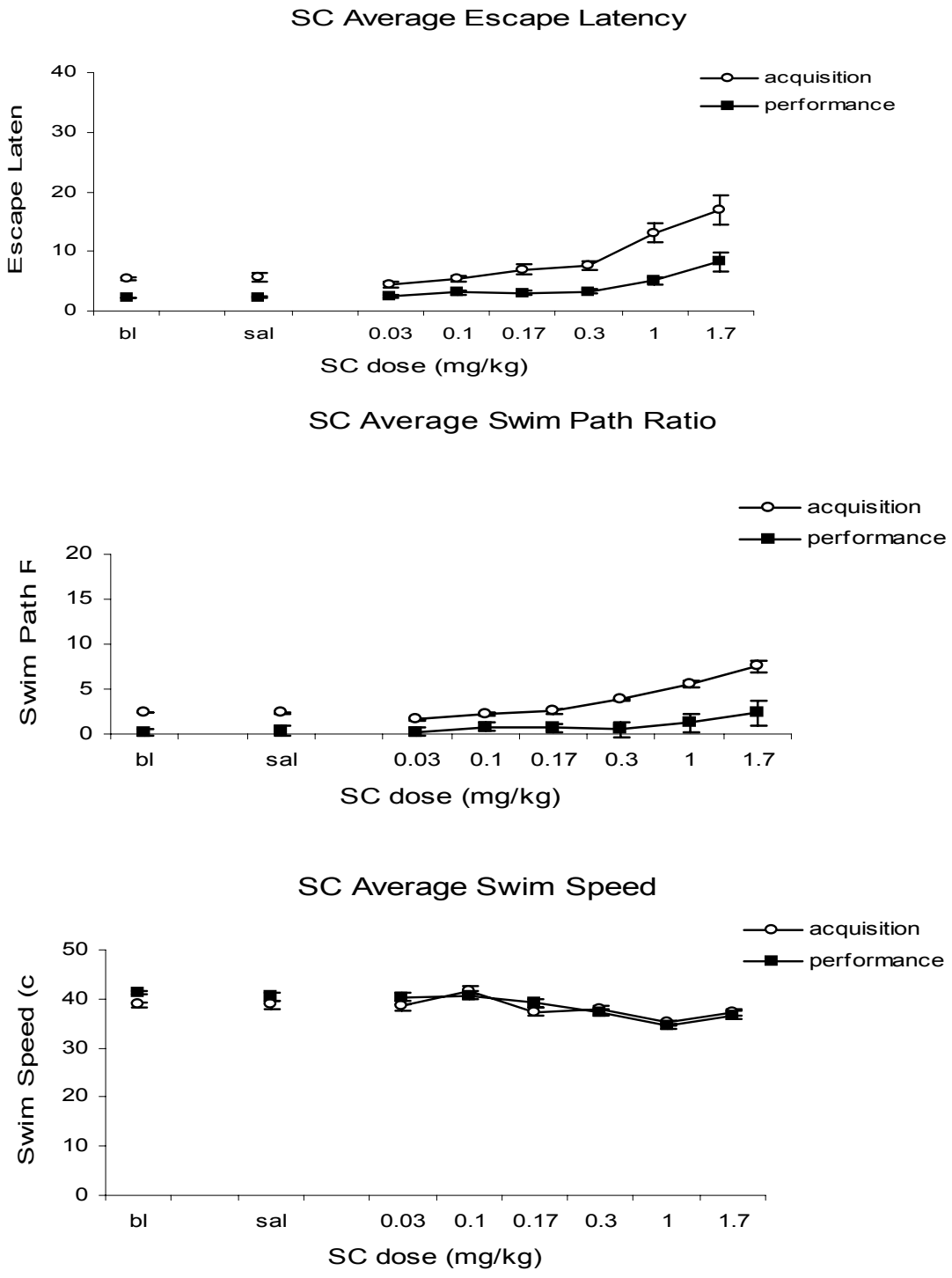

Figure 17. Mean escape latencies (top), swim path ratios (middle), and swim speeds (bottom) as a function of scopolamine dose during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean.

[F(7,329)=5.143, p<.0001], and swim speed [F(7,329)=8.573, p<.0001] but no effect on performance for SPR [F(7,3290=1.307, p=.3164]. It is clear from individual data (Figure 18) that scopolamine effects on the behavior of rats in the task varied greatly between subjects. In terms of latencies, performance component of the rat L4 was affected at 0.17, 0.3, 1.0, and 1.7 mg/kg of scopolamine. K8's performance was hardly affected at all even at 1.7 mg/kg. The performance latencies of K6 were affected on average at 1.7 and latencies of the animal K3 were increased at 1.0 and 1.7 mg/kg on the performance component. Additionally, individual subject raw escape latency data on scopolamine administration is available in the appendix H (raw data on all three dependent variables is available in the electronic version of the present thesis). Figure 17 illustrates that on average, effects of 0.1 mg/kg (p=.0025), 0.17 mg/kg (p<.0001), 0.3 mg/kg (p=.0002), 1.0 mg/kg (p<.0001), and 1.7 mg/kg (p<.0001) on latencies in the performance component were found significantly different from that of saline administration. Acquisition component was affected only at the doses 1.0 mg/kg (p=.0018) and 1.7 mg/kg (p<.0001) suggesting non-selective effect of scopolamine on escape latency.

Figure 17 (middle panel) shows that scopolamine also impaired swim path ratios on the performance component at lower doses than it did on the acquisition component: effects of 0.3, 1.0, and 1.7 mg/kg (all p<.0001) during performance and effects of 1.0 and 1.7 mg/kg (p=.0062 and p=.0007, respectively) during acquisition were significantly higher than those when saline was administered. Rats' individual dose-response curves (Figure 19) show that

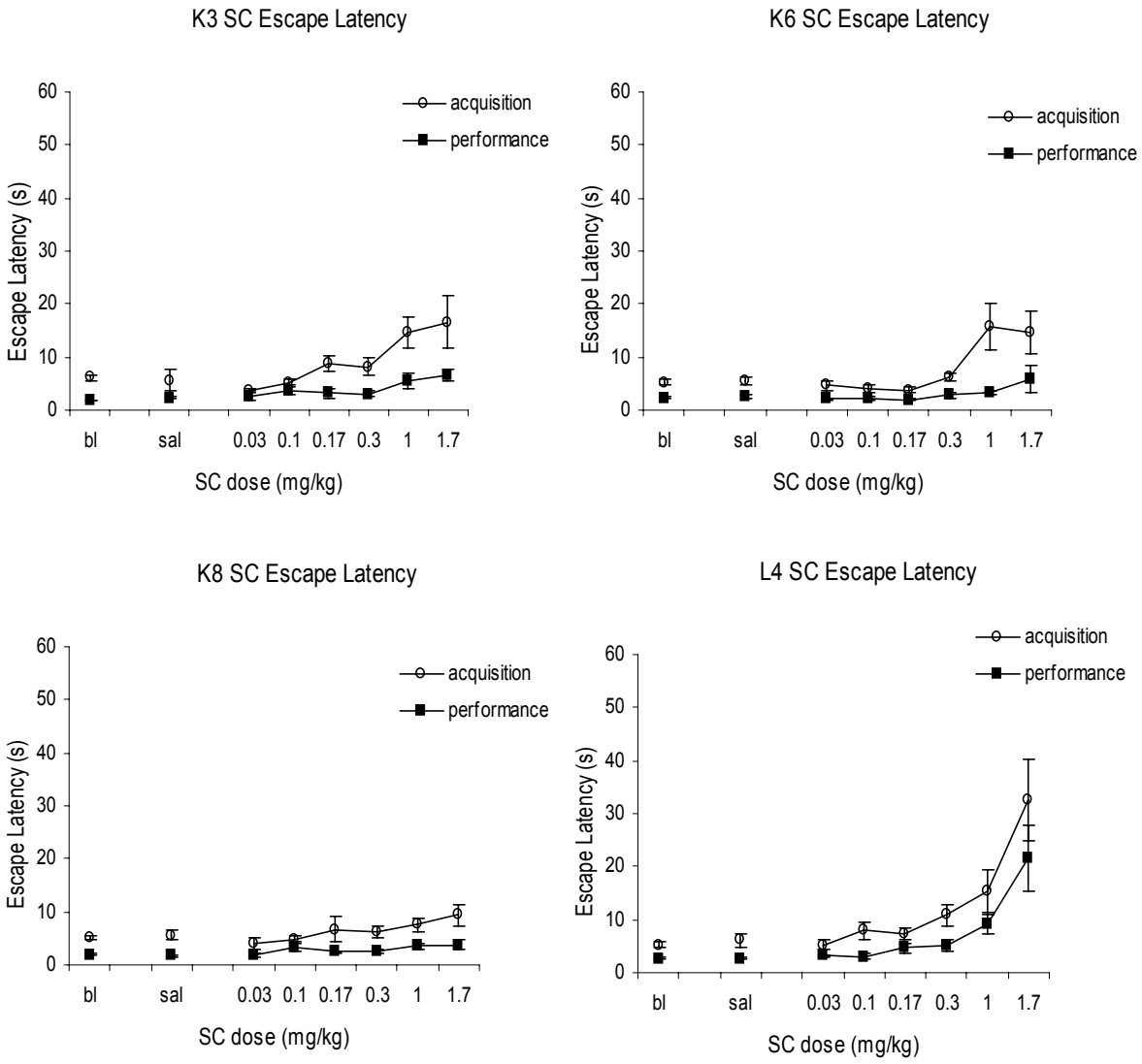

Figure 18. Individual mean subject escape latencies as a function of scopolamine dose during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean of all determinations at a dose.

