A COMPETITIVE NMDA RECEPTOR ANTAGONIST POTENTIATES THE EFFECTS OF MORPHINE ON SPATIAL AND DISCRIMINATION LEARNING

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ABSTRACT

NMDA antagonists have been shown to attenuate the development of tolerance to the antinociceptive effects of morphine, but paradoxically, to potentiate the acute effects of morphine in assays of antinociception. In an effort to characterize the effects of these types of drugs on learning, morphine and the competitive NMDA antagonist, LY235959, were studied alone and in combination in two experiments. The first experiment utilized the Morris Swim Task, a procedure widely used to study spatial learning in rats. The second experiment used an olfactory discrimination procedure for rats. Both experiments involved the use of a within-subject, repeated acquisition and performance procedure (RAP). The RAP procedure allows the researcher to distinguish between a drug's effects on learning versus more general performance effects. In both procedures, morphine produced selective impairments on acquisition, but LY235959 generally affected acquisition only at doses that also produced performance effects. Combinations of selected doses of the two drugs produced effects that suggest a potentiation of the effects that each drug produced alone.

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INTRODUCTION

Opiates have long been used in healthcare for their analgesic properties. Although morphine and other opiate compounds are highly useful analgesics, their effectiveness is limited by the development of tolerance. Specifically, tolerance is a phenomenon where, over repeated administrations of the drug, the analgesic properties of a given dose are decreased (Branch 1991). Tolerance is problematic for the medical application of opiates. Although increasing the dose may surmount the tolerance for a time, ultimately it may not be possible to relieve the patient's pain. Higher doses may increase the abuse potential of the drug in a given individual, and may also increase the risk of side effects such as sedation and respiratory suppression.

Recent studies have shown that competitive N-methyl-D-aspartate (NMDA) receptor antagonists (e.g. LY235959) attenuate morphine tolerance in rats. Allen and Dykstra (2000a) used a rat warm-water tail-withdrawal procedure to study the antinociceptive effects of morphine and LY235959. In this procedure, the rat's tail is immersed in 40^{o} (non-noxious) and 55^{o} C (noxious) water and latency to remove the tail from the water is the dependent measure. Morphine (0.3-10 mg/kg) produced dose-dependent increases in withdrawal latency from the noxious water. Upon determination of the morphine dose-response curves, subjects received one of three doses of morphine (10, 20, or 40 mg/kg) alone or in combination with one of three doses of LY235959 (1.0, 3.0, or 5.6 mg/kg), chronically. Chronically administered morphine produced rightward shifts in the morphine dose response curve, indicating tolerance. However, when administered with LY235959, the development of morphine tolerance was attenuated.

To determine if the tolerance attenuating effects of LY235959 extended to the tolerance and cross tolerance of opiates other than morphine, Allen and Dykstra (2000b) used a rat tail-withdrawal procedure to test the antinociceptive effects of the mu-opioid agonists etorphine, morphine, and dezocine. Dose effect curves for the three compounds were determined, and then the maximally effective dose for each was administered alone or in combination with LY235959 twice daily for seven days. Following chronic administration, the dose effect curves of the opiates were once again determined. After chronic etorphine, the dose effect curves of all three opiates were shifted to the right. LY235959 prevented cross tolerance to morphine and dezocine. After chronic administration of morphine, the etorphine and morphine curves were shifted rightward, while the dezocine curve was flattened. LY235959 prevented morphine tolerance and etorphine cross tolerance, while reducing the magnitude of dozocine cross tolerance. After chronic administration of dozocine, all three curves were shifted to the right, and LY235959 reduced the magnitude of tolerance and cross tolerance. The data shows that the morphine-tolerance-attenuating effects of LY235959 do extend to opiates other than morphine and cross-tolerance between opiates as well.

If these findings were to generalize to human subjects there may be useful implications for healthcare. Bisaga, Comer, Ward, Popik, Kleber, and Fischman (2001) studied the effects of the clinically available, non-competitive NMDA antagonist, memantine, on the expression of opioid physical dependence in humans. Heroin-dependent, non-treatment seeking, inpatients were stabilized on a fixed dose of morphine (30 mg by mouth four times per day). After a series of challenges with 0.4 mg (intramuscular) naloxone the severity of opioid withdrawal was assessed. Prior to the

naloxone challenges, either placebo or memantine (60 mg PO) was given. Naloxone produced results consistent with opioid withdrawal. However, when memantine was given prior to naloxone, withdrawal was reduced in a time-dependent manner. This study extends the preclinical data that suggests that glutamatergic neurotransmission plays a role in opioid tolerance and dependence.

However, little is known about side effects related to NMDA antagonist-opioid combinations. For example, NMDA antagonists and opiates both affect learning and memory processes (Cory-Schlecta, 1994). Any plans to make use of the NMDA antagonists' opiate tolerance attenuating properties may need to be reexamined if NMDA antagonists and/or the combination of those substances with opiates adversely affect learning and memory functions. In order to assess whether or not such unwanted effects might result from administration of the drugs, an experimental design that can distinguish effects on learning and memory from other nonspecific drug effects is needed.

Repeated Acquisitions

The repeated-acquisition procedure has been used to study the effects of various independent variables, such as drugs, on acquisition or learning (Boren, 1963; Boren & Devine 1968; Thompson & Moerschbaecher, 1979). In this procedure subjects are trained to solve a different problem (e.g. sequence of lever presses) from session to session in order to obtain a reinforcer. Eventually, the subject's proficiency in learning to solve these problems from day to day becomes stable. The result is a steady baseline of acquisition that can be used as a tool to examine the effects of a given independent variable on learning.

The procedure can be illustrated through one of the first demonstrations of repeated acquisitions of behavioral chains in rhesus monkeys by Boren and Devine (1968). The monkeys were placed into a metal box where on one wall there were twelve levers mounted in four groups of three. There was a pilot light above each lever. Initially any lever press was reinforced. Subsequently, responses to a given group of levers were reinforced (e.g. 10, 11, and 12). The correct group was specified by the illuminated pilot light. The monkeys were then trained to complete two-response chains; the lights over one group of levers (e.g. 7, 8, and 9) were lit and any response to this group moved the lights to another group (e.g. 10, 11, and 12). A response to any of the levers in the second group was reinforced. This chain was eventually extended to include all four groups, and was reinforced on a fixed-ratio (FR) 2 schedule, so the chain had to be completed twice to produce reinforcement. Then, each lever in the chain had to be pressed five times in order to move on to the next part of the chain. When an inappropriate (unlit) lever was pressed a 15-s time out (TO) followed. During this TO all the lights and levers were off/ineffective. The next step in the procedure was to train the monkeys to press only a single lever from each group (e.g. 2, 5, 8, and 11). The correct sequence always started in the group of levers to the left and worked toward the right. When the correct lever was pressed five times in the first group, the lights over the next group of levers came on. When the monkeys completed the FR 5 on the correct lever in the fourth group a stimulus was presented, but food was only delivered on the second completion of the chain. The correct sequence of lever presses changed from session to session and as a result the monkeys had to learn which four levers made up the response chain at the beginning of each session. This repeated-acquisition of the correct response chain allowed Boren and

Devine to study the effects of an independent variable, in this case TO duration after an incorrect response, on a behavior in transition (measured by the number of incorrect responses). They found that with no TO, subjects made many errors, but with TO from 1 s to 4 min the number of errors was decreased.

Perhaps a more useful tool is the repeated acquisition and performance procedure (RAP). Here the subject is once again trained to solve a problem that changes from session to session, but there is an additional component. The subject also faces a problem that remains the same from session to session, the performance component. For example, a subject may be presented with three press plates each displaying a different shape. In the acquisition or learning component the subject would have to learn to press the plates in a different sequence from session to session. In the performance component, the same response sequence would be reinforced every session.

Thompson and Moerschbaecher (1979) used a repeated acquisition and performance procedure to study the effects of d-amphetamine and cocaine on the completion of response chains in patas monkeys. The monkeys were trained to lever press to complete four-response chains on an FR 5 schedule. The correct response was signaled by the color and geometric form projected behind the response keys. Red levers signaled the acquisition component while the levers were made green in the performance component. The same geometric forms (horizontal line, triangle, vertical line, and circle) were used in both components, and signaled which lever was to be pressed for a correct response (e.g. horizontal line = left lever correct). In the acquisition component the four-response chain was changed from session to session, while in the performance component the same chain of responses produced a reinforcer. Response rate and percent

error were recorded and when behavior stabilized, dose-effect curves for the drugs were determined. Thompson and Moerschbaecher found that both d-Amphetamine and cocaine decreased response rate, impaired accuracy, and decreased within-session error reduction at higher doses, while the performance component was less sensitive to disruptive effects on rate and accuracy.

This repeated acquisition and performance procedure allows one to examine the effects of a drug on behavior in transition and a well-established behavior in individual subjects, within a single session. More specifically, the procedure reveals whether a drug might have a selective effect on behavior in one component but not the other (i.e., disrupt acquisition at doses that do not affect performance).

Effects of Morphine on Learning

Galizio, Keith, Mansfield, and Pitts (2003) used the RAP procedure in an adaptation of the Morris Swim Task (Morris, 1981) to study the effects of morphine on spatial learning. In this task rats were trained to locate a platform submerged underwater in a large circular pool surrounded by curtains displaying distinctive patterns. In the acquisition component the platform changed position in relation to the curtain configuration from session to session, while in the performance component the platform remained in a constant position relative to the configuration of the curtains. As a result the rat was required to swim to a new platform location to solve the acquisition problem each session, while swimming to the same platform position in the performance component. Once trained on this task, rats showed consistent and stable daily learning in the acquisition component. That is, in a given session the time it took the rat to swim to the platform decreased from the first trial, and was stable with a mean under 10 s for the

remaining trials. In the performance component the mean latency to find the platform for all trials was less than 10 s. The researchers were then able to demonstrate that morphine affected rats' behavior in a dose-dependent manner. Acquisition was impaired at doses that did not impair performance. This supported previous work showing that morphine impaired spatial learning. However this work failed to rule out effects on performance (McNamara & Skelton, 1991).

However, studies that have used a repeated acquisitions procedure to study morphine effects on other behaviors have failed to find selective effects on acquisition. Moerschbaecher and Thompson (1983) studied the effects of morphine on the performance of a conditional discrimination in a repeated acquisition and performance design. In the acquisition component the discriminative stimuli for left and right lever presses changed from session to session, while in the performance component the stimuli remained the same from session to session. Morphine produced dose-dependent decreases in overall response rate but did not produce selective effects on acquisition.

Schulze and Paule (1991) used an incremented repeated acquisition (IRA) procedure to examine the effects of morphine in rats. Initially responding to one lever was reinforced (IRA1). After 20 correct responses, a one-minute time-out occurred. Next, the number of responses in the sequences was incremented to two (IRA2). For the IRA2 a response on a lever different from the original lever was required before a response on the original lever was reinforced. After completing the IRA2 20 times with no errors between the first and last correct lever presses of the sequence, the task was incremented to a three-lever sequence. The increments continued in this way on up to a six-lever sequence or until 35 min elapsed.

A progressive ratio (PR) task was used to assess the effect that morphine had on the rats' motivation to work for food reinforcers. In this task subjects were required to increase the number of lever presses required for each reinforcer. Initially one or two lever presses were required for reinforcer delivery, and the response requirement was then increased by the original amount after each reinforcer delivery. Responding generally declined or ceased (breakpoint) during a 10-min PR session.

Morphine produced dose-dependent decreases in IRA percent task completed (1.0, 3.0, and 5.6 mg/kg doses) and in mean response rates (3.0, and 5.6 mg/kg doses). However, no significant increases or decreases in response accuracy occurred. In the PR task morphine produced significant dose-dependent decreases in breakpoint and response rate at doses of 1.0, 3.0, and 5.6 mg/kg.

Thompson and Moerschbaecher (1981) studied the effects of morphine and naloxone on pigeons completing a four-response chain. During each session, subjects completed a different chain by responding on the correct key out of three keys in the presence of one of four colors. Responding was maintained under a FR 5 schedule. Errors produced a brief time-out but did not reset the chain.

Morphine produced dose dependent decreases in response rate and increases in percent error. Naloxone produced dose-dependent decreases in response rate, but did not affect percent error. Both effects of morphine were antagonized by doses of naloxone that were ineffective when administered alone.

As the above studies suggest, there are mixed data on the effects of morphine on acquisition, and those on other non-specific behaviors. It seems that most of the literature suggests that morphine does not selectively impair acquisition. However, most of this

research utilized operant tasks, and with the exception of Schultz and Paule used species other than rats as subjects. In contrast, a study in rats utilizing a spatial acquisition task found selective effects on acquisition (Galizio, et al, 2003).

Effects of NMDA Antagonists on Learning

Repeated-acquisition procedures have also been utilized to study NMDA antagonists. Galizio et al. (2003) found that the competitive NMDA antagonist LY235959, impairs acquisition of a spatial task in rats only at doses that also impaired performance. In addition Keith and Galizio (1997) found that the non-competitive NMDA antagonist MK-801 also had no selective effect on acquisition in the Morris Swim Task in rats.

Moerschbaecher, Thompson, and Winsauer (1985) studied the effects of phencyclidine, a non-competitive NMDA antagonist, on the acquisition and performance of response sequences by patas monkeys. In the acquisition component, completion of four-response sequences was reinforced. The correct sequence changed from session to session. In the performance component the sequence remained the same throughout the study. Phencyclidine was found to disrupt acquisition at doses that did not affect performance.

Moerschbaecher and Thompson (1980) studied the effects of phencyclidine on the acquisition and performance of conditional discriminations in monkeys. Subjects were trained to respond on a right or left lever based on the color and geometric form of the stimuli that were presented. The completion of a two-member chain of discriminations produced a food pellet. In the performance component the discriminative stimuli remained the same from session to session. In the acquisition component, the

discriminative stimuli for left and right responses changed from session to session. The acquisition component always came first and then the components alternated after 50 reinforcers or 25 min, whichever occurred first. Sessions were terminated after 200 reinforcers or 4 hr. Drugs were administered every five days, during which there were baseline and control sessions. The dependent measures were overall response rate, and overall accuracy.

Phencyclidine produced dose-dependent decreases in response in the performance and acquisition components. At high doses the compound produced impairments of accuracy in each component. At lower doses accuracy in the acquisition component was selectively impaired.

The effects of phencyclidine and ketamine on complex operant behavior in monkeys were studied by Thompson, Winsauer, and Mastropaolo (1987). Subjects were trained to complete four-response chains in each of two components of a multiple schedule by responding sequentially on three levers in the presence of different stimuli (numerals). Completion of the four-response chain resulted in food presentation. In the acquisition component, subjects acquired a different chain each session, while in the performance component the chain remained the same from session to session. Each session began with the acquisition component and then alternated with the performance component after 10 reinforcements or 15 min, whichever happened first. Sessions were terminated after 100 reinforcements or 2 hr. Testing was conducted Monday through Friday with drug sessions conducted on Tuesdays and Fridays. The dependent measures were overall response rate and overall accuracy. Phencyclidine and ketamine each produced dose dependent decreases in response rate, and increases in percent error in

each component. However, there was greater impairment of accuracy in the acquisition component relative to control sessions.

Baron and Moerschbaecher (1996) studied the effects of MK-801 and the competitive NMDA antagonist, CGS 19755, on acquisition and performance of response sequences in rats. They found that both compounds impaired acquisition at doses that did not affect performance. However, the design used here studied two groups of rats. The effects of the drugs on the acquisition of one group of rats were compared to the effects on the performance of another group. Although utilizing a true performance component is more desirable than relying on response rates as a measure of performance, the between-group method used here still allows for the possible effects of individual differences on the data.

Wilmore, Bespalov, and Beardsley (2001) examined the effects of NMDA antagonists on a lever-press discrimination task. Rats were trained on a fixed consecutive number task where eight presses on one lever (counting lever) were required before a press on the other lever (reinforcement lever) would produce food reinforcement. The effects of the noncompetitive NMDA antagonists, PCP, MK 801, and memantine and competitive NMDA antagonists, SCZ EAA 494, and NPC 17742 were examined. MK 801 and PCP decreased accuracy at doses that did not affect response rate, however, memantine, SCZ EAA 494, and NPC 17742 reduced accuracy only at doses that also reduced response rates.

Clissold, Ferkany, and Pontecorvo (1991) examined the effects of the competitive NMDA antagonists NPC 12626 and CPP, the noncompetitive NMDA antagonists PCP and MK 801, scopolamine, and haloperidol in a non-spatial operant discrimination task.

Rats were trained to discriminate two stimuli using two levers during each problem. Responses to the right lever were reinforced in the presence of the stimuli designated S1 and responses to the left lever were reinforced in the presence of the stimuli designated S2. Sessions were terminated after 60 min, following 400 trials, or when the discrimination criteria, of 8 of the last 10 trials correct, was met. New problems were presented from session to session. The total number of responses to criterion was used as an index of acquisition. Though in this design there was no performance component as in the repeated-acquisition and performance procedures discussed above, the researchers attempted to distinguish between acquisition and performance effects by only including data if the rat responded on 50% of the trials or if criterion was met in that session. The competitive NMDA antagonist CPP, the noncompetitive NMDA antagonist MK 801, and scopolamine increased the number of responses necessary to attain discrimination criterion. Only the noncompetitive NMDA antagonist MK 801 also reduced the probability of a response during the trial and also increased the probability of inter-trial interval responses. Although measures such as response probability during and between trials are of some worth, a performance component like those used in RAP procedures might better distinguish between true acquisition effects, and non-specific drug effects.

Gerak, Stevenson, Winsauer, and Moerschbaecher (2004) studied the effects of ketamine on repeated acquisitions and performance in rats. Rats were trained to respond on one of three keys in the presence of one of three colors. Upon making the correct response the color and correct key changed. Rats were required to make three correct responses in order to complete a chain (e.g. keys white, center correct; keys red, left correct; keys amber, right correct). Every second completion of the chain produced

access to a reinforcer. In the acquisition component, within a given session the correct response associated with a particular color did not change, and the sequence did not change. Across sessions the response/color pairing and sequence changed. In the performance component the sequence remained the same across sessions. Gerak et al. found that ketamine produced dose-dependent decreases in response rate and increases in errors in both components. Acquisition was only disrupted at doses that also disrupted performance.

As was the case with the morphine studies discussed earlier, the data are mixed in regard to the effects of NMDA antagonist's effects on acquisition versus performance. Most of the literature, which comes from studies using operant tasks with subjects other than rats, suggests that NMDA antagonists selectively impair acquisition. However, in spatial learning studies and the Gerak et al. operant study using rats, NMDA antagonists only affected acquisition at doses that also disrupted performance.

It is notable that there are contrasting reports on the effects of both morphine and NMDA antagonists on repeated acquisition and performance. Most of the RAP work discussed above either employs the Morris Swim Task, repeated discriminations, and/or the completion of response chains. In addition, it seems that, by and large, it is the data from the swim task studies that is in disagreement with the other methods discussed. It may be true that some of the differences between these studies play a significant role in producing the divergent data. Response modes differ, with swimming in the swim task as opposed to key or lever manipulation in the operant tasks. The type of reinforcer, escape from the water in the swim task and food in the operant studies, also is a difference between these procedures. Spatial and non-spatial elements of the procedures vary as

well. In the swim task the subject must use spatial cues that are not contiguous with the hidden platform to find it and escape. On the other hand in the operant procedures, correct responses rely on characteristics of the stimuli such as color and shape. Perhaps most striking is that while rats are widely used as subjects in the swim task, they less frequently used in RAP procedures, perhaps because it is difficult to train stable behavior in these tasks in rats. As a result, the contradictory data discussed above come from studies that differ not only across procedures, but often across species as well. There have been attempts to study drug effects on acquisition by rats in operant procedures, but, with the exception of the Gerak et al. study, these studies generally lack the simultaneous performance component that benefit the RAP procedures.

Olfactory Learning

The limited literature on repeated acquisition of operant behavior in rats likely stems from difficulty in training the rats to stability on these relatively complex tasks. However, it has been pointed out that rats, as well as many other mammals, rely much more on their superior sense of smell to survive in the world as opposed to the inferior modality of vision (See Jennings and Keefer, 1969). Intuitively then, it seems that if rats have trouble performing visual non-spatial repeated acquisition tasks, they might perform better on a task that capitalizes on their superior olfactory abilities. Indeed, in the past, it has been reported that rats can acquire a learning set with olfactory stimuli (Jennings and Keefer, 1969). More recently researchers such as Slotnick (1993) and Eichenbaum (e.g., (Eichenbaum & Otto, 1993) have demonstrated that olfactory stimuli can be very useful in the study of learning in the rat.

One drawback that is independent of whether or not olfaction is a privileged sensory modality with respect to learning for rats is that olfactory stimuli are difficult to control relative to visual or audio stimuli. To halt presentation of a visual stimulus to the subject a researcher only has to remove the stimulus from sight, to remove olfactory stimuli from *smell* is a bit more challenging. Berger-Sweeny, Libbey, Arters, Junagadhwalla, and Hohmann (1998) developed a very simple odor discrimination procedure for mice. Various spices were mixed with sterilized sand, and presented to mice in cups, two at a time. One of the cups, scented with a given spice, was baited with chocolate (S+), while the other, scented with a different spice, was not (S-). Latency and accuracy in retrieving the chocolate from the baited cup improved over trials and sessions respectively. Another study, by Mihalick, Langlois, Krienke, and Dube (2000), also used scented sand baited with chocolate to study olfactory discrimination in mice. Once again mice were trained to dig in one of the cups in a pair that was designated as S+, and were able demonstrate improvement of the task. In addition, Mihalick et al. were able to perform a reversal of the baited and non-baited stimuli, once the mice met a designated criterion on the initial discrimination. Mice were able to get back to criterion performance levels on these and subsequent reversals. These studies offer encouragement to those seeking inexpensive, yet useful ways to study olfactory learning in rats.

For instance, Galizio, Miller, Ferguson, McKinney and Pitts (in preparation) utilized an olfactory repeated discrimination reversal task to study the effects of the benzodiazepine, chlordiazepoxide and the non-competitive NMDA antagonist, MK-801 in rats. In each session rats were exposed to two different 2-choice discrimination problems with food reinforcement for correct responses. In the performance component

one odor was always correct (S+) and the other was never correct (S-). In the acquisition component stimuli changed every session. Six different scents were used throughout the experiment in the acquisition component. In each session acquisition could be studied along with the performance of a well-learned discrimination. After extensive training, consistently high accuracy could be observed in the performance component, and consistent, rapid learning could be seen within session in the acquisition component. Chlordiazepoxide interfered with reversal learning at doses that had no effect on the performance discrimination. MK-801 also impaired reversal learning, but its effects were generally less selective.

Studying the Effects of Drug Combinations

Because of the interest in the therapeutic value of combinations of opiates and NMDA antagonists, it seems important to examine the effects of the combination of these compounds on learning and complex behaviors as well. For example, if combinations of the drugs disrupt learning to a greater extent than the two substances alone, it might limit therapeutic use of such combinations.

Administering drugs in combination can produce various consequences. The effect may simply be additive, where the result of the combination of two drugs is what would be expected if the effects of the two drugs when given alone were simply added to one another. For instance, imagine an experiment where the selected dependent variable was response rate and the control and baseline rate was 100 hundred lever presses in one minute. A given dose of drug “A” produced only 70 responses per minute, 30 data points out of control range. A given dose of drug “B” produced a response rate of 90 responses per minute, 10 data points out of control range. If combining drugs “A” and “B”

produced a simple, additive effect the data point resulting from the two given doses above be expected to fall at about 60 responses per minute, or 40 data points out of the range of the control. On the other hand if the combination produced a response rate of 20, the effect would be said to be supra-additive, that is, the effect of the two drugs in combination is greater than that of either drug alone. The effect of the combination could also be antagonistic in nature, or produce an effect that is less than one or both of the drugs given alone. For example, if a combination of the previously mentioned doses of drugs “A” and “B” produced a response rate of 95, the fact that the rate decreasing effects of the drugs had been suppressed would be considered antagonistic (Tallarida, 2001).

The terms additive, supra-additive, and antagonist are often used when discussing these types of effects. Synergism and potentiation are also commonly used terms. While there does not seem to be a standard terminology in the world of drug interaction and combination studies, for the purposes discussing the results of the current study two terms will be focused on: antagonism and potentiation. These terms have been chosen as a result of the limitations of the current study in regard to quantifying the effects of a given dose combination. For instance, it would not require an analysis of the data that is out of the scope of this study to determine that the effect that is produced by a combination is less than what would be expected if the effects were additive in nature. Such an effect would be antagonistic. However, further analysis would be required to delineate between an additive effect, and a supra-additive effect. As a result, either of these effects will be referred to as potentiation. Specifically, if a dose of a drug that had no effect when administered alone produced an effect greater than that of the other drug when it was administered alone the nature of the effect would be determined to be potentiation.

In a study examining the effects of phencyclidine and pentobarbital in combination, Chait and Balster (1978) trained squirrel monkeys to lever press on a schedule of continuous food presentation (FR 1). The animals were then trained to press on a variable interval (VI) 15 s schedule, which was eventually extended to a VI 100 s schedule. Animals were tested on this schedule for one additional month after initial training to allow the baseline to stabilize and for the animals to habituate to injections. The experimental sessions generally lasted about 2 h and were run on a 4-day cycle. The first day was a "warm up" day, the second day was used to record baseline data, on the third saline or the drug(s) were given, and on the fourth the animal was not tested.

After the training period a dose response curve was obtained for PCP, with one determination at each dose (0.02, 0.04, 0.08, 0.16, 0.32, and 0.64 mg/kg). Next a dose response curve for PB was obtained in the same manner, with doses of 2.0, 4.0, 8.0, and 16.0 mg/kg. Finally a series of combination injections were given starting with PCP in combination with saline, and then PB and PCP combinations given in ascending order of PCP dose.

Only data from the first hour of the session were analyzed because baseline responding decreased and became more variable toward the end of the session. Responses per minute were recorded, and response rates were expressed as percent of baseline ((response rate during first hour of drug session / response rate during first hour of baseline) X 100). Response rates after the combinations were compared to those that would have been expected if the effects of each drug alone were additive in nature.

Low doses of PCP produced small increases in response rate. On the other hand, higher doses produced dose-dependent decreases in rates of responding with the two

highest doses (0.32 and 0.64 mg/kg) producing an abrupt drop in the curve. PB produced a dose-related decrease in the rate of responding as well, and the highest doses almost completely suppressing responding.

In one animal, the intermediate doses of PCP (0.16 and 0.32 mg/kg), when combined with the doses of PB, produced higher rates of responding than would be expected than if the response decreasing effects of the drugs were additive when combined. In this same animal, 0.08 mg/kg PCP, a dose that had no effect or even slightly increased responding when administered alone, when in combination with PB, consistently produced response rates less what would be expected if the drugs were simply additive. While the highest dose of PCP, in combination with PB, resulted in little deviation from a purely additive effect. In the other animal all doses of PCP except the high dose of 0.64 mg/kg yielded rates of responding greater than what would be expected if there was simply an additive effect.

Another study by Thompson & Moerschbaecher (1982) yielded somewhat different results. They used a repeated acquisition and performance procedure to study the effects of phencyclidine (PCP), and pentobarbital (PB) alone and in combination on learning and memory in patas monkeys. Monkeys were trained to complete four response chains that produced a food reward. In the learning or acquisition component one of four geometric forms (horizontal line, triangle, vertical line, or circle) were projected onto three red response keys. The color of the key (red) indicated that the acquisition component was in effect, and the form indicated which key to press for a correct response (e.g. horizontal line = left correct, triangle = right correct, vertical line = center correct, circle = right correct). When a four-response chain was correctly completed five times

(FR 5) a lamp above the reward dispenser was illuminated and when pressed the food was delivered. The reinforced response chain in the acquisition component changed from session to session. In the performance component, in which the chain remained the same from session to session. The chain was to be completed as in the acquisition component with the only difference being that the pads were illuminated in green and the geometric forms projected onto them indicated a different correct response than that of the acquisition component (e.g. horizontal line = right correct, triangle = left correct, vertical line = right correct, circle = left correct). Each session began with the learning component, which alternated with the performance component after 10 reinforcements or 15 minutes, whichever came first. Sessions were terminated after 100 reinforcements or 2 hours. The behavior was measured in terms of overall response rate (responses / minute) and percent error ((errors / total responses) X 100).

Once behavior was stable, dose effect data was obtained for PCP. Generally there were two determinations at each effective dose and the highest ineffective dose. Doses included in the study were 0.03 mg/kg, 0.056 mg/kg, 0.1 mg/kg, and 0.17 mg/kg. Three mg/kg of PB was then administered alone. Once all animals had completed their cycle of 3 mg/kg of PB, the varying doses of PCP were administered in combination with PB with determinations of each effective dose combination. Upon obtaining the combination dose effect curves 3 mg/kg of PB was administered alone again. Then using the same procedure, doses of 7.5 mg/kg and 10 mg/kg PB were administered alone and in combination with the varying doses of PCP. Finally the dose effect data of PCP alone were again determined.

Thompson and Moerschbaecher found that PCP alone decreased response rate and increased errors in a dose dependent manner with the lowest dose having little effect. The lowest dose of PB, 3 mg/kg also had little or no effect. However, in the presence of 3 mg/kg PB, the PCP dose effect curve shifted to the left relative to PCP alone (i.e. though PB had little effect alone, the effect of the combination of PB and PCP was greater than that of PCP alone). The high doses of PB (7.5 mg/kg and 10 mg/kg) when administered alone decreased response rate while increasing the percent error. In addition, these higher doses of PB in combination with PCP shifted the PCP dose effect curves to the left relative to the 3 mg/kg dose.