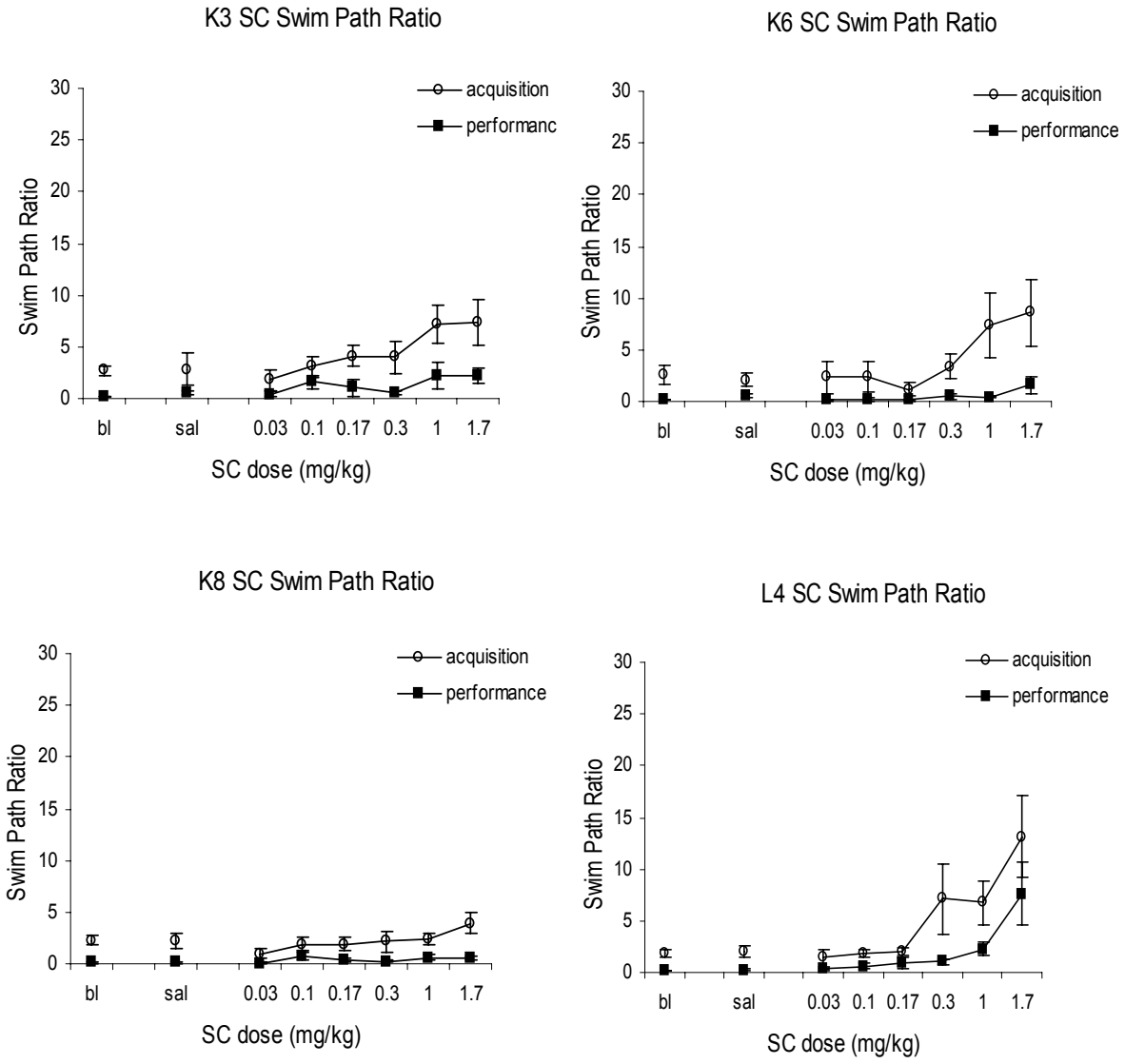

Figure 19. Individual mean subject swim path ratios as a function of scopolamine dose during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean of all determinations at a dose.

only K6's swim path ratio was selectively impaired (at 1.0 mg/kg) out of four experimental animals.

Figure 17 demonstrates that administration of scopolamine had also caused non-selective disturbance of the swimming speed. Although during performance component the speed was significantly lowered by doses 0.3 mg/kg ($p=.0041$), 1.0 mg/kg ($p<.0001$) and 1.7 mg/kg ($p=.0003$), acquisition component was affected only by 1.0 mg/kg ($p<.0001$).

Discussion

Present results contradict the findings from the chain-response studies that argued for the selective nature of anticholinergic agents. Research published by Savage et al. (1996) and Higgins et al. (1989) on monkey and humans provided evidence that anticholinergic drugs, when administered under RAP procedure, disrupt the acquisition component at doses lower than required to disrupt the performance component. In the present experiment, doses that produced impairments in the acquisition component also caused disruption of the performance. The results of Experiment 4 appear to be in agreement with reports by Saucier et al. (1996), Cain et al. (2000), and Whishaw (1989). These studies have produced evidence that cholinergic muscarinic antagonists do not block spatial learning but rather can disrupt appropriate strategies necessary to perform the task. Data averaged across the subjects indicates that the performance component was impaired at as low dose of scopolamine as 0.1 mg/kg, whereas the acquisition was affected at a higher dose (1.0 mg/kg). Swim path ratios and swim speeds were affected in the same manner.

Together, these findings suggest that scopolamine altered elements (accuracy and speed) of the well established behaviors. Indeed, the most consistent observation during this experiment was perseverance of rats when they received scopolamine in circling along the walls of the pool. If in the beginning of the swimming rats' activity was not reinforced by encountering and mounting the platform, the rest of the behavior during the trial often degenerated to in general ineffective patterns (swimming about the perimeter of the pool in the same direction). Data plotted as a function of trial (Figure 20) indicate that new locations were never acquired by rats subjected to the doses 1.0 and 1.7 mg/kg of scopolamine.

Experiment 5: Scopolamine-Chlordiazepoxide

Figure 21 presents the effects of Scopolamine-CDZ dose combinations on the three dependent variables. Data of only three out of originally four subjects were included in the analysis. Escape latency and swim path ratio data were transformation to a logarithmic scale. The repeated-measures ANOVAs revealed significant effects of combination dose on acquisition for SPR [$F(5,120)=10.245$, $p<.0001$], escape latency [$F(5,115)=13.239$, $p<.0001$] and swim speed [$F(5, 45)=14.947$, $p<.0001$], and on performance escape latency [$F(5,140)=10.568$, $p<.0001$], and swim speed [$F(5,55)=8.471$, $p<.0001$] but no effect on performance for SPR [$F(5,145)=1.038$, $p=.4464$]. As Figure 21 demonstrates escape latencies were significantly higher than those of saline-saline administration during performance component only once – after 1.0 SC-5.6 CDZ combination ($p=.0002$). During acquisition component latencies were higher after

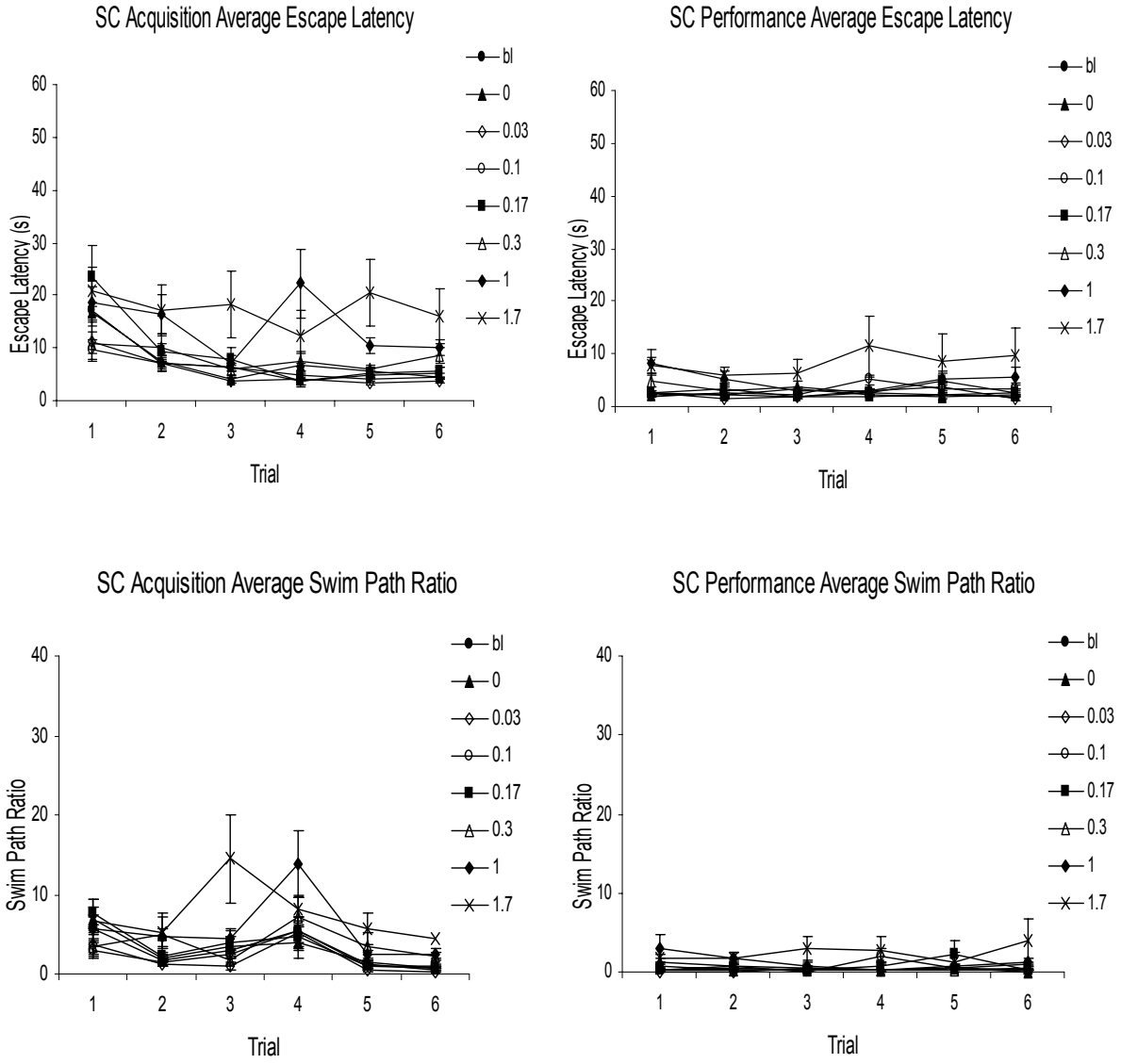

Figure 20. Mean escape latencies during acquisition (top left) and performance (top right), swim path ratios during acquisition (bottom left) and performance (bottom right) components as a function of trials for all dizocilpine doses tested. Bars indicate standard error of the mean.

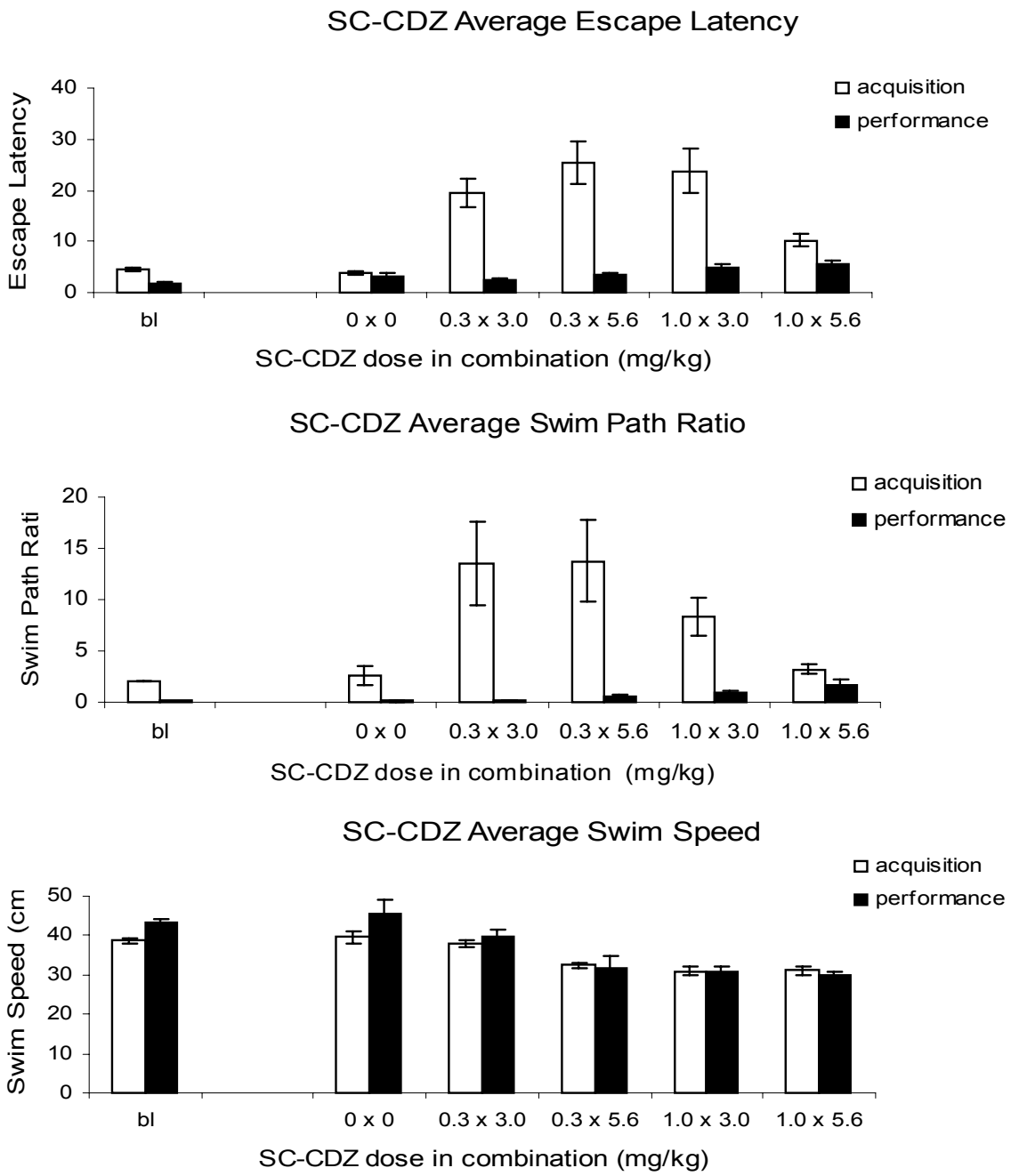

Figure 21. Mean escape latencies (top), swim path ratios (middle), and swim speeds (bottom) as a function of scopolamine-chlordiazepoxide dose combination during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean.

administration of all four combinations: 0.3-3.0 ($p<.0001$), 0.3-5.6 ($p<.0001$), 1.0-3.0 ($p=.0024$), and 1.0-5.6 ($p<.0001$).

Swim path ratios were selectively disrupted only at the 0.3–3.0 mg/kg doses combination, after which only acquisition component's ratio was significantly higher than that of saline-saline administration ($p<.0001$). Further administration of the combinations significantly increased swim path ratios at 0.3-5.6 ($p=.0053$), 1.0-3.0 ($p=.0047$), and 1.0-5.6 mg/kg ($p=.0080$) during the performance component and at 0.3-5.6 ($p=.0004$) and 1.0-3.0 mg/kg ($p=.0046$) during the acquisition component.

Figure 21 shows that swimming speeds were selectively impaired only when the 0.3 SC-5.6 CDZ combination was administered, i.e. only acquisition speed was significantly lower than that after saline-saline administration $p=.0014$). Further administration of 1.0 SC-3.0 CDZ and 1.0 SC-5.6 CDZ mg/kg significantly decreased swimming speed during both components (all p's<.0001).

Rats' individual data suggest that escape latencies of the animal K3 only were selectively impaired at all four combinations (see Figure 22). The behavior of the rat K6 was selectively disrupted at 0.3 SC-3.0 CDZ and 0.3 SC-5.6 CDZ. Administration of 0.3 SC-3.0 CDZ and 1.0 SC-3.0 CDZ selectively affected escape latencies of animal K8. Additional results that include individual subject swim path ratios and the effects combinations presented as a function of trial are shown in the appendix of the present thesis (appendices C and D, respectively). Furthermore, individual subject raw escape latency data on scopolamine-chlordiazepoxide administration is available in the appendix I (raw data on all

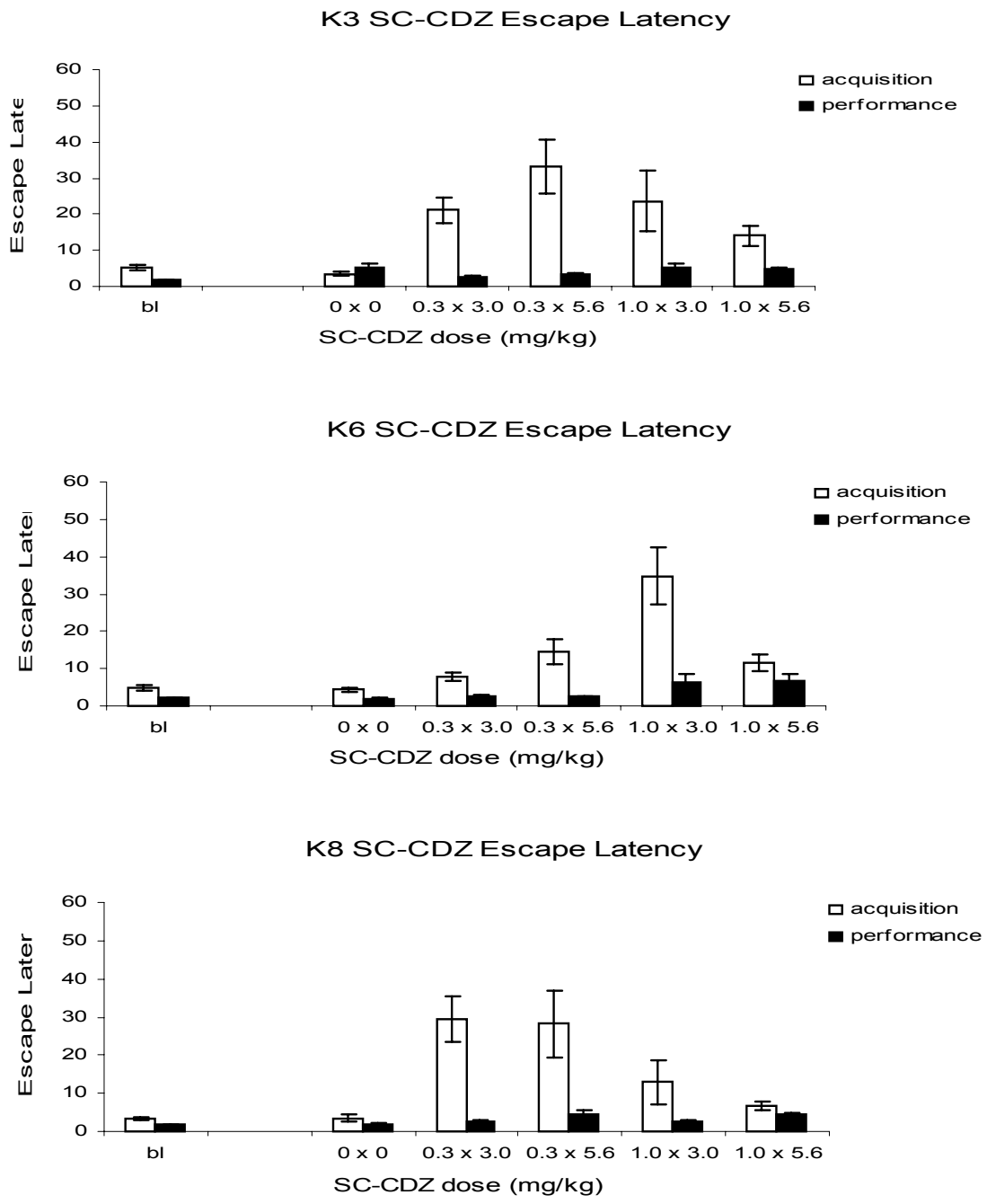

Figure 22. Individual mean subject escape latencies as a function of scopolamine-chlordiazepoxide dose combination during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean of all determinations at a dose.

three dependent variables is available in the electronic version of the present thesis).