If the effect of the combinations of these drugs were simply additive, the sum of the difference of the effect of a given PCP dose from the control data and the difference of a given PB dose from the control data would indicate where one would expect the data point for that combination to fall. However, this was not the case. The difference between the control and the combinations were generally greater than if you simply added the difference of control and the given doses of PB and PCP.

Preclinical Studies of Opiate-NMDA antagonist combinations

Carlezon, Kosten, and Nestler (2000) studied the analgesic effects of combinations of MK-801 and morphine in rats. A tail immersion assay was used to assess analgesia. The tail withdraw latency was taken 30 min and 60 min after drug administration. In a pilot study the effects of morphine (10 mg/kg) alone, MK-801 (0.25 mg/kg) alone, and morphine preceded by MK-801 on withdraw latency were examined over 10 days. MK-801 was shown to potentiate morphine analgesia. In a follow up study, morphine (10 mg/kg) and lower doses of MK-801 (0.031, 0.063, and 0.125 mg/kg)

were tested in the same manner as above. The highest of the three doses of MK-801 potentiated the analgesic effects of morphine, but the lower doses of MK-801 had no effect on morphine analgesia. On day 11 all rats were treated with saline followed by morphine (10 mg/kg). Tail withdraw latencies depended on the subjects' previous treatments (vehicle + vehicle, vehicle + morphine, MK-801 (0.125 mg/kg) + morphine, MK-801 (0.063 mg/kg) + morphine, and MK-801 (0.031 mg/kg) + morphine). Morphine increased latencies in rats that had received 10 days of treatment with the vehicle, but not in rats that received 10 days of morphine. In addition, rats that had received the MK-801 doses had higher latencies than those that had received morphine. This study suggests that NMDA antagonists are not only capable affecting the development of tolerance to morphine, but might also potentiate morphine induced analgesia.

NMDA antagonists have been shown to potentiate the antinociceptive effects of opiates in other studies as well. For example, Allen, Granger, and Dykstra (2003) studied the antinociceptive effects of competitive NMDA antagonist LY235959 in combination with morphine as well as other opioid receptor agonists *l*-methadone, levorphanol, butorphanol, and buprenorphine using a squirrel monkey shock-titration procedure. Increasing shock was delivered to the tail every 15 s in 30 increments. Five lever presses during the shock period (FR 5) produced a 15-s shock-free period, after which shock resumed at the next lower intensity. All of the opiates, but not LY235959, increased median shock level in a dose- and time-dependent fashion. When given concurrently with the opioid agonists, LY235959 potentiated the antinociceptive effects in a dose-dependent fashion.

Baker, Hoffmann, and Meert (2002) studied the effects of the clinically available NMDA antagonists ketamine and dextromethorphan in combination with various opiates in a mouse hot-plate test. Mice were placed on a hotplate, and response (licking of paws or jumping) latencies were measured. The NMDA antagonists potentiated the effects of morphine, fentanyl, and sufentanil. The hot plate test has also been used in a similar manner for rats (Bulka, Wiesenfeld-Hallin & Xu, 2002) revealing that dextromethorphan potentiated the antinociceptive effects of morphine and methadone.

Thus, NMDA antagonists have been shown, not only to attenuate the development of opiate tolerance, but, paradoxically, to potentiate the antinociceptive and locomotor stimulating effects of opiates as well. Given that both NMDA receptor antagonists and opiates have been implicated in the disruption of learning, it seems possible that combinations of these drugs could produce a potentiation of effects of this kind as well. This would obviously not fall into the realm of desirable effects in the clinical application of combinations of NMDA antagonists and opiates, and an important goal of the present study was to determine the nature of NMDA-opiate interactions on learning.

The present study offers a systematic replication of the Galizio et al. (2003) study of spatial learning, adding a morphine/LY235959 combination dose, as well as a non-spatial olfactory discrimination learning task that is analogous in its use of the RAP procedure. In this latter procedure, rats were presented olfactory stimuli, scented sand, two at a time, with one of the stimuli designated S+. This designation remained unchanged throughout the session, but changed across sessions. This was the acquisition component. Proficiency in this component resulted in presentation of the performance

component where the positive/negative stimulus designation remained constant throughout the study. Upon achieving stability in each component, morphine, LY235959, and a combination of the drugs were administered providing a dose-response curve for a single subject.

It seems reasonable that the repeated acquisition and performance procedure applied to the Morris Swim Task, and an olfactory discrimination task, may be able to shed light on some of the questions concerning drug effects on learning in rats. This will allow a within species comparison of the effects of morphine, LY235959, and their interaction on spatial versus non-spatial learning.

Experiment 1

Effects of morphine and LY235959 alone and in combination on behavior in the Morris Swim Task

METHOD

Subjects

Seven male Holtzman Sprague-Dawley albino rats served as subjects for the morphine study, five for the LY235959 study and three for the combination study. Subjects were between 90 and 150 days old at the start of testing and were housed individually in a temperature and humidity controlled vivarium with other rats. The rats were kept in a cycle of 12 h light, 12 h dark (simulated with red light), and had continuous access to food and water.

Apparatus

The apparatus was circular white fiberglass pool (1.5 m diameter, 45.7 cm deep) filled with 22.5 cm water. A cylindrical white plastic platform (10 cm diameter, 20 cm high) was submerged so that its major surface was 2.5 cm below the surface of the water.

The water was made opaque with white, non-toxic paint. Water temperature was maintained at 30°C (+/- 3°). Four shower curtains, each capable of displaying two patterns, surrounded the pool (see Figures 1 and 2). A digital video camera was mounted, centrally, above the pool, and connected to a contrast tracking system (Polytrack, San Diego Instruments). Data recorded by this system included escape latencies, and path distance. A video monitor was connected to the system allowing for observation of the subject during a trial. Non-toxic black dye was be used to mark the subjects for contrast tracking.

Procedure

Preliminary training. Subjects began the experiment exposed only to the performance component. The curtain configuration and platform position remained the same (see Figure 1) across sessions that consisted of 6 trials each. To begin a session, subjects were placed into the water, facing the pool wall at one of four randomly determined start locations (North, South, East, or West), at this point the experimenter also started the tracking system. The rats were given 60 s to swim and find the platform submerged under the water, if the rat did not do so within this period of time it was placed onto the platform by the experimenter. Once it reached the platform, whether on its own or with the help of the experimenter, the rat remained there for 15 s. The rats were then removed from the pool and returned to the holding cage for an inter-trial interval (ITI) of 2 min. Sessions continued in the performance component until a criterion of three consecutive sessions with mean escape latencies under 10 s was met.

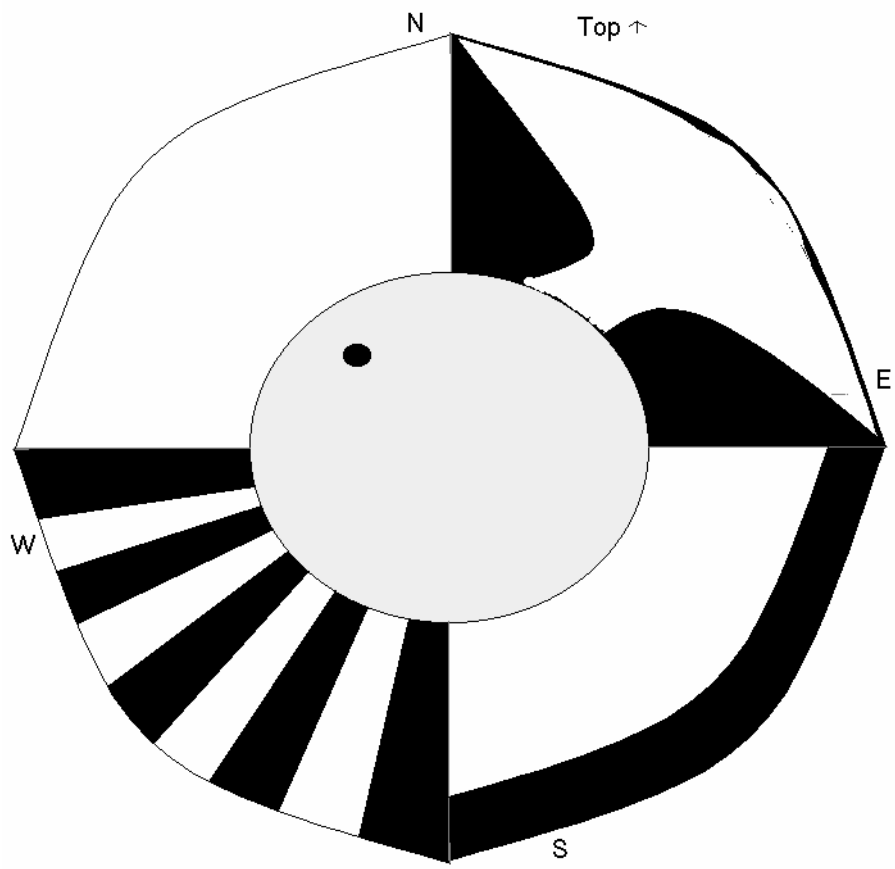

Figure 1. Escape platform position and stimulus arrangements used in the performance component of the swim task.

Multiple-component training. Upon satisfying the criterion for completion of preliminary training, subjects were exposed to the performance and acquisition components. Under the performance component conditions, the curtain configuration and platform location remained as they were in preliminary training across sessions. Alternating with the performance component was the acquisition component, in which the curtain configuration remained the same from session to session, but the platform location was randomly selected from 11 possibly locations for each session with the constraint that the platform location could not be that of the previous session (see Figure 2).

The first trial in a session was under performance component conditions. Subjects entered the pool in the same manner as in preliminary training, and once again, upon reaching the platform remained there for 15 s. During the 2 min ITI, the rat remained in its home cage while the experimenter recorded data, and prepared the apparatus for the next, acquisition trial. With the exception of curtain configuration and platform location, acquisition trials were identical to performance trials. Performance and acquisition trials alternated until the session was terminated after 12 total trials. Multiple-component sessions continued until subjects mean escape latencies for both components (based on all six trials for performance and last 5 trials for acquisition) was under 10 s for ten consecutive sessions, and a stability criterion (the difference between the last 5 sessions and previous 5 sessions was less than 15% of the mean of the final 10 session (Perone 1991)), was met. Once the criteria were met, the drug administration phase of the study began.

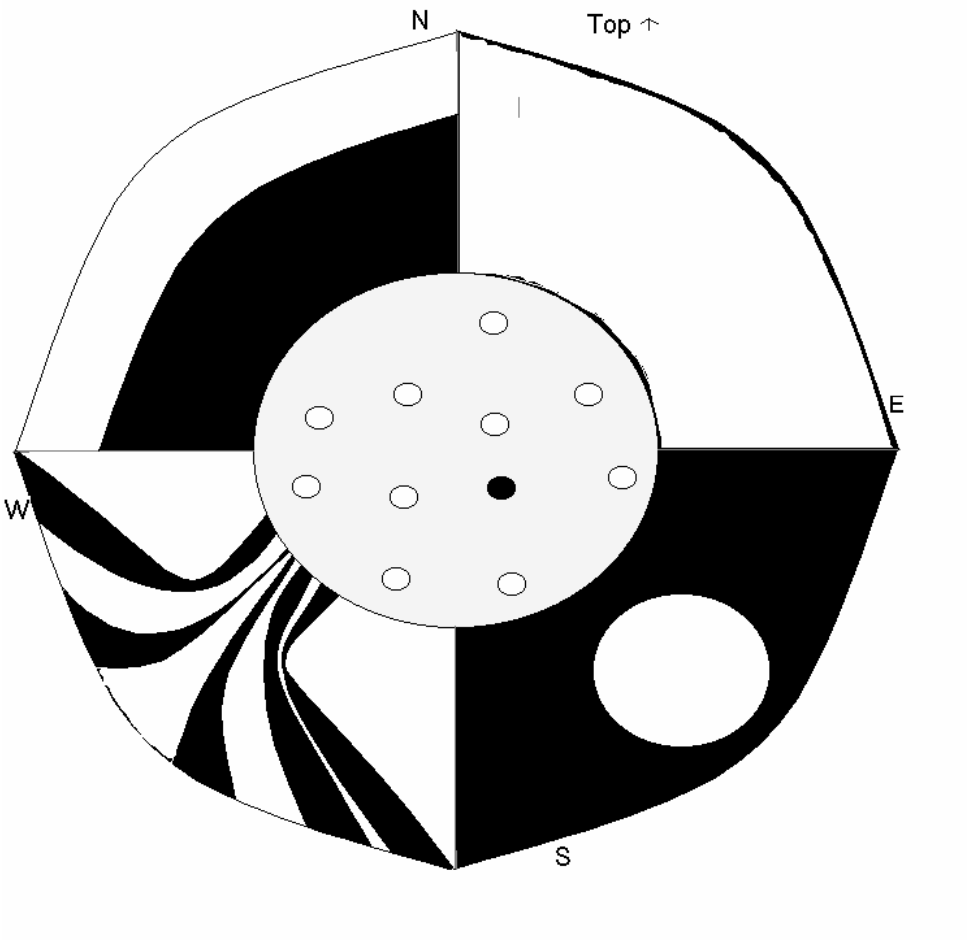

Figure 2. Escape platform position and stimulus arrangements used in the acquisition component of the swim task.

Drug Preparation and Administration. Drug solutions were prepared within 24 h of administration. Each compound was dissolved in an isotonic (0.9%) sodium chloride solution, and refrigerated. Solutions were prepared by the experimenter, at a volume of 1 ml/kg, on the day of the session, and the solution was allowed to approach room temperature for approximately 5 min prior to injection. Injections were administered twice per week, on Tuesdays and Fridays, 15 min prior to testing. All rats were tested with morphine in doses of 1.0, 3.0, 5.6, and 10.0 mg/kg; one subject was tested with 17.0 mg/kg. LY235959 doses of 0.3, 1.0, 1.7, and 3.0 mg/kg were tested in all rats; two subjects also received 5.6 mg/kg. Finally, combinations of morphine and LY235959 were tested. The doses selected for the combination phase of the experiment were selected based on the individual subject's dose effect curves in addition to the initial determinations of the doses when given in combination with saline. For each drug the highest dose with no effect was selected as the low dose, and the lowest dose with an effect was selected as the high dose. Morphine doses of 1.0, 3.0, 5.6, and 10.0 mg/kg were selected along with LY235959 doses of 1.0 and 1.7 mg/kg. Most doses were administered two to three times unless the dose severely impaired the animals, or variability required further determinations. Separate injections of each drug were given, with the order of these injections determined randomly.