Figure 23 indicates that significant impairments of the performance component under 1.0-5.6 mg/kg doses combination suggest additivity of pharmacological interaction between the two compounds. However, impairment of the acquisition component under this combination was yet more modest than that under 1.0 mg/kg of scopolamine alone. Nevertheless, administration of 0.3-3.0, 0.3-5.6, and 1.0-3.0 mg/kg produced a profound evidence for less than additive effect of psychopharmacological action of scopolamine and chlordiazepoxide in combination.

Discussion

Data described above suggest that simultaneous administration of chlordiazepoxide and scopolamine produced selective impairment across three tested combinations (0.3 SC-3.0 CDZ, 0.3 SC-5.6 CDZ, 1.0 SC-3.0 CDZ) but not in 1.0 SC-5.6 CDZ combination. The effect additivity would predict that in all of these cases reliable performance disruption should occur because the doses of scopolamine that alone produced performance impairment (0.3 and 1.0 mg/kg) were paired with either no-impairment (3.0 mg/kg) or performance-impairment (5.6 mg/kg) dose of chlordiazepoxide. Clearly, three out of four effects of combinations do not agree with such prediction. Perhaps, it is more correct to say in this instance that the additive effect occurred in terms of acquisition latencies and less than additive effect occurred in terms of the performance latencies. The last combination (1.0 SC-5.6 CDZ) suggests the reversed

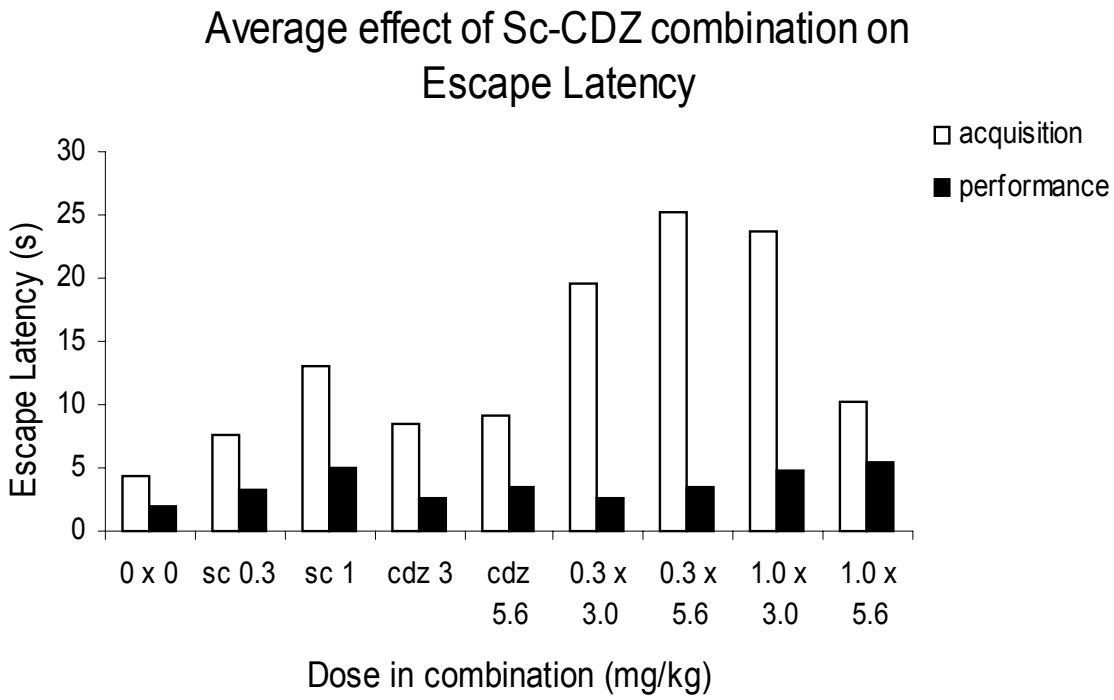

Figure 23. Mean escape latencies during acquisition (white bars) and performance (black bars) of selected dose of scopolamine and chlordiazepoxide given alone and in combination.

interpretation: less-than additive effect occurred during the acquisition component and the additivity effect occurred during the performance component. The reasons for such effect in the results are not clear. Most likely it indicates the fact of the pharmacological interaction that exists between the two neurotransmitter systems. One can assume that possible explanation for such interaction may lay in the fact that these systems play different roles in spatial learning processes.

Overall, the outcome of this experiment is quite unique: the particular doses of the compounds from two different classes were found which caused unselective performance disturbance when given alone but produced a selective impairment in spatial learning when given simultaneously. At this point it is safe to say that this effect warrants further investigation.

GENERAL DISCUSSION

The Morris Swim Task is frequently used by neuroscientists to study spatial learning and memory. Over the last two decades numerous studies have used this task to study the effects of various drugs on spatial navigation. Initially, the general consensus among researchers was that certain drugs, such as NMDAR antagonist, cholinergic drugs, and $GABA_A$ agonists, can impair spatial learning at doses that do not disrupt general performance processes (Ahlander et al., 1999; Broekkamp et al., 1984; Danysz et al., 1988;McLamb et al.,1990; McNamara and Skelton, 1991 Morris et al., 1986). Recent studies, however, suggest that conclusions reached regarding the effects of these drug classes on spatial learning in the MST may have been premature. It is now clear that drugs

can disrupt performance in the Morris Swim Task by interfering with the general organization of rats' behavior in the pool. During the initial stages of training on the Morris Swim Task, typically rats primarily swim around the edge of the pool scratching at the wall. When they do encounter the submerged escape platform during the early stages of training rats often swim over it or even jump off of it. Typically, as training proceeds these behaviors are replaced by behaviors that increase successful escape from the water. Such as swimming away from the pool wall and climbing on the escape platform when it is encountered. Studies reported by Peter Cain and his colleagues (Saucier et al., 1996; Cain, 1997) show that NMDAR antagonists, $GABA_A$ modulators, and cholinergic drugs, when given to behaviorally naïve rats, cause the ineffective behavior patterns to persist longer then they do in non-drugged rats. Furthermore, when given to behaviorally pretrained animals, the same drugs typically do not cause such sensorimotor impairments, and drug effects on spatial learning are diminished. To summarize, dose of NMDAR and cholinergic antagonists, and $GABA_A$ modulators that produced non-specific disturbances and acquisition impairments in naïve rats did not produce those in subjects that received pretraining.

The procedure used in the present thesis allows precisely such a comparison in the subjects that received extensive spatial learning. The RAP methodology made it possible to determine whether the observed impairments in acquisition were due to the non-specific effects of compounds on general performance or if these compounds specifically cause the impairment of spatial learning. Stable baselines on the non-drug sessions insured that the procedure

continuously maintained reliable performance behaviors and rapid acquisitions. Overall, throughout the present study the subjects demonstrated evidence of acquisition of a new location during baseline sessions.

Three experiments of the present thesis investigated the effects of a single drug administration on spatial learning and memory. An important finding of the present study was the confirmation of the previous report by our laboratory relating to the effects of dizocilpine and chlordiazepoxide (Keith and Galizio, 1997). Keith and Galizio (1997) found that $GABA_A$ agonist but not NMDAR antagonist disrupts acquisition of a new spatial location at the doses that do not affect general performance.

It appears clear that the NMDA non-competitive receptor antagonist does not disrupt place learning without also producing general performance disturbances. In agreement with the previous reports by our laboratory, results from dizocilpine manipulations contradict those obtained from the serial response chains (Moerschbaecher and Thompson, 1980; Thompson and Moerschbaecher, 1981; Thompson and Moerschbaecher, 1982). Generally, these studies suggested that NMDAR antagonists can produce learning impairments at the dose that spare ability to perform the task. In our view, this contrast in obtained results can be explained by the between-task differences (spatial versus non-spatial, positive versus negative reinforcement tasks), between-drug differences (competitive versus non-competitive NMDAR antagonists), and between-species differences (monkeys and pigeons versus rodents).

The findings from the Experiment 1 contribute to the current reports on effects of NMDAR antagonists on spatial learning and corroborate Saucier et al. (1996) and Cain's (2000) reports that NMDAR antagonists do not impair learning of a new spatial location.

Another important outcome of this study was replication of the effects of $GABA_A$ agonist administration that was previously reported by our lab (Keith and Galizio, 1997; Keith et al., 2003). In the present study three out of four subjects produced evidence of selective disruption of place navigation after manipulation with chlordiazepoxide. The present results contradict Cain's findings that after stabilizing general performance on the spatial navigation task, GABA agonists do not produce acquisition impairments in the pretrained animals. Subjects in the present study were behaviorally stabilized before entering the drug study. Thus, overall, the present findings support the evidence that came from research that also employed multiple-component RAP procedure. These reports used the RAP procedure to study non-spatial acquisition of the response sequences. Research in the operant settings has consistently found that GABA agonists disrupted acquisition responses at the doses that did not disrupt performance responses (Moerschbaecher and Thompson, 1980; Thompson and Moerschbaecher, 1981; Thompson and Moerschbaecher, 1982). The present results appear in agreement with the pattern of findings obtained in these studies.

Another intriguing result of the experiment on chlordiazepoxide was the evidence that suggested the possibility that high doses of CDZ (10 and 17

mg/kg) produced retrograde amnesia. Human studies have shown that both antero- and retrograded amnesia are often observed as effects of benzodiazepine administration (Ott et al., 1988; Netter, 1988). In the present study careful observation of data plotted as a function of trial revealed that under the highest doses of CDZ performance component was significantly disrupted on the first trial. Thus, our understanding of the effects at these doses is currently limited and more research on that is needed to confirm whether this disruption reflects retrograde amnesia or non-cognitive sensorimotor disturbances.

To our knowledge, the present thesis reports the first attempt to investigate the effects of muscarinic cholinergic antagonist scopolamine under RAP procedure adapted to the Morris Swim Task. In the manner similar to dizocilpine, the present data on scopolamine manipulations disagree with the studies that employed repeated acquisitions procedure to study the effects of scopolamine and atropine on serial response chains (Savage et al., 1996; Higgins et al., 1989). These studies showed that both scopolamine and atropine impaired acquisition of response sequences at the doses that did not affect response accuracies during the performance component. However, the evidence obtained in the Experiment 4 points in favor of non-selective nature of pharmacological action of scopolamine. The results support the Whishaw's (1989) observations in which he found that scopolamine intervened with acquisition of effective behavioral strategies during the navigational task. Data on scopolamine effects presented here demonstrated that the performance component was reliably affected by scopolamine at lower doses than those

required to disrupt acquisition. The present findings are also in agreement with Cain (1997) and Cain et al. (2000) reports in which the investigator was able to demonstrate that muscarinic cholinergic manipulations do not impair navigation to the hidden platform.

Overall, the data on a single drug administration presented here further contribute to the literature on effects of NMDAR and ACh muscarinic antagonists and GABA agonists.

The present thesis also makes one very important methodological addition in the field of testing the combined actions of different drugs. To our knowledge this was the first study that looked at the effects of simultaneous administration of drugs from different classes using the benefits of the RAP methodology that were named above.

A great number of studies that are related to the problem of co-administering numerous pharmacological agents are reported periodically. Studies on patients with neurodegenerative dementia frequently produce evidence of dysfunction in multiple (i.e., cholinergic, glutamatergic, benzodiazepine, and serotonergic) neurotransmitter systems, sometimes suggesting that simultaneous damage to the transmitter functioning constitutes such conditions (Cain et al., 2000).

The initial assumption for both experiments on drug combination was based on the results obtained by Thompson and Moerschbaecher (1981 and 1982). These two studies obtained the identical results with two species

(pigeons and monkeys): the effect of PCP administration was potentiated by PB administration and overall produced overadditive effect on learning.

The results of combined administration of NMDAR antagonist-GABA agonist (Experiment 3) differed from those of Thompson and Moerschbaecher, and therefore, did not support the hypothesis held before entering the drug combination study. Findings of Experiment 3 resembled more the pattern obtained by Chait and Balster (1978) who also reported antagonism between NMDAR antagonist and GABA agonist. Chait and Balster argued that less-than additive results could be attributed, among the other reasons, to the different species used in their and Thompson and Moerschbaecher's studies (squirrel monkeys versus pigeons). Chait and Balster also assumed the possibility of behavioral measure employed in their study (the rate of lever pressing for food presentation) being "not a suitable measure for detecting a drug interaction which manifests itself in other forms of behavior". The between-species differences remain a valid reason for the divergent outcomes of the present and the Thompson and Moerschbaecher's studies (rats versus pigeons). Additionally, between-task and between-drug differences are also plausible.

Generally, to determine whether effects of drug combination are additive, synergistic or antagonistic two requirements must be met. First, the dose-response for each drug alone and combinations of the drugs must be obtained. Second, one should have knowledge of what to expect in the case of additive drug effects. According to Woolverton (1987), with respect to the second requirement there are two basic approaches that make prediction about

the additive outcome: a prediction based on dose addition and a prediction based on effect addition. Dose addition is best conceptualized as the result of combining a drug with itself and it is clear that a drug is additive with itself. On the other hand, effect addition is thought to exist when effect of the combination of two drugs is equal to the sum of the individual effects of each drug given alone. In spite of the fact that each model is based on the comparisons of experimental results to predicted additive drug effects, the two approaches make different predictions of additivity. In order to perform the quantitative analysis of drug interactions it is necessary to use isobolographic method (for details, see Woolverton, 1987). To do so, one would need to conduct a greater number of determinations of each dose combinations. Such analysis was beyond the scope and expertise of the present study.

Nevertheless, the finding of Experiment 3 is very important and worth to be explored with further thoroughness, namely, that impairment in both task components produced by 0.1 mg/kg DZP was abolished by the co-administration of 1.0 mg/kg CDZ to the saline-saline level. The concept of effect-additivity predicts that combined effects of such combination would produce even greater deficit than 0.1 mg/kg did alone. This was not the case. Moreover, when combined with 3.0 mg/kg CDZ (which alone produced a selective impairment in half of the subjects), effects of the 0.1 DZP continued to be abolished.

In case of scopolamine-chlordiazepoxide combination the same less-than additive pattern reoccurred. The most significant outcome of Experiment 5 was the finding of doses from two different classes that alone caused

impairments in both components but, when given together, produced a selective deficit. The results of this study seemed to be in accord with Cain et al.'s (2000) report. As shown by Cain and his colleagues, combined administration of scopolamine and diazepam spared behavioral strategies used by the rats in their experiment but blocked learning of a new platform location. A single drug administration produced no impairment in either of these two elements. As the authors admit, the reasons for sparing the behavioral strategies are not clear. The possibility for such a pattern was attributed to the neurotransmitter systems interaction. Interestingly, the implication made by Cain et al. was that potentiation happened between the two systems (Cain et al., 2000). In this way, the present study makes a unique contribution to the assumptions behind the spared performance and impaired acquisition. It follows from Experiment 5 that it was less-than additive pattern rather than potentiation (more-than additive) causing such outcome.

Another unique implication from Experiment 5 came as a result of using the procedure that allows a direct comparison of the drug effects on the behavior during the performance and acquisition components. As was stated before, not “pure” less-than additive effect occurred at 0.3-3.0, 0.3-5.6, and 1.0-3.0 mg/kg doses combinations. More precisely, a less-than additive effect occurred during the acquisition component and additive effect during the performance component. With the highest doses in combination, the pattern was reversed. A likely conclusion that follows from this finding can be that effect of interaction depends on the measured behavior. It appears that the effects of drugs in

combination are different depending upon whether well-learned behavior or acquisition of a new information is measured.

The conclusions that can be reached from the present study on drug interactions would be stronger if the original single-drug dose-response curves had been redetermined after the drug combinations have been studied to confirm that rats' sensitivities to dizocilpine, chlordiazepoxide, and scopolamine did not change over the course of the experiments. Nonetheless, the present thesis produced two novel findings that warrant further investigation. First, the benzodiazepine, chlordiazepoxide, ameliorated the adverse effects of dizocilpine, an NMDAR antagonist, on spatial behavior. Second, a cholinergic antagonist, scopolamine, amplified the disruptive effects of chlordiazepoxide on acquisition without causing an impairment in the general performance.

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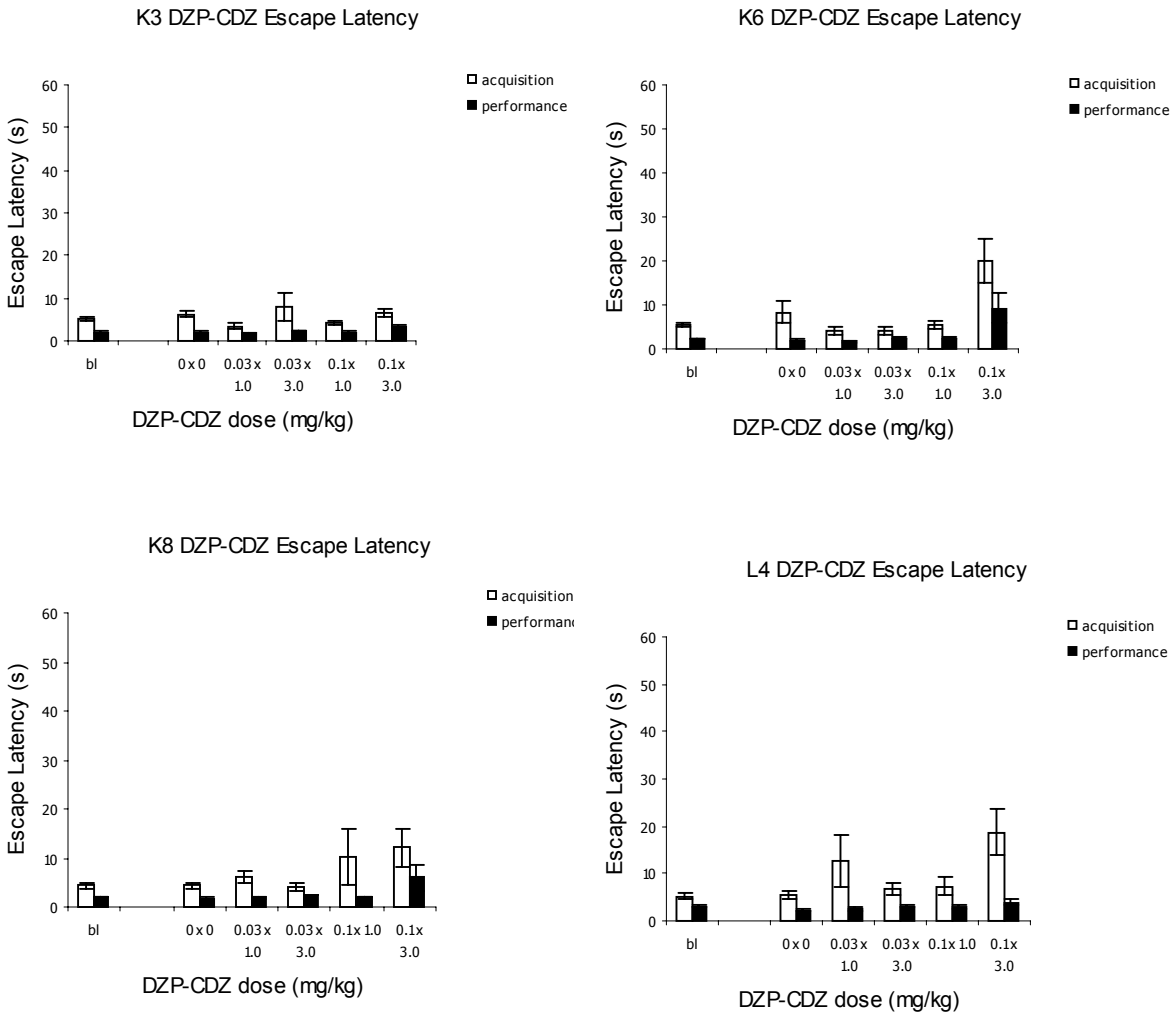
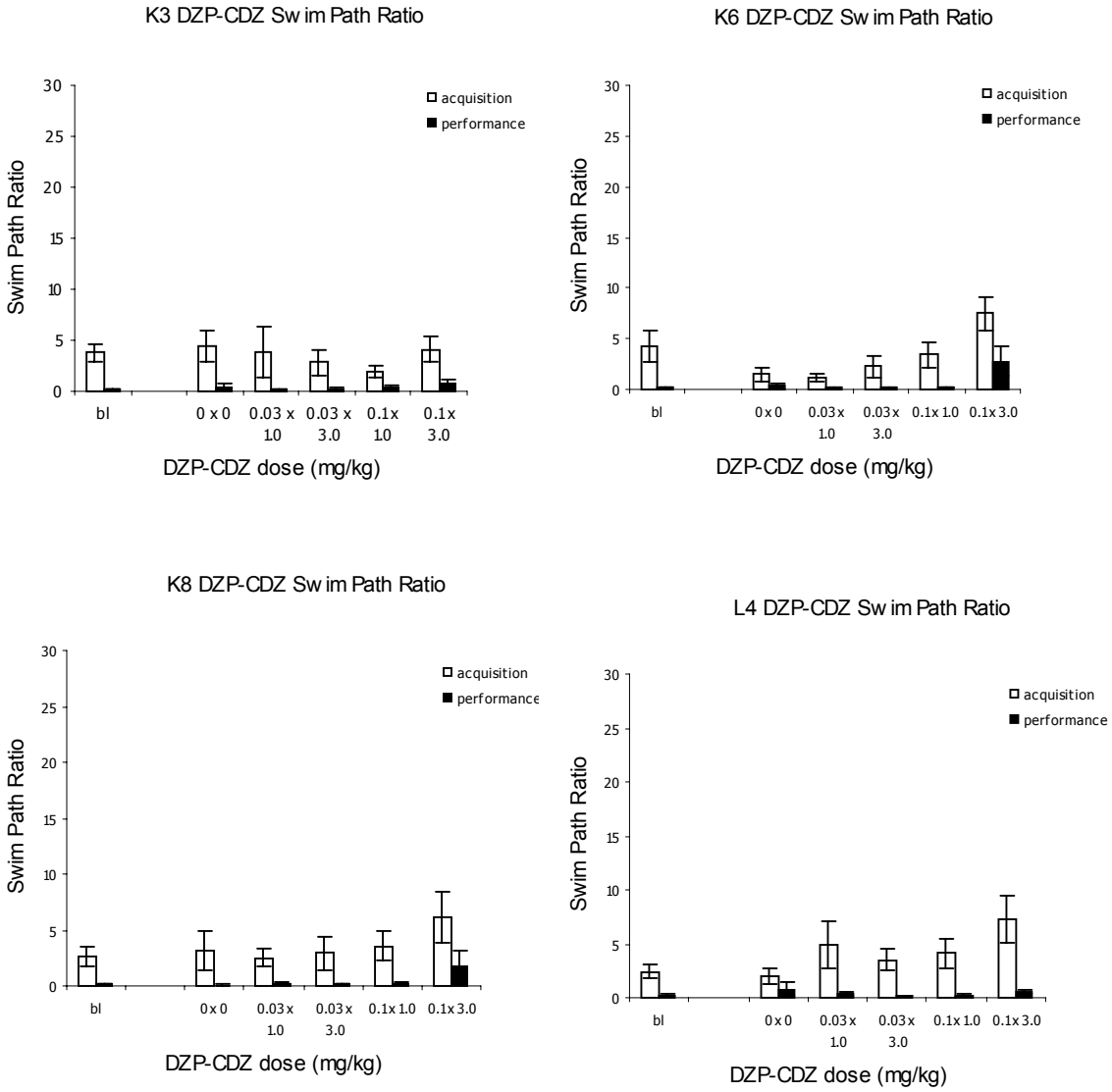
APPENDICES