Dependent Variables

The measure that perhaps most readily reveals the rats' performance in the task is the mean escape latency. This measure is related to the two other measures: swim speed and mean path ratio. Swim speed was calculated by dividing the swim distance by the latency. Mean path ratio was calculated by subtracting the optimal or minimal distance

(MD) that the subject could have traveled from the actual distance (AD) that it traveled, and then dividing this number by the minimal distance, thus: (AD-MD)/MD. Swim speed and path ratio can help reveal whether a long escape latency is the result of slow swimming or poor navigation. Means for the performance component were calculated based on all six trials, while acquisition latencies were based on only the final five trials. This is a result of the fact that the first acquisition trial is essentially a novel problem, and including this data in the mean would misrepresent the animals' behavior in the task.

RESULTS

Rats rapidly learned to swim to the fixed performance location during preliminary training. Criterion level performances (three consecutive sessions with escape latencies averaging less than 10 s) were reached by all subjects between 4 and 15 sessions (mean = 7.71). The multiple component task required between 14 and 47 sessions (mean = 31.14) for rats to meet criteria (10 consecutive sessions with mean escape latencies in both components averaging less than 10 s (mean of all trials for performance and last five trials for acquisition--see Table 1).

Effects of morphine on behavior in the Morris Swim Task

Figure 3 shows the effects of morphine on escape latency (top panel), swim path ratio (middle panel), and swim speed (bottom panel). Only doses (1.0, 3.0, 5.6, and 10.0 mg/kg) that were tested in all subjects are shown. The closed circles represent the group mean for the performance component and open circles represent the group mean for the acquisition component. The error bars represent the standard error of the mean. Escape latency was calculated by averaging all six, and the last five trials, respectively for the performance and acquisition components.

Table 1

Number of Sessions in Each Component Prior to Drugs in Experiment 1

Subject	Single Component	Multiple Component	First Drug	Second Drug	Combination?
K5	6	14	Morphine	-	-
N24	8	46	LY235959	-	-
O1	8	24	LY235959	Morphine	Yes
O21	15	47	Morphine	-	-
O22	7	41	LY235959	Morphine	-
R6	4	31	LY235959	Morphine	Yes
T1	6	15	Morphine	LY235959	Yes

Table 1. Number of sessions in each component prior to receiving drugs and drug conditions for each rat in experiment 1.

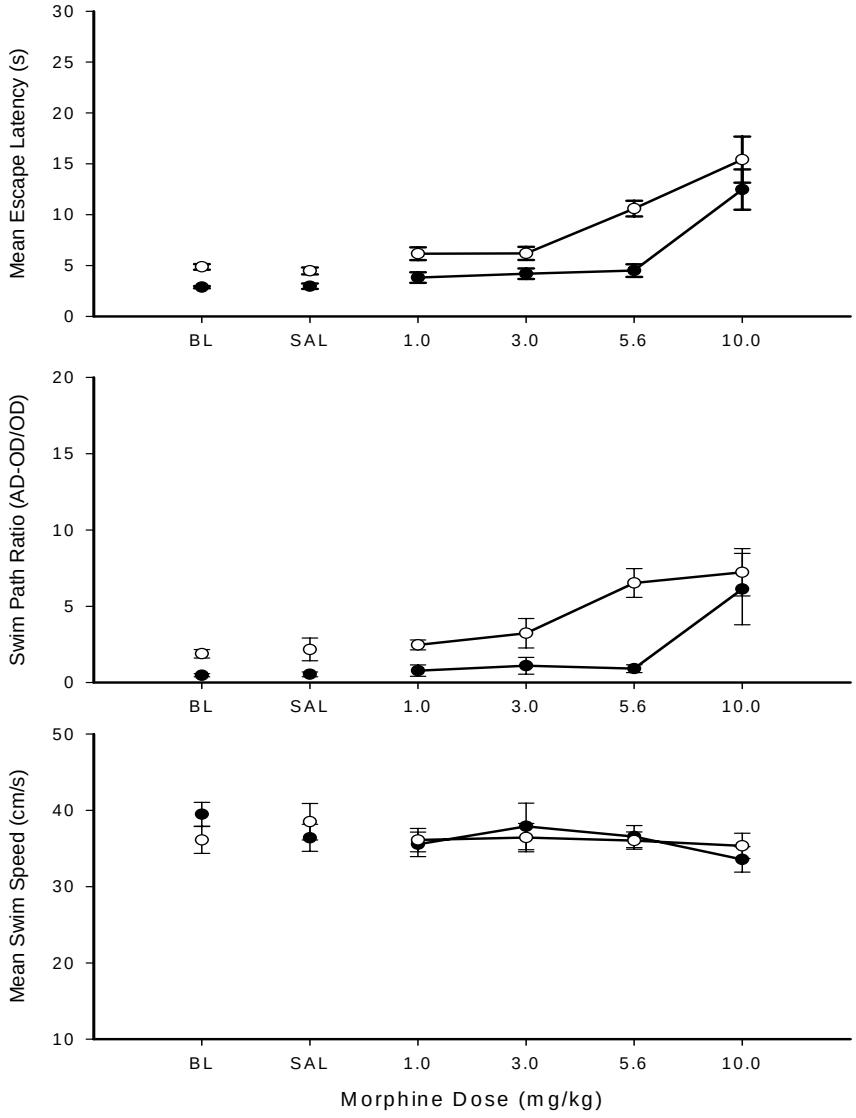

Figure 3. Mean escape latencies (top), swim path ratios (middle), and swim speeds (bottom), as a function of morphine dose during performance (closed circles) and acquisition (open circles) components.

Morphine produced dose-dependent increases in escape latency. This effect was confirmed by statistical analysis showing a main effect for morphine Dose: [$F(4, 16) = 9.72$, $p < .05$]. There was also a main effect of Component: [$F(1,4) = 51.10$, $p < .05$] with acquisition requiring longer latencies than performance. Figure 3 (top panel) shows no morphine effects at the lower doses of 1.0 and 3.0 mg/kg. However, at 5.6 mg/kg, escape latency was elevated in the acquisition component, but not in the performance component. This selective effect on acquisition was confirmed by a significant Dose X Component interaction [$F(4,16) = 3.36$, $p < .05$]. At the highest dose tested in all rats, 10.0 mg/kg, acquisition and performance were both disrupted.

The results for swim path ratio are similar in nature to that of escape latency. Morphine also produced dose-dependent increases in swim path ratio. This effect was confirmed by statistical analysis [$F(4, 16) = 6.75$, $p < .05$]. There was again an effect of component as well: [$F(1,4) = 79.40$, $p < .05$]. Figure 3 (top panel) shows that at the lower doses of 1.0 and 3.0 mg/kg, neither performance nor acquisition were disrupted. However, at 5.6 mg/kg, escape latency was elevated in the acquisition component, but not in the performance component. This selective effect on acquisition was confirmed by a significant dose X component interaction [$F(4,16) = 3.74$, $p < .05$]. The highest dose tested in all rats, 10.0 mg/kg, acquisition as well as performance are impaired.

The final dependent variable, swim speed, is shown in the bottom panel of figure 3. There was a decreasing trend in swim speed as a function of dose, but the effect was not statistically significant [$F(4, 16) = 0.64$, $p > .05$]. Importantly, there was also no effect of component [$F(4,16) = 0.67$, $p > .05$], and there was no dose X component

interaction [F (4,16) = 1.07, p > .05]. Differences in drug effects on the performance and acquisition component were not a result of differential swim speeds.

Figure 4 shows individual subject data for the effects of morphine on escape latency. This figure shows that the selective effect of 5.6 mg/kg on acquisition was fairly consistent across rats. Four of the five rats tested seem to exhibit this effect. Rats K5, O1, O21, and O22 were largely unaffected by 1.0 and 3.0 mg/kg. However, at the 5.6 dose, all four of those subjects showed elevations in acquisition latencies, but no effect on latency in the performance component. The higher doses tested continued to disrupt acquisition, as well as impairing performance. For all rats, performance was disrupted along with acquisition at the higher dose of 10.0 mg/kg.

Morphine clearly disrupted acquisition at a dose (5.6 mg/kg) that did not affect performance in this task. This effect was present for escape latency, and swim path ratio. This effect was not a result of differential swim speed between the two components.

Effects of LY235959 on behavior in the Morris Swim Task

Figure 5 shows the effects of LY235959 on escape latency (top panel), swim path ratio (middle panel), and swim speed (bottom panel). Only doses (0.3, 1.0, 1.7, and 3.0 mg/kg) that were tested in all subjects are shown. The closed circles represent the group mean for the performance component and open circles represent the group mean for the acquisition component.

LY235959 produced dose dependent disruptions of behavior in both components. There was a statistically significant main effect of dose for latency, [F (4, 16) = 6.82, p < .05]. There was no effect of component [F (1,4) = 3.11, p > .05]. There appeared to be a trend towards a selective effect of 1.7 mg/kg, however there was no statistically

significant dose X component interaction [$F(4,16) = 0.44$, $p > .05$]. Latencies were clearly disrupted in both components at the 3.0 mg/kg dose.

There was a statistically significant main effect of Dose on swim path ratio as well [$F(4,16) = 9.36$, $p < .05$]. Unlike escape latency, there was an effect of Component on swim path ratio [$F(1,4) = 11.20$, $p < .05$]. The apparent trend toward selectivity on acquisition revealed by the escape latency data is less pronounced here, although arguably still present. Once again a statistically significant dose X component interaction is absent [$F(4,16) = 0.41$, $p < .05$]. As with the escape latency data, 3.0 mg/kg clearly disrupts both components.

Unlike in the morphine data, there was a statistically significant decrease in swim speed as a function of LY235959 Dose [$F(4, 16) = 10.40$, $p < .05$]. However, once again there was no main effect of Component [$F(4,16) = 0.90$, $p > .05$], or Dose X Component interaction [$F(4,16) = .90$, $p > .05$]. This reveals that the trend toward a selective effect of 1.7 mg/kg is not a result of differential swim speeds the acquisition component versus the performance component.

Figure 6 shows that LY235959 produced varying effects at certain doses in individual rats. For instance, in rat O1 there is little disruption of either component until both components are clearly disrupted at 5.6 mg/kg. In Rat O22 1.7 mg/kg seemed to disrupt behavior slightly in the acquisition component without impairing behavior in the performance component, but 3.0 mg/kg clearly disrupted behavior in both components (performance actually to a greater extent than acquisition). In Rat N24 there was disruption of both components at the relatively low dose of 1.0 mg/kg. The clearest selective effect is seen in rat R6 where 3.0 mg/kg disrupted behavior in the acquisition

component to a much greater degree than in the performance component. Finally, Rat T1 shows no selective effect at any dose.

LY235959 only affected spatial learning at doses that also disrupted performance. There was a dose (1.7 mg/kg) that seemed to affect acquisition to a slightly greater extent than performance, however this was not consistently the case, and the performance data were slightly elevated as well.

Effects of morphine and LY235959, in combination, on behavior in the Morris swim task.

Figure 7 shows the mean latency for all three rats that received drug combinations. Since the three rats did not receive the same morphine dose the figure shows the effects of the low and high dose of morphine each animal received in combination with 1.0 and 1.7 mg/kg LY235959. The left panel shows the control data and saline in combination with the two LY235959 doses. Relative to the control data, the low LY235959 dose (1.0 mg/kg) did not produce an effect when given in combination with saline. The high LY235959 dose (1.7 mg/kg) did produce a slight trend toward a selective effect on acquisition when given with saline.

The middle panel shows the low morphine dose in combination with saline, and the two doses of LY235959. The low dose of morphine in combination with saline produced a slight selective effect on acquisition. The low morphine dose in combination with 1.0 mg/kg LY235959 produced data similar to that when the morphine dose was given with saline. When the low morphine dose was given in combination with the high LY235959 dose, acquisition was impaired considerably compared to when the two drugs were given with saline.

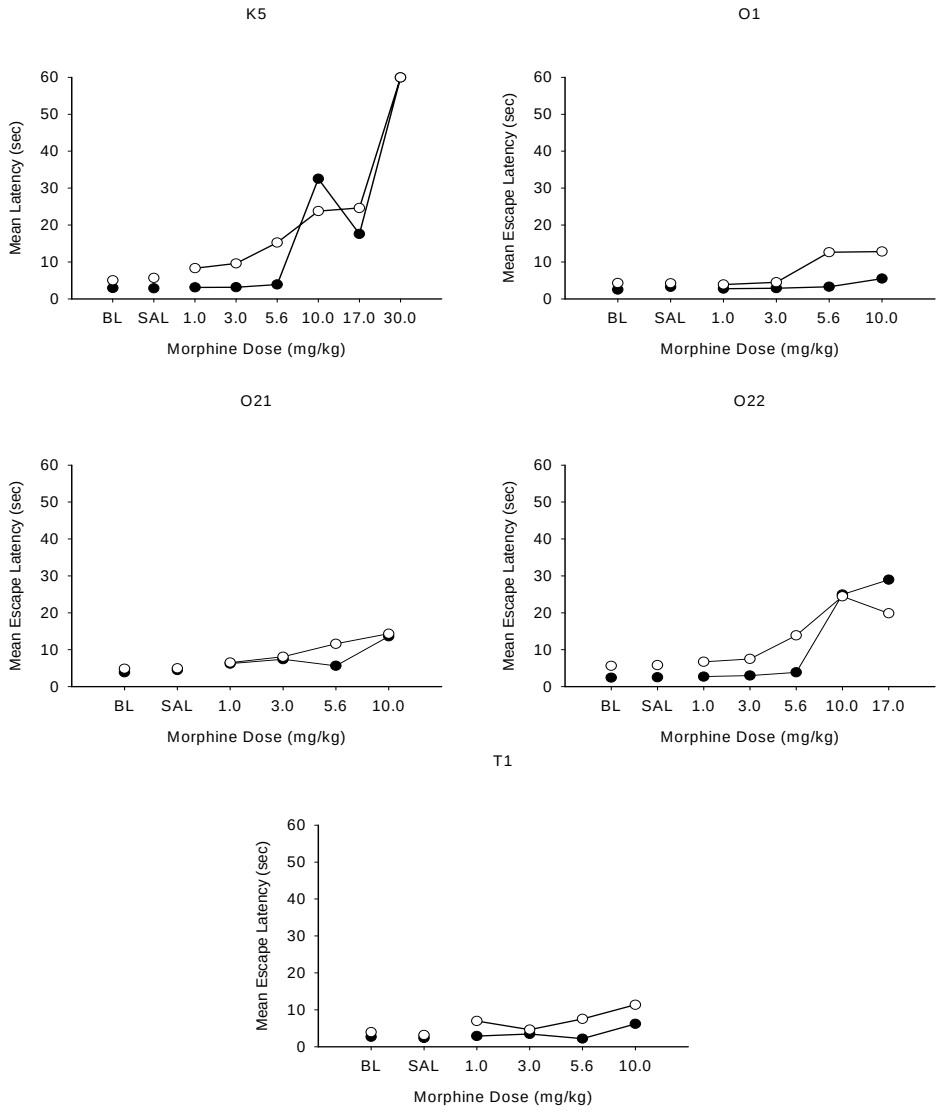

Figure 4. Individual subject mean escape latencies as a function of morphine dose during performance (closed circles) and acquisition (open circles) components.

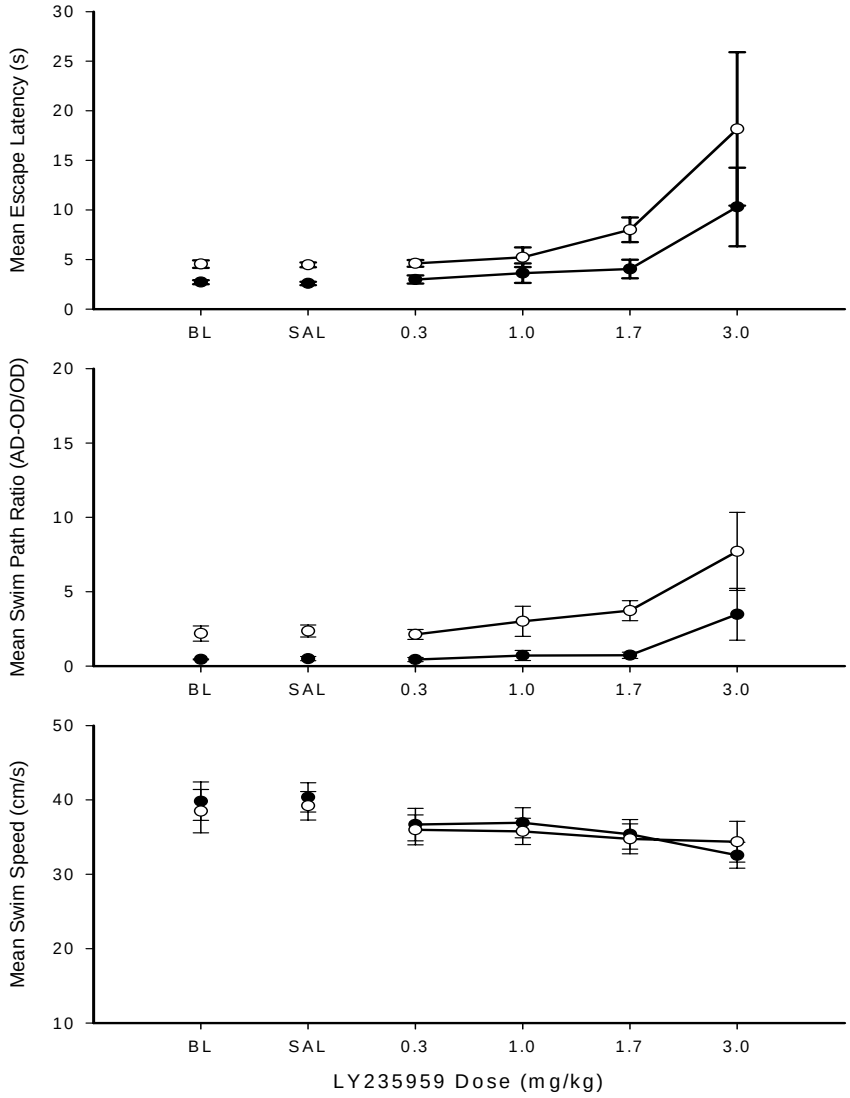

Figure 5. Mean escape latencies (top), swim path ratios (middle), and swim speeds (bottom) as a function of LY235959 dose during performance (closed circles) and acquisition (open circles) components.

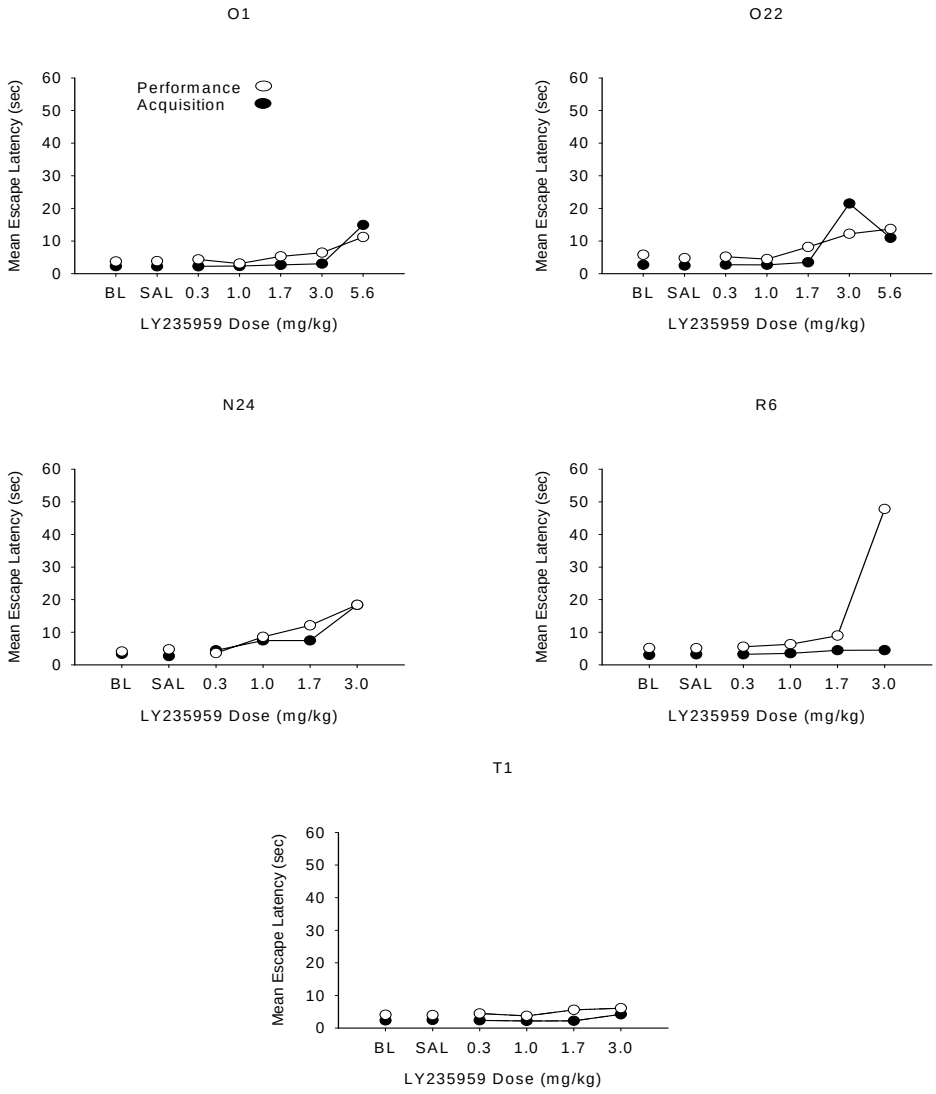

Figure 6. Individual subject mean escape latencies as a function of LY235959 dose during performance (closed circles) and acquisition (open circles) components.

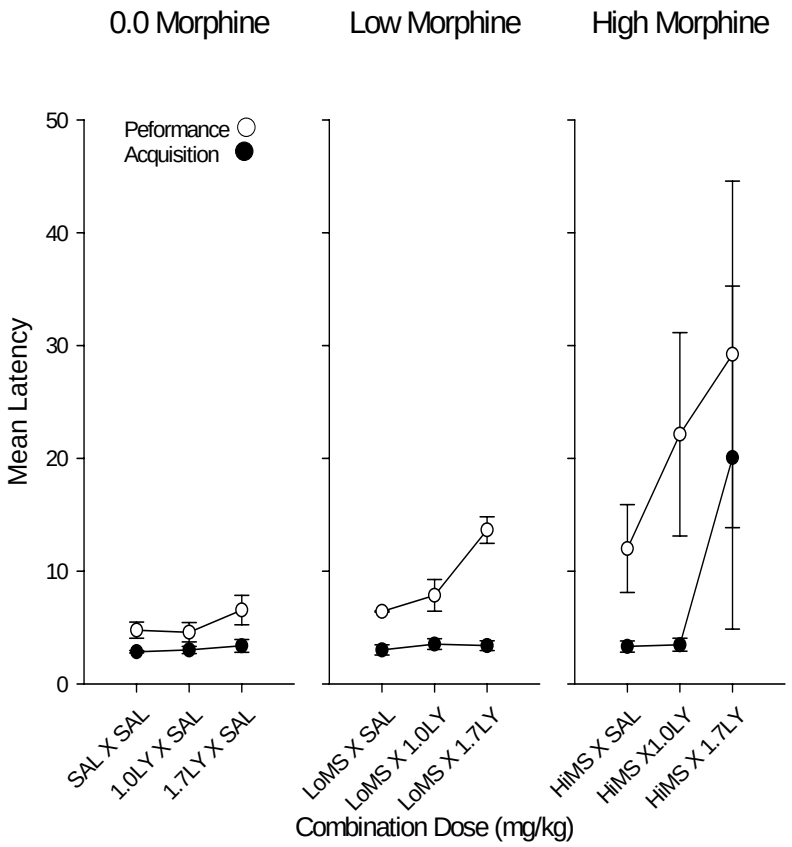

Figure 7. Mean escape latencies during performance (closed circles) and acquisition (open circles) for selected doses of morphine and LY235959 given in combination.

In the right panel are latencies for the high morphine dose in combination with saline and the two LY23595 doses. The high morphine dose produced a clear selective effect on acquisition when given in combination with saline. When this dose of morphine was given in combination with the ineffective dose of LY235959 (1.0 mg/kg), the acquisition latency was elevated considerably relative to when the low morphine dose was given in combination with saline. Finally, when the high dose of morphine was given in combination with the high dose of LY235959 (1.7 mg/kg), acquisition was impaired to a much greater extent than when either drug was given alone. In addition, while neither of these doses impaired performance when given alone, performance was severely impaired when the two doses were given in combination.

Figure 8 shows the group combination data for swim path ratio. In the left panel are the data for the saline/saline, saline/1.0 mg/kg LY235959, and saline/1.7 mg/kg LY235959 dose combinations. It is clear that there was no effect of either dose of LY235959 when given in combination with saline relative to the saline/saline combination. The path ratios for the low morphine dose combinations are in the middle panel. When the low dose of morphine is given in combination with saline the acquisition data point was slightly elevated relative to the saline/saline data. However, there is no effect when this dose of morphine is given in combination with the low dose of LY235959 (1.0 mg/kg). However, as with the escape latency data, when the low dose of morphine was given in combination with the high dose of LY235959 (1.7 mg/kg) the acquisition point was elevated considerably. In the right panel are the data for the high morphine dose combinations. Acquisition was slightly disrupted when the low dose of morphine was given in combination with saline and was selectively affected to an even

greater extent when the high dose of morphine was given in combination with the low dose of LY235959 (1.0 mg/kg). When the high doses of both drugs were given in combination, as was the case with escape latency, there was a disruption of both acquisition and performance.

Figure 9 shows the swim speed data for the combination doses. While swim speed in the performance component tended to be higher than the acquisition component this difference was not significant. Consistent swim speeds were maintained in both components until the highest doses of both drugs were given in combination.

Figure 10 shows the effects of morphine and LY235959 combinations for Rat O1. In the left panel are the data saline alone and in combination with the two doses of LY235959 (1.0 and 1.7 mg/kg). It is clear that the effects of the doses of LY235959 do not differ from the control data when given in combination with saline.

The middle panel shows the effects of 1.0 mg/kg morphine in combination with saline, 1.0 mg/kg LY235959 and 1.7 mg/kg LY235959. This dose of morphine produced a slight trend toward a selective effect on acquisition when given in combination with saline relative to the saline-saline combination data. However, when this dose of morphine was combined with the two doses of LY235959 there seems to be an exaggerated effect on acquisition relative to the data produced by the morphine dose and by each LY235959 dose in combination with saline. Similar data are produced by the higher dose of 3.0 mg/kg morphine (right panel). This dose produced a clear selective effect on acquisition when given in combination with saline. However, given that the two LY235959 doses had no effect when given in combination with saline it seems that combinations of 3.0 morphine and the LY235959 doses resulted in potentiation. This was

most clear with the 1.0 mg/kg morphine and 1.7 mg/kg LY235959 dose combination where there was an elevation in acquisition with little variation in the data.

The same dose combinations were used for rat T1 (figure 11). The left panel shows the control data, as well as saline in combination with 1.0 and 1.7 mg/kg LY235959. There is clearly no effect of 1.0 mg/kg when given in combination with saline. However, 1.7 mg/kg does seem to at least produce a trend toward a selective effect on acquisition.

The middle panel shows latencies after 1.0 mg/kg morphine given in combination with saline, and 1.0 and 1.7 mg/kg LY235959. The figure shows that 1.0 mg/kg morphine in combination with saline seems to produce a slight elevation in acquisition. A similar acquisition effect is seen when 1.0 mg/kg morphine is given with 1.0 mg/kg LY235959. The combination of 1.0 mg/kg morphine and 1.7 mg/kg LY235959 produced an acquisition disruption that was consistently greater than that produced by either drug dose in combination with saline.

The right panel shows the effects of 3.0 mg/kg morphine in combination with saline, and 1.0 and 1.7 mg/kg LY235959. There was little effect, if any, when 3.0 mg/kg morphine was given in combination with saline or 1.0 mg/kg LY235959. However, there was a consistent disruption of acquisition by the combination of 3.0 mg/kg morphine and 1.7 mg/kg LY235959. In addition, while neither of these two doses of the drugs produced performance effects when given with saline, there is clearly a performance disruption with this combination.

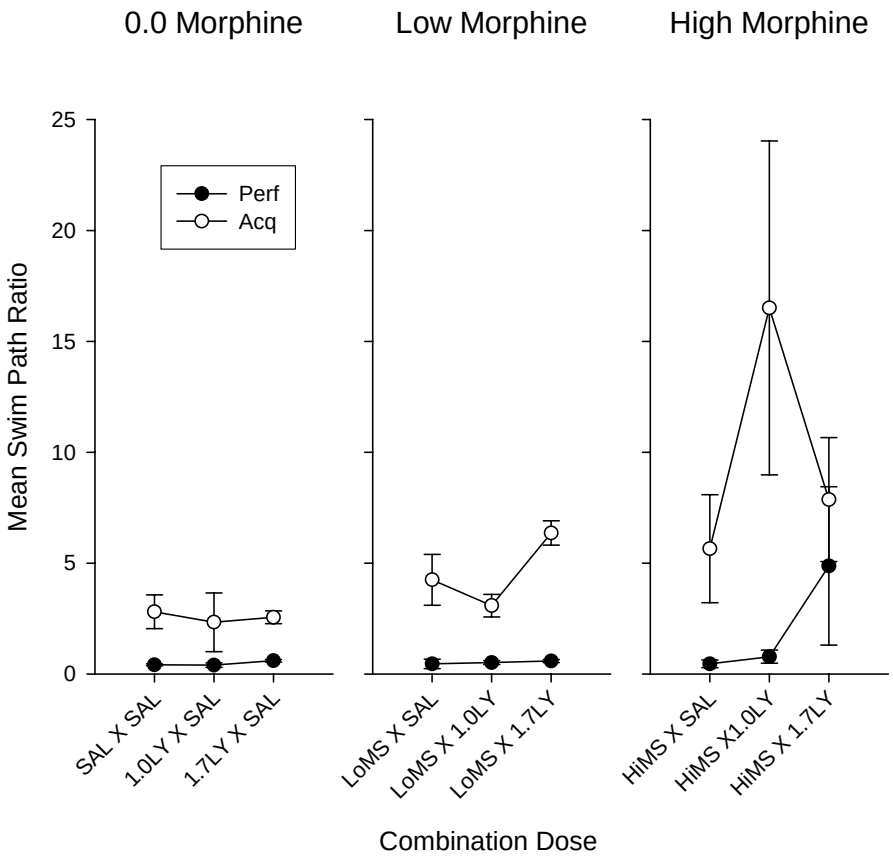

Figure 8. Mean swim path ratio during performance (closed circles) and acquisition (open circles) for selected doses of morphine and LY235959 given in combination.