Appendix A



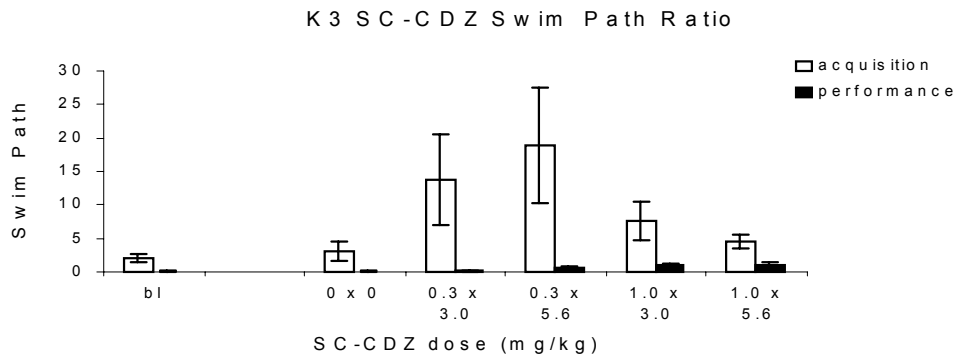
Individual mean subject escape latencies as a function of dizocilpine-chlordiazepoxide dose combination during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean of all determinations at a dose.

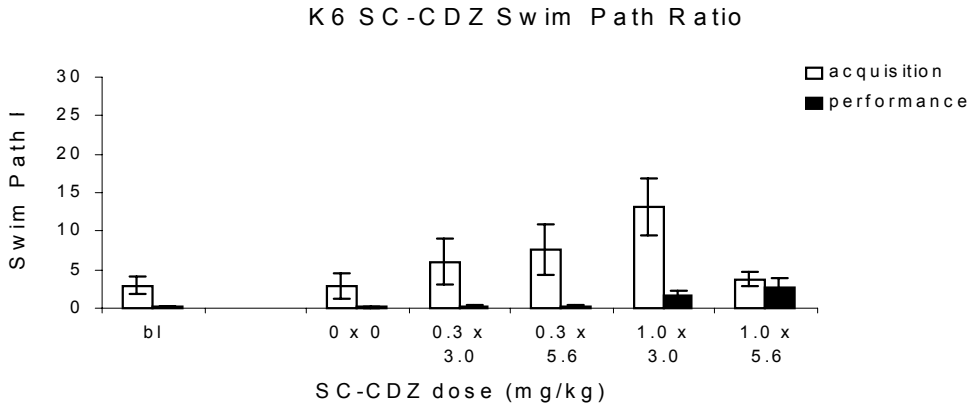
Appendix B

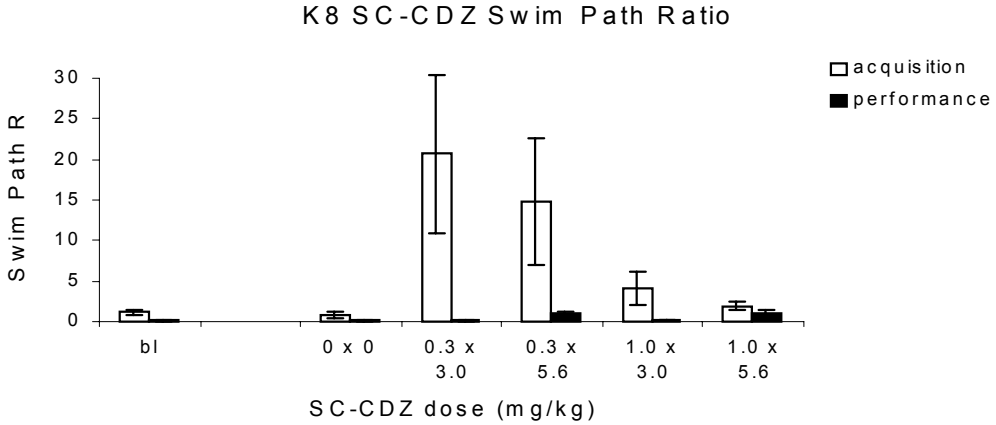


Individual mean subject swim path ratios as a function of dizocilpine-chlordiazepoxide dose combination during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean of all determinations at a dose.