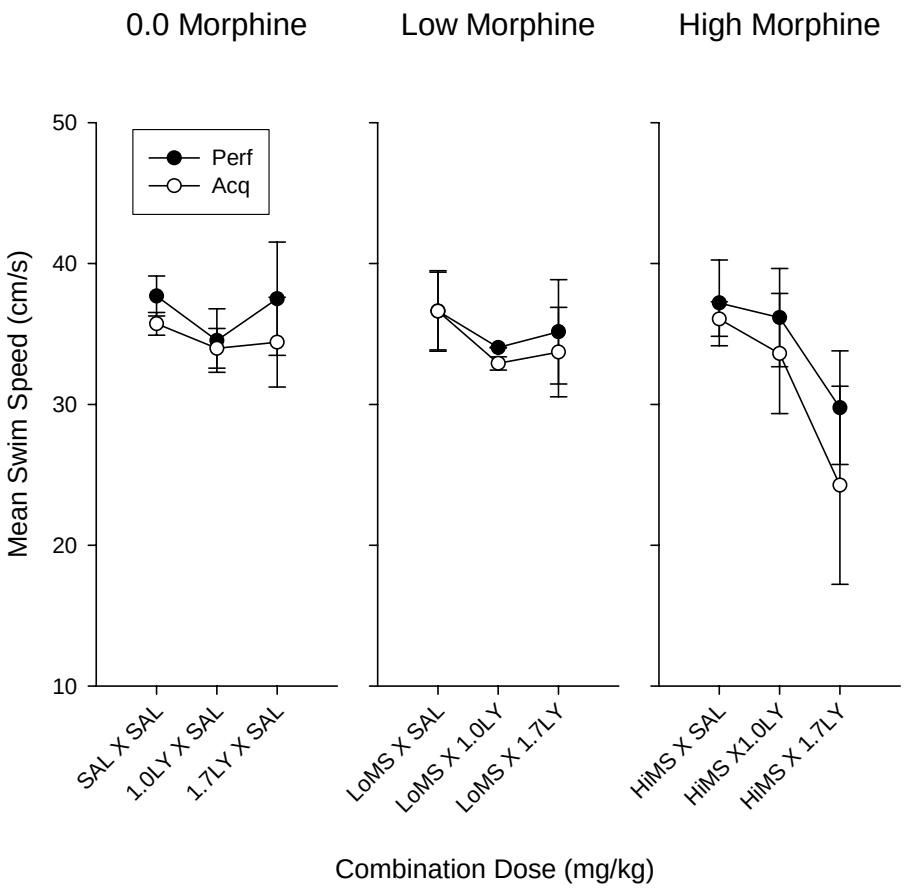

Figure 9. Mean swim speed during performance (closed circles) and acquisition (open circles) for selected doses of morphine and LY235959 given in combination.

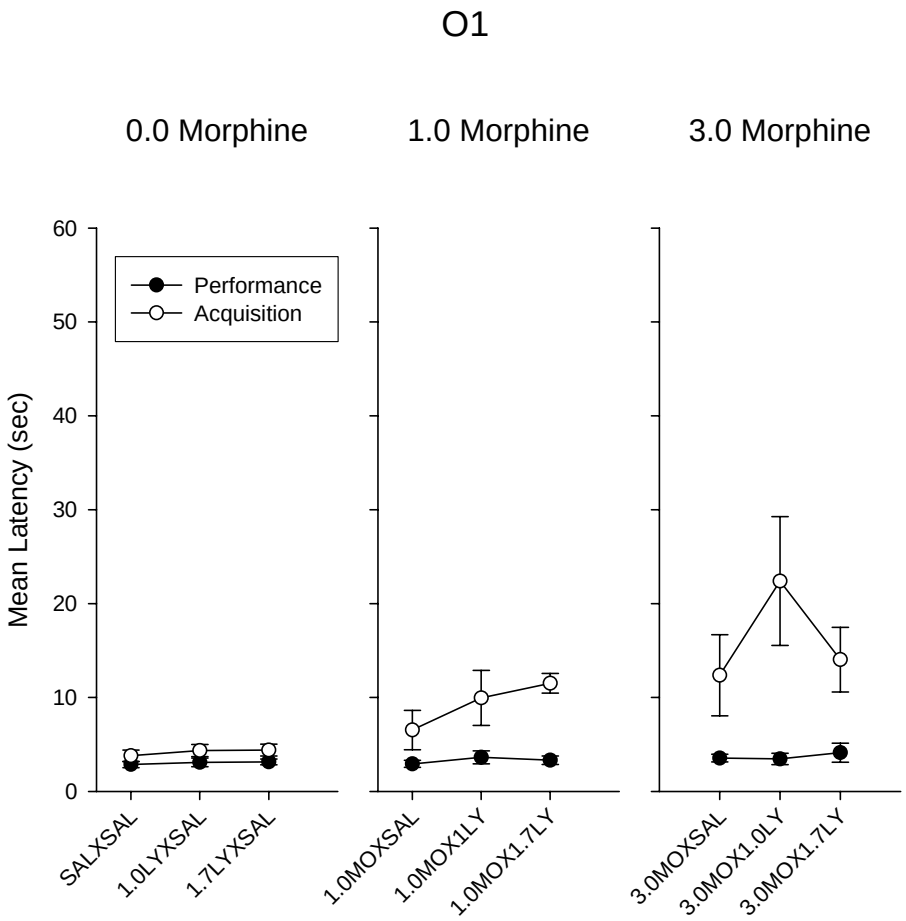

Figure 10. Mean escape latencies for Rat O1 during performance (closed circles) and acquisition (open circles) for selected doses of morphine and LY235959 given in combination.

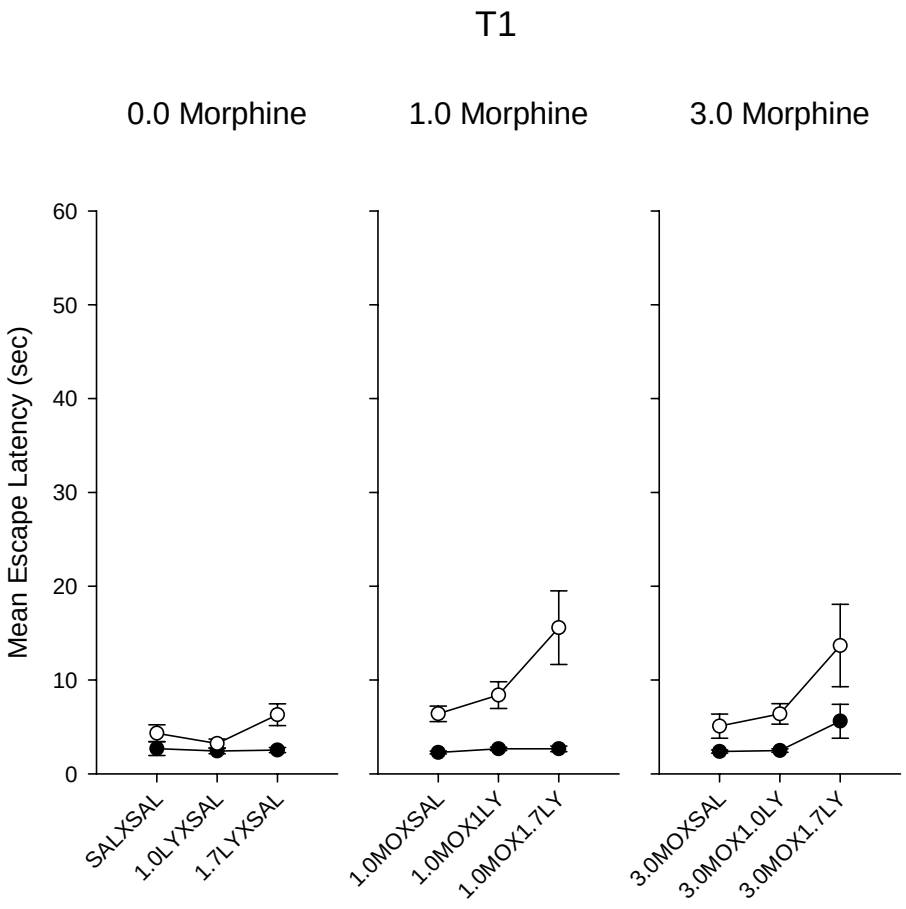

Figure 11. Mean escape latencies for Rat T1 during performance (closed circles) and acquisition (open circles) for selected doses of morphine and LY235959 given in combination.

Figure 12 shows the effects of morphine and LY235959 combinations on escape latency for Rat R6. This rat was tested with the same doses of LY235959 as the other rats (1.0 and 1.7 mg/kg), along with doses of 5.6 and 10.0 mg/kg morphine which were higher than those used with the previous two rats. The left panel shows the results for doses of 1.0 and 1.7 mg/kg LY235959 in combination with saline. Again, there was no effect of the low dose of LY235959 and the higher dose of LY235959 produced a slight and variable increase in acquisition latencies.

The middle panel shows latencies for 5.6 mg/kg morphine in combination with saline, 1.0 and 1.7 mg/kg LY235959. There was no effect of 5.6 mg/kg morphine when given in combination with saline. In addition, there was no effect of 5.6 mg/kg morphine when given in combination with the low dose of LY235959. When 5.6 mg/kg morphine was given in combination with the high dose of LY235959 there was an increase in the acquisition latency relative to when either of the respective two drug doses were given in combination with saline.

The right panel shows the data for 10.0 mg/kg morphine in combination with saline and the two LY235959 doses. 10.0 mg/kg produced a very selective effect when given in combination with saline. However, when this dose of morphine was given in combination with the ineffective dose of LY235959 (1.0 mg/kg) the latency for the acquisition component was close to two-fold higher than that observed when the morphine dose was given with saline. When this dose of morphine was given with the high dose of LY235959 (1.7 mg/kg) there was not only a severe impairment in the acquisition component, but in the performance component as well. Interestingly, neither of these two doses produced performance effects when given in combination with saline.

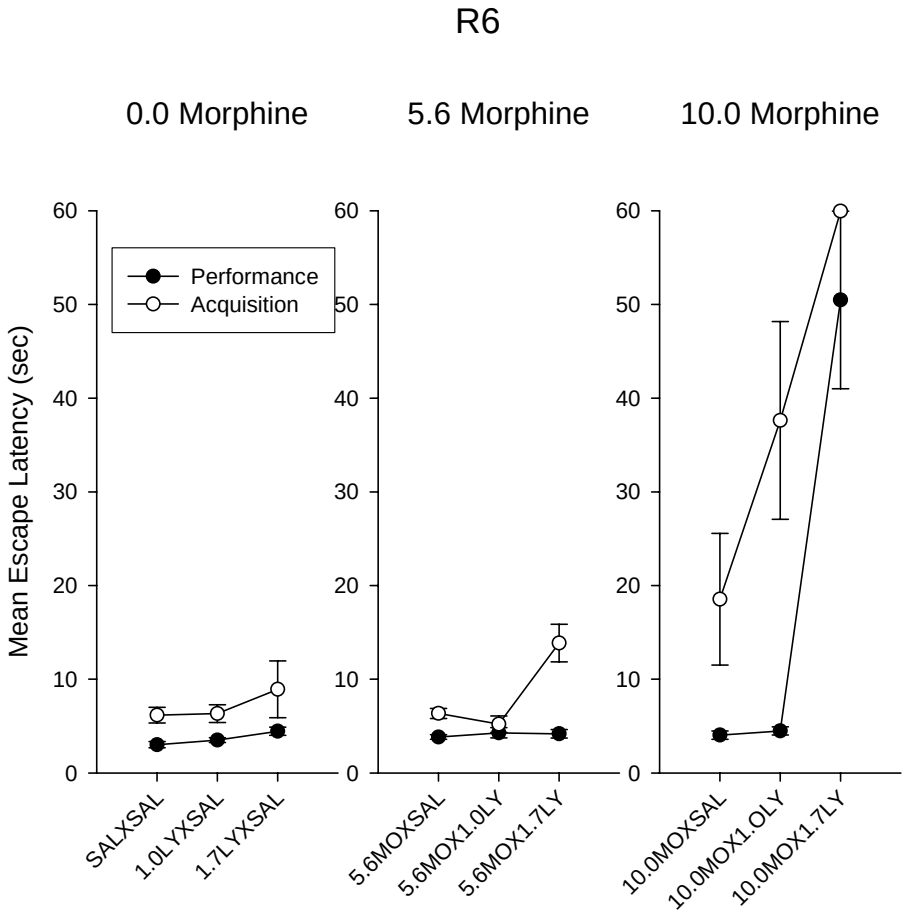

Figure 12. Mean escape latencies for Rat R6 during performance (closed circles) and acquisition (open circles) for selected doses of morphine and LY235959 given in combination.

In summary, doses of morphine and LY235959 that were ineffective when given in combination with saline seemed to selectively affect acquisition when given together. In addition, higher doses of the drugs that did not affect performance when given in combination with saline did produce disruption of performance when given together. Thus, these data suggest that the LY235959/morphine combinations potentiated one another.

DISCUSSION

Under control conditions in the acquisition component, rats were able to drastically reduce the amount of time it took to find the platform, and optimize their swim path from the first trial to the subsequent trials of the session. In the performance component rats were consistently able to swim quickly and directly to the platform. Morphine consistently increased escape latency and swim path ratio in a dose dependent manner. There were no increases in latency and swim path ratio in the acquisition and performance components at the 1.0 and 3.0 mg/kg doses. Morphine consistently increased latency and swim path ratio in acquisition at the 5.6 mg/kg dose. However, at this dose neither of these measures was impaired in the performance component. At the 10.0 mg/kg dose both accurate swimming was disrupted in both acquisition and performance components. There was a slight trend for decreases in swim speed as dose increased, but the acquisition and performance speeds never differed. This is important because it ensures that the selective effect on learning at 5.6 mg/kg was not due to swim speed difference between the two components.

The results of the experiment replicated the results from previous work (Galizio, Keith, Mansfield, and Pitts, 2003) that revealed selective effects of morphine on

acquisition in the MST. However, it must be said that the data differed in that a lower dose (3.0 mg/kg) of morphine produced more consistent selective effects in the Galizio et al. study than in the present study. Galizio et al. did not use a 5.6 mg/kg morphine dose, the dose that was most selective in the present study. Two rats in the present study (K5 and O22) showed tendencies towards selective effects on acquisition at 3.0 mg/kg of morphine. Two other rats (O1 and T1) showed no acquisition effects at 3.0 mg/kg, and finally, in one rat (O21) there seemed to be a slight acquisition effect, which was accompanied by a performance disruption. In both studies 10.0 mg/kg morphine produced acquisition and performance effects, so an overall conclusion would be that morphine doses between 3 and 5.6 mg/kg produce selective effects on spatial acquisition.

Though the present study is in agreement with the Galizio et al. study in regards to the effects of morphine on learning, these results were different from those of some other investigators (Moerschbaecher & Thompson, 1983 and Moerschbaecher et al., 1985). These studies found opiates to produce non-selective effects on learning in monkeys. In addition to the species differences, these studies involved a different type of learning task. The MST is a spatial navigation task whereas the research of Moerschbaecher and his colleagues that found non-selective effects of morphine involved non-spatial procedures.

The second phase of Experiment 1 studied the effects of the competitive NMDA antagonist LY235959 on acquisition and performance in the MST. LY235959 produced dose-dependent decreases in escape latency and swim path ratio. There was no effect in either component of 0.3 and 1.0 mg/kg. At the 1.7 mg/kg dose there was a very small increase in latency and swim path ratio in the performance component. In addition there

was a slightly greater, but nonsignificant, latency increase in the acquisition component. The 3.0 mg/kg dose produced disruption of escape latency and path ratio in both components. There was also a dose dependent decrease in swim speed.

As did Phase 1 of the experiment, this phase replicated the results from Galizio et al. which found that the NMDA antagonist LY235959 generally impaired acquisition only at doses that also interfered with performance. It was the case that the present study found a slight trend toward a selective acquisition effect at the 1.7 mg/kg that was not statistically significant. This dose was not studied by Galizio et al. The results were also consistent with the finding of non-selective effects of non-competitive NMDA antagonist, MK-801, on spatial acquisition (Keith & Galizio, 1997).

Though the present study is in agreement with the Galizio et al. study in regards to the effects of LY235959 on learning, once again these results differed from other work involving non-spatial learning (Moerschbaecher et al., 1985). This study found NMDA antagonists to produce selective effects on learning in monkeys. These discrepant outcomes may reflect the species differences or the type of learning task employed.

The final phase of this experiment examined the effects of combinations of selected doses of morphine and LY235959 on behavior in the MST. Three rats were used in this phase of the experiment. The doses were selected on the basis of the individual dose-response curves for each drug in addition to the effects of the doses of the drugs in combination with saline. The initial aim was to select the highest dose of each drug that had no effect and the lowest dose that produced an acquisition effect. There were no morphine doses tested in combination that were common to all the rats. However, the LY235959 doses that were tested were common to all rats. This allowed us to look at the

combination data in terms of low and high morphine doses in combination with the two LY235959 doses (1.0 and 1.7 mg/kg).

The group combination data showed that the low dose of morphine and the two doses of LY235959 produced little if any disruption of learning. However, when the low dose of morphine was given in combination with the high dose of LY235959, acquisition was considerably impaired. In addition, when the high dose of morphine was given in combination with the high dose of LY235959 there was a disruption of performance. None of the doses of either drug produced such effects when given in combination with saline. These results suggest that the effects of the two drugs are potentiated to some degree when given in combination.

Experiment 2

Effects of morphine, and LY235959, alone and in combination on behavior in an olfactory discrimination procedure

METHOD

Subjects

Eight male Holtzman Sprague-Dawley albino rats served as subjects. Subjects' age and housing was the same as in the first experiment. However, subjects in this experiment were only allowed access to food for one hour per day (established 5 days prior to the beginning of testing), in addition to the sucrose pellets used as reinforcement during testing.

Apparatus

The apparatus was a modified Gerbrands operant chamber. The interior dimensions were 28 cm long X 26 cm wide X 30 cm high. Walls used for food

presentation, and subject apparatus manipulation, that run the width of the chamber, were adjusted so as to offer no distraction to the subjects, and served as the side walls of the apparatus. The floor of the apparatus consisted of stainless steel bars that ran the length of the apparatus, while the roof was a hinged Plexiglas lid. The clear Plexiglas walls that ran the length of the chamber served as the front and back. The front wall has a 5 cm section of the Plexiglas removed, allowing for presentation and removal of a stimulus tray. This tray was 28 cm long X 12 cm wide X 4 cm high, with two 5 cm holes cut out of the top surface. These holes were separated by 5 cm, and provided a place for cups of sterile sand scented with common household aromatics (e.g. spices, extracts, etc.) that served as the olfactory stimuli (see Figure 13). A speaker in the testing room, and adjacent to the chamber provided white masking noise.

Stimuli

Olfactory stimuli were produced by mixing common household spices (Great American Spice Co.-- celery, cinnamon, garlic, ginger, mustard, onion, paprika, and sage) or coffee (Folgers) with sterilized play sand. Stimulus cups were filled to approximately 1 cm below the rim with scented sand and the cup designated as correct (S+) on a particular trial was baited by placing a sucrose pellet 1 cm below the surface of sand with tweezers. A pellet was inserted and removed from the stimulus cup that was designated as incorrect on a trial (S-) to insure that neither displacement of the sand nor the scent of the pellet or tweezers would serve as potential cues. A ratio of 1 g of spice per 100 g of sand was used because pilot research suggested that these spice levels were sufficient to mask the scent of the sucrose pellet. In these pilot studies, the rats of the present study as well as additional rats were exposed to multiple trials on which they

could choose between two cups filled with sand with a specified concentration of one of the above spices, one baited with a sucrose pellet and the other not. No evidence of above-chance pellet detection was obtained at the 1 g/100 g concentrations with any of spices used in the present study. Within each experimental session, different cups were used on each trial.

Procedure

Pre-training. Rats were placed on a food restriction schedule such that they were allowed free access to food for one hour per day for about one week prior to the beginning of testing. The initial test session was used to allow the subject to acclimatize to the chamber. The subject remained in the chamber for 30 min. In the following sessions the rats were presented with 45 mg sucrose pellets in the stimulus presentation cups presented in the tray. The subjects remained in the chamber in these sessions until consuming 50 pellets. Upon completing this phase of pre-training subjects were exposed to a shaping procedure to initiate digging for the pellets in sand. First the pellets were presented on the surface of sand presented in the stimulus presentation cups. Gradually, the pellets were placed deeper and deeper in the sand until eventually the rats dug for the pellet when it was completely covered by sand. There was no designated timeline for this process as there were individual differences in the acquisition of this skill; however, these procedures were continued until the rat consumed 25 pellets that were completely buried under the sand in one 20-min session.

Single-Component. In this phase of the experiment subjects were presented with only one pair of olfactory stimuli within a given session, one of which was designated as the S+ for that session, and the other was designated S-. Such sessions consisted of 20

trials with the stimuli counterbalanced so that they each occurred on the left and the right side of the tray, however the same stimulus was not allowed to appear on the same side more than two times consecutively. Within a session the stimuli remained the same, and maintained the same value (S+ or S-). However, across sessions the stimuli and value changed. That is, if stimuli "A" and "B" were presented in session 1, with "A" being designated S+, then session 2 would consist of presentation of "C" and "D" or "Y" and "Z" or "C" and "Y", with S+ and S- designations made respectively. More specifically there were constraints on the presentation of stimuli: if a stimulus was designated as S+ then it served as S- before it could serve as S+ again, and all of the stimuli had to serve as S+ prior to one stimulus being used as S+ again. Two paired stimuli were not paired again until they had been paired with other stimuli and, regardless of pairing or S+/S- status, a stimuli were not used in consecutive sessions. With these contingencies in place the subject had to learn a different olfactory discrimination in each session. This is analogous to the acquisition component of the Morris Swim Task where the subject has to learn a new platform location from session to session, and will be referred to as the reversal discrimination. These 20 trials per session were broken down into four bins of five trials. The subject was considered to have met criterion in the single component stage when 90% accuracy was achieved in the final ten trials for five consecutive sessions.

Multiple-component. In this stage a novel pair of stimuli, which were not presented in the reversal discrimination discussed above, was added into the session. These stimuli maintained the same S+ and S- designations from session to session, and were presented everyday in conjunction with the changing acquisition stimuli. These

stimuli were designated as the performance stimuli. The number of trials in a multiple-component session was 24: 16 acquisition trials with stimuli that change from day to day under the constraints discussed above, and 8 performance trials with stimuli that remained the same across sessions. The trials were broken down into four bins of four acquisition trials, and four bins of two performance trials that were distributed together throughout the session. Upon maintaining 100% accuracy for performance throughout the session, and 87.5% correct in the last two bins of acquisition trials, subjects were considered to have met criteria to move on to the drug administration phase.

Drug Preparation and Administration. Drug preparation and administration was identical to that of experiment 1. Rats were tested with morphine at doses of 1.0, 3.0, 5.6, and 10.0 and 17.0 mg/kg, and LY doses of 0.3,1.0, 1.7, 3.0, 5.6, and 10.0 mg/kg. Finally, combinations of morphine and LY235959 were tested. The doses selected for the combination phase of the experiment were selected based on the individual subject's dose effect curves in addition to the initial determinations of the doses when given in combination with saline. For each drug the highest dose with no effect was selected as the low dose, and the lowest dose with an effect was selected as the high dose. Morphine doses of 3.0, 5.6, and 10.0 mg/kg were selected along with LY235959 doses of 1.7, 3.0, and 5.6 mg/kg.

Dependent Variable

The percent of correct trials for each bin (four trials in the acquisition component and two trials in the performance component), for each component was calculated. This revealed retention on the performance component problem throughout the session, while demonstrating learning of a relatively new discrimination in the acquisition component.

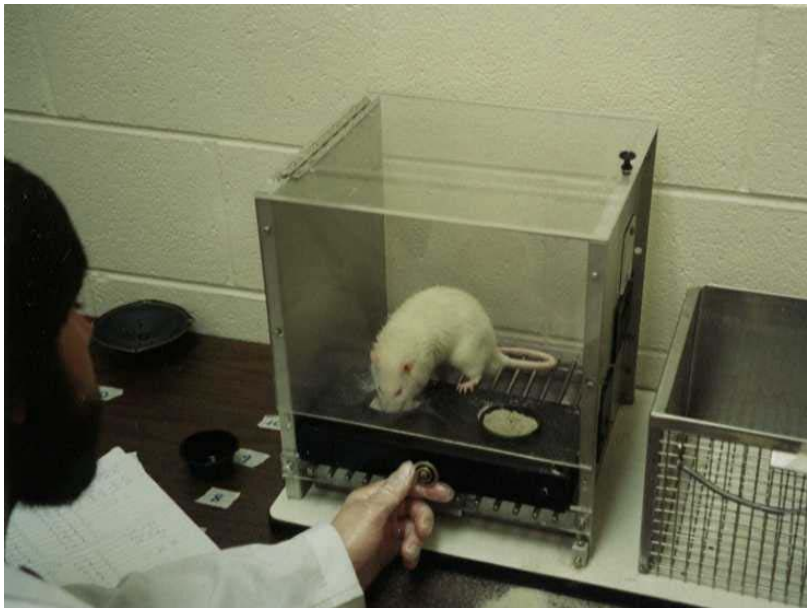

Figure 13. Photograph of olfactory discrimination apparatus during a session.

RESULTS

Rats required from 15 to 35 sessions (M = 22.88) to reach the criterion of 90% correct or better on the final ten acquisition trials of each of five consecutive sessions. Fewer sessions were required for rats to reach mastery criteria for the multiple component phase (100% correct throughout the session for performance and 90% correct for the last two bins of acquisition trials for eight consecutive sessions), with a range of 12 to 51 sessions (M = 23.50--See table 2).

Effects of morphine on behavior in an olfactory discrimination procedure

Figure 14 shows the effects of morphine on percent correct responses in the olfactory discrimination. The closed circles represent the group mean for the performance component and open circles represent the group mean for the acquisition component. The error bars represent the standard error of the mean. In each panel there are four data points per component, this is a result of breaking up the sessions into four blocks of trials. There are two performance trials per block for a total of eight in a session, and there are 4 acquisition trials per block for a total of 16 in a session. This method allows the reader to see behavior in transition within a session.

In the performance component morphine produced dose-dependent decreases in percent correct. The effect was confirmed by statistical analysis [$F, (5, 30) = 35.55, p < .05$]. Figure 14 shows that performance was disrupted by the 17.0 mg/kg ($p < .05$). There was no effect of Bin [$F, (3,18) = .52, p > .05$], and there was no Dose by Bin interaction [$F, (15,90) = 1.22, p > .05$]. In the acquisition component morphine again produced dose-dependent decreases in percent correct [$F, (5,30) = 38.39, p < .05$]. Acquisition was significantly impaired at the 10 and 17 mg/kg morphine dose ($p < .05$). There was also an

effect of Bin [$F, (3,18) = 137.21, p < .05$]. Percent correct was consistently lower in the first bin ($p < .05$). There was a significant Dose X Bin interaction [$F, (15,90) = 4.32, p < .05$]. The fact that morphine produced an impairment in acquisition at a dose (10.0 mg/kg) that produced no effect in performance reveals a selective effect on acquisition.

Figure 15 shows that there were doses that produced selective effects on acquisition for individual rats. Unlike when saline was administered, rat M7 did not reach 100% correct in the acquisition component at the 1.0 mg/kg dose of morphine. However, two higher doses of morphine, 3.0 mg/kg and 5.6 mg/kg, did not produce such an impairment in this animal. In addition, 1.0 mg/kg did not produce learning deficits in any other animals in the study suggesting that this effect was an anomaly. It seems more likely that the delay in learning at the 10.0 mg/kg morphine dose is a real effect.

Three animals, I7, M11, and O2 showed selective effects on acquisition at the 3.0 mg/kg dose of morphine. At this dose, Rat I7 was able to reach the level of accuracy that was attained when saline was administered. However, the rat's learning seemed to be delayed by the 3.0 mg/kg dose as evidenced by the fact that it did not reach 100% correct in a bin until the final bin. Under control conditions the rat reached this level of accuracy by the second bin of trials. When administered 3.0 mg/kg, rat M11 never reached the level of accuracy that was attained when saline was administered. At this dose percent correct was lower in the three final bins relative to when saline was administered. Similarly to Rat I7, Rat O2 was delayed in reaching the level of accuracy that was attained when saline was administered, as accuracy was much lower in the second bin of trials relative to the saline condition. In addition, this animal was not able to maintain this level of accuracy. Importantly, in all three of these rats, performance was unaffected.