Appendix C

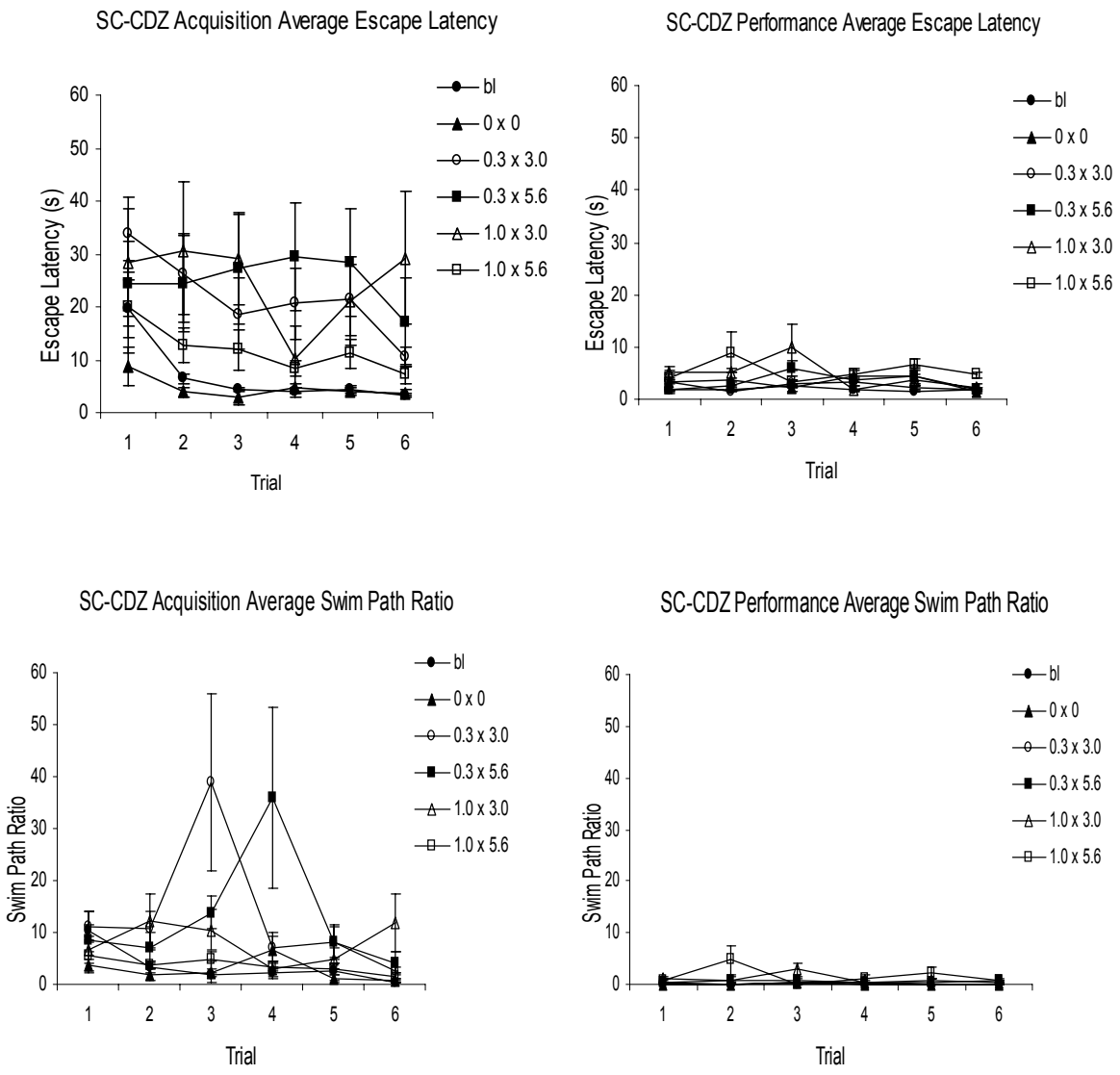






Individual mean subject swim path ratios as a function of scopolamine-chlordiazepoxide dose combination during acquisition (open circles) and performance (closed squares) components. Bars indicate standard error of the mean of all determinations at a dose.

Appendix D



Mean escape latencies during acquisition (top left) and performance (top right), swim path ratios during acquisition (bottom left) and performance (bottom right) components as a function of trials for all scopolamine-chlordiazepoxide dose combinations tested. Bars indicate standard error of the mean.

Appendix E

Average of Latency			Component	
Rat	Dose	Date	acquisition	performance
K 3	0	7/27/2001	5.64	2.15
		8/24/2001	6.71	2.70
	0.01	9/4/2001	5.53	2.03
	0.03	8/17/2001	6.77	2.79
		9/21/2001	5.48	1.47
	0.1	8/28/2001	42.32	4.79
		9/7/2001	8.35	2.12
		9/11/2001	5.32	2.06
		9/25/2001	48.15	18.95
	0.18	8/14/2001	24.32	9.82
		9/18/2001	49.07	30.49
	0.3	8/10/2001	45.02	24.88
	baseline	7/26/2001	5.88	2.05
		8/9/2001	6.30	4.03
		8/16/2001	7.19	2.84
		8/23/2001	5.30	3.42
		9/6/2001	5.34	1.94
		9/20/2001	3.60	1.90
K 6	0	7/17/2001	6.62	2.34
		8/24/2001	7.47	2.45
	0.01	9/4/2001	5.27	1.88
	0.03	7/20/2001	3.82	2.25
		8/17/2001	5.15	2.33
	0.1	7/24/2001	4.86	2.04
		8/21/2001	4.15	6.90
		9/11/2001	13.77	2.90
		9/21/2001	7.93	2.21
		9/25/2001	59.91	14.49
	0.18	8/14/2001	39.43	15.29
		8/28/2001	19.21	3.09
		9/14/2001	18.78	6.82
		9/18/2001	48.15	5.27
	0.3	7/27/2001	41.33	17.94
	baseline	7/19/2001	6.04	1.99
		7/26/2001	6.58	3.78
		8/16/2001	6.53	8.31
		8/23/2001	9.39	2.15
		9/13/2001	6.88	2.38
		9/20/2001	3.03	2.23
K 8	0	6/22/2001	3.73	1.94
		7/3/2001	3.39	2.15
		8/24/2001	5.11	2.06
		9/18/2001	2.13	2.01

	0.01	8/28/2001	5.00	1.82
		9/4/2001	4.80	1.75
	0.03	7/13/2001	7.18	1.74
		7/27/2001	2.84	2.84
		8/17/2001	6.53	2.34
		9/21/2001	5.40	1.93
	0.1	7/10/2001	4.58	1.57
		7/24/2001	4.00	2.08
		8/21/2001	42.28	33.33
		9/7/2001	5.69	1.74
		9/11/2001	6.94	2.02
	0.18	7/20/2001	9.45	8.84
		8/14/2001	31.52	46.25
		9/14/2001	8.12	4.95
	0.3	6/29/2001	41.27	7.34
		7/17/2001	11.61	7.36
	baseline	6/21/2001	3.86	1.85
		6/28/2001	7.18	1.91
		7/5/2001	3.37	2.21
		7/12/2001	3.92	1.94
		7/19/2001	6.46	2.29
		7/26/2001	2.59	2.61
		8/16/2001	5.65	2.67
		8/23/2001	4.31	2.00
		8/30/2001	5.51	1.98
		9/6/2001	4.02	1.80
		9/13/2001	7.57	1.94
		9/20/2001	4.27	1.86
L 4	0	7/20/2001	3.83	2.48
		8/28/2001	6.73	2.30
		9/21/2001	3.70	2.33
	0.01	9/4/2001	9.46	2.10
		9/28/2001	4.65	2.13
		10/5/2001	3.90	2.11
	0.03	7/27/2001	3.71	2.36
		8/17/2001	6.12	2.40
		9/14/2001	5.37	2.44
	0.1	7/24/2001	11.97	2.37
		8/21/2001	49.90	51.21
		9/7/2001	5.28	5.82
		9/11/2001	5.64	2.78
		9/25/2001	36.05	15.51
	0.18	8/14/2001	38.64	50.47
		8/24/2001	20.09	23.01
		9/18/2001	25.71	29.60
	0.3	8/10/2001		
	baseline	7/19/2001	4.23	2.49
		7/26/2001	4.87	3.45
		8/9/2001	3.60	2.37
		8/16/2001	5.89	2.64

		8/23/2001	5.19	3.32
		9/6/2001	4.79	2.26
		9/13/2001	3.36	2.75
		9/20/2001	8.41	2.31
		9/27/2001	6.37	2.45
		10/4/2001	7.46	2.59
		10/11/2001	5.82	2.86

Individual subject escape latency raw data of dizocilpine administration

Appendix F

Average of Latency			Component	
Rat	Dose	Date	acquisition	performance
K 3	0	10/12/2001	5.85	1.96
		10/23/2001	8.48	1.91
		11/30/2001	4.68	2.13
	1	11/6/2001	4.71	1.87
		11/20/2001	5.04	2.11
	3	10/16/2001	5.06	2.01
		11/13/2001	18.47	2.54
	5.6	11/2/2001	7.54	3.02
		11/16/2001	4.26	2.91
		11/27/2001	8.40	2.48
	10	10/26/2001	7.59	3.35
		12/4/2001	15.76	3.70
	17	11/9/2001	13.89	13.31
	baseline	10/11/2001	4.34	1.81
		10/22/2001	5.88	1.96
		10/25/2001	6.30	2.21
		11/1/2001	3.67	1.86
		11/8/2001	6.12	2.81
		11/15/2001	5.68	2.11
		11/29/2001	2.52	1.66
K 6	0	9/28/2001	10.44	2.62
		10/23/2001	4.17	2.62
		11/30/2001	7.91	2.61
	1	10/9/2001	4.96	2.61
		11/16/2001	2.89	1.97
	3	11/13/2001	10.05	2.42
		11/20/2001	5.14	2.75
	5.6	10/12/2001	6.83	3.28
		11/2/2001	15.86	5.18
		11/27/2001	7.76	3.80
	10	10/26/2001	10.30	8.54
		11/6/2001	5.00	7.19
		11/9/2001	26.58	13.73
	17	12/4/2001	13.57	14.73
	baseline	9/27/2001	5.80	3.07
		10/11/2001	8.82	2.94
		10/25/2001	8.44	2.53
		11/6/2001	8.04	3.29
		11/8/2001	4.22	2.39
		11/15/2001	9.82	2.41
		11/29/2001	4.17	2.78
K 8	0	10/23/2001	3.86	1.92
		11/30/2001	4.38	2.09

	1	10/9/2001	3.70	2.13
		11/6/2001	3.57	2.14
	3	10/16/2001	8.46	2.16
		11/9/2001	12.24	2.30
	5.6	10/12/2001	6.20	3.97
		11/2/2001	6.79	3.24
		11/16/2001	9.47	2.89
	10	9/28/2001	3.86	2.66
		10/26/2001	16.51	3.72
		11/13/2001	14.96	3.25
	17	10/5/2001	37.81	8.46
	baseline	9/27/2001	9.62	1.87
		10/4/2001	6.31	1.98
		10/11/2001	2.78	2.10
		10/25/2001	4.87	2.03
		11/8/2001	3.92	2.21
		11/15/2001	5.63	1.98
		11/29/2001	2.91	1.92
L 4	0	10/12/2001	4.21	2.94
		10/23/2001	6.58	2.54
		12/8/2001	6.71	2.90
	1	11/6/2001	3.68	2.41
		11/20/2001	4.81	2.15
	3	10/16/2001	4.71	2.70
		11/13/2001	3.70	3.21
	5.6	11/2/2001	6.62	3.17
		11/16/2001	14.52	3.25
		11/27/2001	15.26	3.69
	10	10/26/2001	6.56	5.77
		12/21/2001	16.16	8.86
	17	11/9/2001	19.55	7.02
		12/18/2001	39.07	30.07
	baseline	10/11/2001	5.82	2.86
		10/25/2001	6.71	2.63
		11/1/2001	5.44	2.24
		11/8/2001	5.20	2.66
		11/15/2001	5.49	2.55
		12/7/2001	7.65	2.83
		12/20/2001	4.85	4.96

Individual subject escape latency raw data of chlordiazepoxide administration

Appendix G

Average of Latency			Component	
Rat	Dose	Date	acquisition	performance
K 3	0 x 0	12/7/2001	5.85	2.65
		1/8/2002	6.59	1.44
	0.03 x 1.0	12/28/2001	3.76	1.72
		1/18/2002	3.22	1.71
	0.03 x 3.0	12/18/2001	11.74	2.45
		1/11/2002	4.31	2.22
	0.1 x 1.0	12/21/2001	3.76	2.48
		1/4/2002	4.56	1.74
	0.1 x 3.0	12/11/2001	6.31	4.95
		1/1/2002	8.76	2.30
		1/15/2002	4.59	2.58
	baseline	12/6/2001	3.89	1.71
		12/20/2001	6.43	2.30
		12/27/2001	6.88	2.19
		1/3/2002	5.83	2.11
		1/10/2002	5.91	2.14
		1/17/2002	2.58	2.09
K 6	0 x 0	12/7/2001	13.14	1.86
		1/8/2002	3.64	2.13
	0.03 x 1.0	12/28/2001	5.03	1.76
		1/18/2002	3.04	1.82
	0.03 x 3.0	12/18/2001	3.37	2.40
		1/11/2002	4.85	2.46
	0.1 x 1.0	12/21/2001	5.39	2.28
		1/4/2002	5.45	2.69
	0.1 x 3.0	12/11/2001	22.98	21.47
		1/1/2002	27.34	2.99
		1/15/2002	10.04	3.42
	baseline	12/6/2001	5.93	2.12
		12/20/2001	6.70	2.20
		12/27/2001	7.69	2.58
		1/3/2002	4.13	2.47
		1/10/2002	3.92	2.15
		1/17/2002	4.40	1.92
K 8	0 x 0	12/4/2001	3.56	1.97
		1/8/2002	5.07	1.54
	0.03 x 1.0	12/7/2001	5.42	2.08
		12/28/2001	6.60	1.88
	0.03 x 3.0	12/18/2001	4.04	2.38
		1/11/2002	4.38	2.52
	0.1 x 1.0	12/21/2001	5.92	2.03
		1/4/2002	14.74	2.04
	0.1 x 3.0	12/11/2001	22.86	11.62

		1/1/2002	8.96	2.52
		1/15/2002	4.66	3.63
	baseline	12/6/2001	3.61	1.96
		12/20/2001	4.66	2.19
		12/27/2001	5.99	2.07
		1/3/2002	3.62	2.26
		1/10/2002	3.79	1.69
L 4	0 x 0	1/8/2002	7.59	2.23
		1/29/2002	4.67	2.50
		2/12/2002	4.11	2.24
	0.03 x 1.0	12/28/2001	7.06	2.62
		1/18/2002	18.01	2.25
	0.03 x 3.0	1/11/2002	9.32	3.09
		1/22/2002	3.55	3.05
		2/8/2002	7.21	2.87
	0.1 x 1.0	1/4/2002	11.95	3.40
		2/1/2002	3.21	3.01
		2/15/2002	6.88	2.53
	0.1 x 3.0	1/1/2002	28.14	4.62
		1/15/2002	5.46	4.23
		2/5/2002	22.25	3.19
	baseline	12/27/2001	5.80	4.33
		1/3/2002	5.15	3.11
		1/10/2002	4.44	2.59
		1/17/2002	3.59	2.69
		1/31/2002	9.58	3.47
		2/7/2002	4.23	2.68
		2/14/2002	4.16	2.77

Individual subject escape latency raw data of dizocilpine-chlordiazepoxide administration

Appendix H

Average of Latency			Component	
Rat	Dose	Date	acquisition	performance
K 3	0	2/5/2002	4.75	2.20
		4/9/2002	3.30	2.37
		2/12/2202	8.97	2.36
	0.03	1/29/2002	3.61	1.58
		3/26/2002	3.88	3.65
	0.1	2/1/2002	4.89	3.32
		2/22/2002	5.64	4.08
	0.17	2/8/2002	13.84	5.50
		3/29/2002	6.79	1.84
		4/2/2002	9.00	2.80
		4/23/2002	4.78	2.75
	0.3	2/15/2002	5.05	4.39
		3/19/2002	10.11	2.00
		4/12/2002	12.21	3.98
		4/26/2002	5.11	1.88
	1	2/19/2002	6.91	3.52
		3/15/2002	24.57	5.24
		4/16/2002	8.33	3.52
		4/19/2002	18.79	9.35
	1.7	2/26/2002	22.46	6.47
		3/22/2002	10.69	6.90
	baseline	1/31/2002	7.01	1.93
		2/7/2002	7.64	1.99
		2/14/2002	5.46	2.07
		2/21/2002	5.70	1.70
		3/14/2002	6.27	1.78
		3/21/2002	6.31	1.84
		3/28/2002	4.71	1.84
		4/11/2002	5.96	2.11
K 6	0	2/5/2002	6.10	3.51
		2/12/2002	5.74	1.73
		4/9/2002	4.45	2.58
	0.03	1/29/2002	4.89	2.09
		3/26/2002	4.29	2.01
	0.1	2/1/2002	3.05	2.72
		2/22/2002	5.24	1.65
	0.17	2/8/2002	4.44	1.99
		3/29/2002	2.93	1.92
	0.3	2/15/2002	3.98	1.81
		3/1/2002	6.76	5.50
		3/19/2002	6.16	1.93
		4/12/2002	8.58	2.19
	1	2/19/2002	13.96	3.69

		3/15/2002	15.67	4.19
		4/5/2002	7.79	2.90
		4/19/2002	25.31	2.40
		3/22/2002	4.54	2.49
		4/2/2002	3.51	2.35
		4/16/2002	34.23	14.69
	baseline	1/31/2002	4.26	2.50
		2/7/2002	8.93	1.72
		2/14/2002	4.63	2.00
		2/21/2002	5.11	2.07
		2/28/2002	3.50	2.51
		3/14/2002	3.88	2.75
		3/21/2002	3.93	3.05
		3/28/2002	5.26	1.89
		4/4/2002	7.75	2.51
		4/11/2002	6.10	2.38
		4/18/2002	3.83	2.35
K 8	0	2/2/2002	7.36	1.69
		2/12/2002	4.57	1.69
		4/9/2002	4.95	1.99
	0.03	1/29/2002	4.73	1.77
		3/26/2002	3.27	1.88
	0.1	2/1/2002	3.09	6.61
		2/22/2002	6.07	2.43
		4/19/2002	5.84	1.82
		4/26/2002	4.67	2.45
	0.17	2/8/2002	40.82	
		3/29/2002	3.52	2.17
		4/2/2002	4.70	2.85
		4/23/2002	4.84	2.35
	0.3	2/15/2002	14.51	
		3/19/2002	6.45	2.16
		4/12/2002	4.26	2.79
	1	2/19/2002	3.08	
		3/15/2002	8.86	5.72
		4/5/2002	9.77	3.85
		4/16/2002	6.76	2.28
		4/30/2002	5.88	2.31
	1.7	3/22/2002	8.12	6.25
		5/3/2002	15.97	2.68
		5/7/2002	4.14	2.55
	baseline	1/31/2002	6.94	2.01
		2/7/2002	5.36	1.92
		2/14/2002	3.30	2.45
		2/21/2002	6.65	1.88
		3/14/2002	3.20	2.07
		3/21/2002	4.68	2.00
		3/28/2002	3.12	2.09
		4/4/2002	6.42	1.80
		4/11/2002	5.24	2.10

		4/18/2002	9.11	1.98
	1	3/15/2002	16.55	14.07
		4/5/2002	10.30	4.80
		4/30/2002	19.30	8.46
	1.7	3/22/2002	16.82	8.64
		4/16/2002	48.26	34.45
	baseline	2/28/2002	4.02	2.76
		3/14/2002	4.27	2.75
		3/21/2002	4.74	2.46
		3/28/2002	7.50	2.41
		4/4/2002	6.08	2.27
		4/18/2002	4.41	2.96
		4/25/2002	5.08	2.67
		5/2/2002	6.27	2.81

Individual subject escape latency raw data of scopolamine administration

Appendix I

Average of Latency			Component	
Rat	Dose	Date	acquisition	performance
K 3	0 x 0	5/7/2002	2.32	8.32
		5/28/2002	4.74	2.22
	0.3 x 3.0	5/21/2002	21.58	2.82
		6/11/2002	20.80	2.43
	0.3 x 5.6	5/17/2002	53.44	3.58
		5/31/2002	12.97	3.02
	1.0 x 3.0	5/14/2002	9.16	4.96
		6/4/2002	41.79	5.44
	1.0 x 5.6	5/25/2002	8.64	4.98
		6/8/2002	19.35	4.83
	baseline	5/16/2002	4.89	1.98
		5/23/2002	6.49	1.90
		5/30/2002	4.08	1.60
		6/7/2002	5.00	1.56
K 6	0 x 0	4/26/2002	3.99	1.86
		5/28/2002	4.83	1.87
	0.3 x 3.0	5/3/2002	7.06	2.83
		5/21/2002	8.68	2.20
	0.3 x 5.6	5/17/2002	19.03	2.82
		5/31/2002	9.90	2.23
	1.0 x 3.0	5/14/2002	36.14	3.88
		6/4/2002	33.49	8.98
	1.0 x 5.6	4/30/2002	9.38	9.14
		5/24/2002	6.43	2.81
		6/8/2002	18.41	8.54
	baseline	4/25/2002	3.68	2.11
		5/2/2002	5.74	2.24
		5/16/2002	4.03	2.26
		5/23/2002	6.80	1.98
		6/6/2002	4.14	1.70
K 8	0 x 0	5/28/2002	3.53	1.77
	0.3 x 3.0	5/21/2002	43.84	2.80
		6/11/2002	14.85	2.25
		5/31/2002	5.35	2.67
	1.0 x 3.0	5/14/2002	4.38	2.49
		6/4/2002	21.34	2.88
	1.0 x 5.6	5/24/2002	6.03	3.35
		6/8/2002	7.22	5.05
		6/14/2002	6.46	4.63
	baseline	5/16/2002	4.10	2.19
		5/23/2002	2.66	1.71
		5/30/2002	2.89	1.50
		6/7/2002	2.74	1.43

		6/13/2002	4.85	2.00

Individual subject escape latency raw data of scopolamine-chlordiazepoxide administration