Table 2

Number of Sessions in Each Component Prior to Drugs in Experiment 2

Subject	Single Component	Multiple Component	First Drug	Second Drug	Combination?
I6	15	12	Morphine	-	-
I7	15	12	Morphine	-	-
I94	30	19	Morphine	-	-
M7	30	51	Morphine	LY235959	Yes
M11	21	20	LY235959	Morphine	Yes
N22	19	35	Morphine	-	-
O2	18	16	Morphine	LY235959	Yes
T2	35	23	-	LY235959	-

Table 2. Number of sessions in each component prior to receiving drugs and drug conditions for each rat in experiment 2.

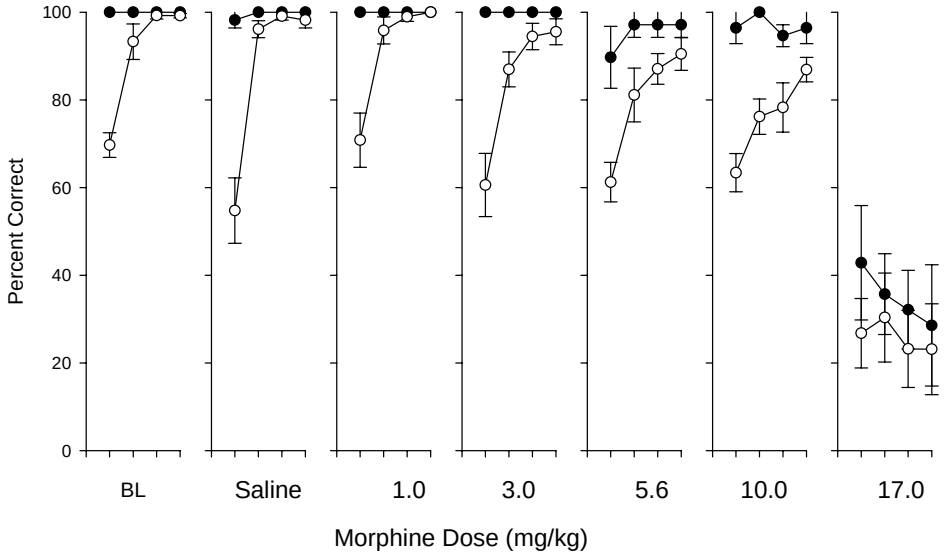

Figure 14. Mean percent correct as a function of morphine dose on performance (closed circles) and repeated reversal learning/acquisition (open circles).

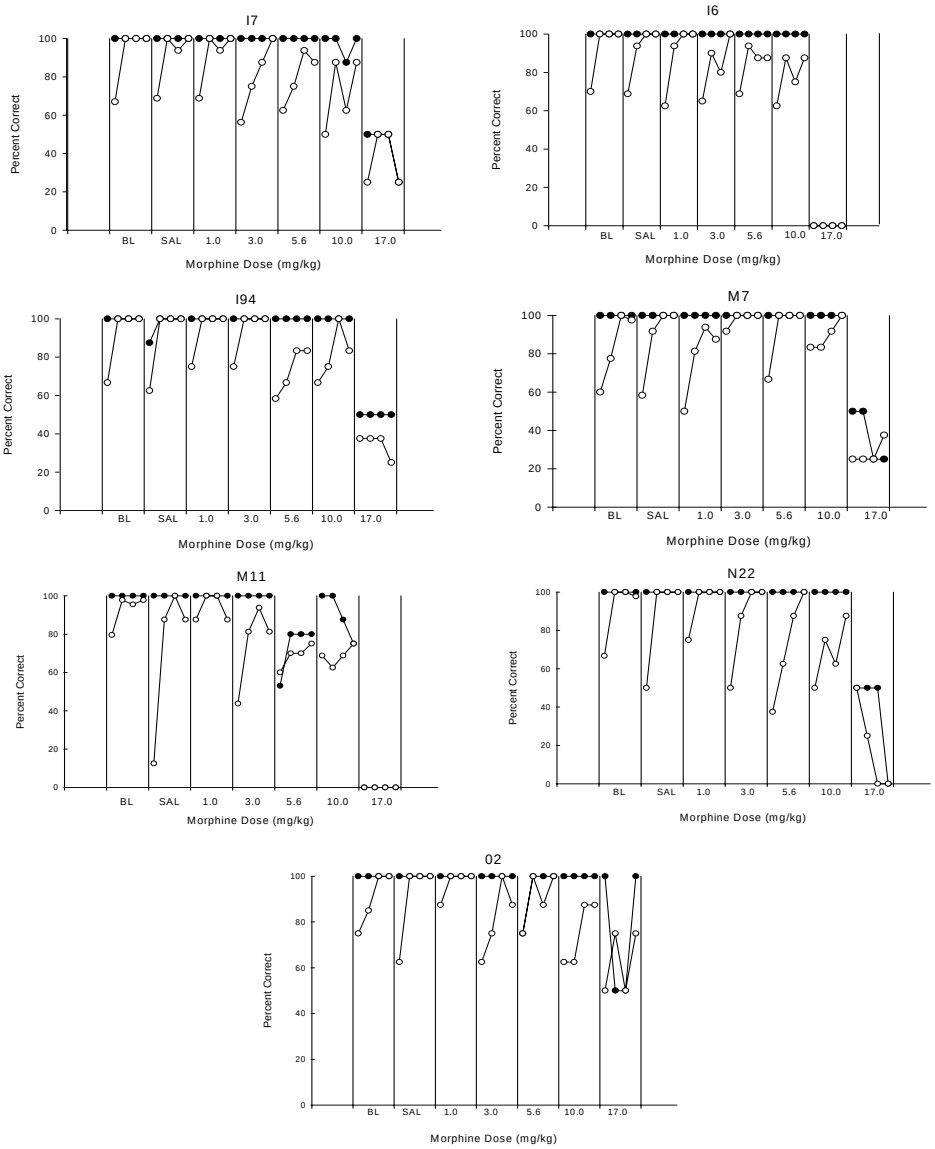

Figure 15. Individual subject mean percent correct plotted as a function of morphine dose on performance (closed circles) and repeated reversal learning/acquisition (open circles).

The dose of 5.6 mg/kg morphine produced selective effects on acquisition in numerous animals. Rats I6, I7, and I94 failed to reach the level of accuracy that was reached when saline was administered. Rat N22 was able to reach the level of accuracy that was attained when saline was administered. However, the rat's learning seemed to be delayed by the 5.6 mg/kg dose. In the saline condition, rat N22 reached and maintained 100% accuracy in the acquisition component by the second bin of trials. However, at the 5.6 dose 100% accuracy was not attained until the final bin of trials.

Rats I6, N22, and O2 never attained the level of accuracy that was attained in the saline condition when administered the 10.0 mg/kg dose of morphine. Rat M7 was able to reach the peak level of accuracy that was attained when saline was administered. However, the rat's learning seemed to be delayed by the 10.0 mg/kg dose as evidenced by a lower percent correct in bins two and three. Rat I94 was also delayed in reaching the level of accuracy that was attained when saline was administered. In addition, this animal was not able to maintain this level of accuracy.

Effects of LY235959 on performance in an olfactory discrimination procedure

In the performance component there was a significant effect of LY235959 dose [$F(5,15) = 3.48$, $p < .05$]. Performance was significantly impaired at the 5.6 mg/kg LY235959 dose. However there was no effect of Bin [$F(3,9) = 2.06$, $p > .05$], and the Dose X Bin interaction only approached significance [$F(15,45) = 1.88$, $p > .05$]. In the acquisition component there was no effect of Dose [$F(5,15) = .896$, $p > .05$]. However there was an effect of Bin [$F(3,9) = 22.32$, $p < .05$] with percent correct significantly lower in bin 1. There was a Dose X Bin interaction [$F(15,45) = 2.87$, $p < .05$]. Figure 16 shows that at the 5.6 dose acquisition is disrupted in the last two bins relative to saline.

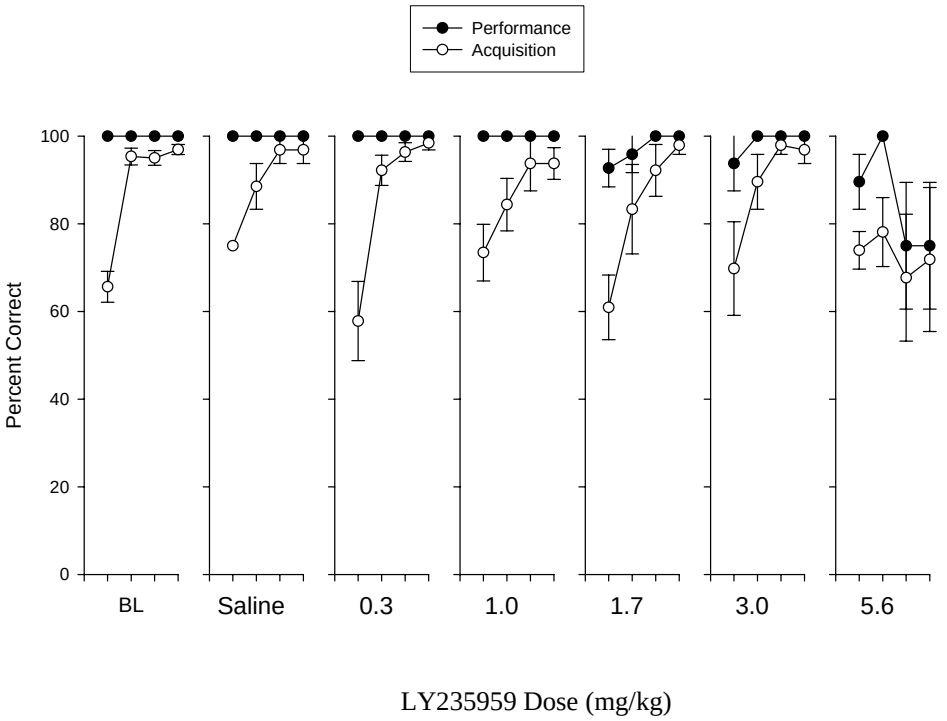

Figure 16. Mean percent correct as a function of LY235959 dose on performance (closed circles) and repeated reversal learning/acquisition (open circles).

Figure 17 reveals that the lack of a main effect of dose is likely due to the data produced by rat T2. This rat was unusual in that it showed relatively poor performance in the saline condition. Most of the rats in the study reached a level of 100% correct in the acquisition component when administered saline. Figure 16 shows that rat T2 never achieved a percent correct above 87.5% in the saline condition. In addition, the other rats in the study did show dose dependent disruptions of behavior. Rat T2 always showed greater accuracy, under the LY235959 doses tested, relative to when saline was administered.

Some of the LY235959 doses tested produced slightly selective effects on acquisition in some of the subjects. Rat M11 was delayed in reaching the level of accuracy that was attained in the saline condition when administered the 0.3 mg/kg dose. This was also the case for rat M7 in the 1.0 mg/kg condition. The 1.0 mg/kg dose also disrupted accuracy in the acquisition component for rat M11. This rat attained a similar level of accuracy as in the saline condition, but was not able to maintain this accuracy. The 3.0 mg/kg dose also disrupted learning for rats M7 and O2.

LY235959 did not consistently affect acquisition without affecting performance at any dose. The group data shows that the doses that are high enough to reliably affect acquisition, also tended to disrupt performance.

Effects of morphine and LY235959, in combination, on performance in the olfactory discrimination.

Figure 18 shows the effects of combinations of the doses of each drug that were common to each rat. The first panel shows the effects of the saline/saline combination on percent correct. Surprisingly, while the performance data were as expected, the rats did

not reach the level of accuracy in the acquisition component in this condition that they had in the previous phases of the study. However, when the selected doses of LY235959 (3.0 mg/kg) and morphine (5.6 mg/kg) were given in combination with saline the accuracy in the performance component was maintained, and accuracy in the acquisition component improved (relative to the saline/saline combination). In fact, accuracy under these conditions closely resembled the control data from the previous two phases of the study where there was clearly within session learning in the acquisition component.

When the two doses of the drugs were given in combination with each other the data was much different than the previous control data, the data for each of these dose given in combination with saline, and the control data from this phase (with less within session learning). There is little, if any, evidence of learning in the acquisition component. In addition, there is also a disruption of performance when this combination was given, whereas neither dose of either drug produced performance impairments when given in combination with saline.

Figure 19 shows the effects of morphine and LY235959 combinations for rat M7. The top panel shows that the data for 3.0 and 5.6 mg/kg LY235959 were similar to that produced by saline alone. The two LY235959 doses did delay learning relative to the saline condition, however, accuracy in the saline condition fluctuated. The middle panel shows the data for combinations of 5.6 mg/kg morphine with saline, and 3.0 and 5.6 mg/kg LY235959. When administered with saline, 5.6 mg/kg morphine produced a slight learning deficit. When 3.0 mg/kg LY235959 was administered with 5.6 mg/kg there was no effect relative to saline. However, when 5.6 mg/kg LY235959, a dose that produced data similar to saline alone, was administered with 5.6 mg/kg morphine learning was

disrupted to a greater degree than when 5.6 mg/kg morphine was administered with saline. The bottom panel shows that the 10.0 mg/kg morphine and saline combination greatly disrupted learning relative to saline alone. Similarly, the administration of 3.0 mg/kg LY235959 and 10.0 morphine produced disruptions in the acquisition component, although, M7 did achieve 100% in the second bin of trials in this condition.

The top panel of figure 20 shows that rat M11 never reached the optimal level of accuracy in the saline only condition. However, when both LY235959 doses, 3.0 and 5.6 mg/kg were administered with saline rat M11 was able to attain 100% accuracy. The middle panel of figure X shows that the combination of saline and 3.0 mg/kg morphine produced data similar to that of saline alone. The combination of 3.0 mg/kg LY235959 and 3.0 mg/kg morphine produced data similar to that produced by 3.0 mg/kg LY235959 alone. However, the combination of 5.6 mg/kg LY235959 and 3.0 mg/kg morphine, two doses that alone, did not produce impairments of learning, disrupted both performance and acquisition in this rat. Similarly, 5.6 mg/kg morphine produced no acquisition impairment when administered with saline (bottom panel). However, when 5.6 mg/kg morphine was combined with the two ineffective doses of LY235959, there were disruptions in both components.

Figure 21 shows the drug combination data for rat O2. The top panel shows that two dose of LY235959, 1.7 mg/kg and 3.0 mg/kg, when administered with saline did not affect learning relative to the saline alone condition. The middle panel shows that 3.0 mg/kg morphine also did not disrupt acquisition when administered with saline. However, when this ineffective dose of morphine was administered with the two doses of LY235959, which also did not disrupt learning when administered with saline, there was

a delay in acquisition. The rat achieved 100% correct but not until the final bin of trials. The higher dose of morphine tested in this rat, 5.6 mg/kg, also produced not acquisition impairment. However, when administered with 1.7 mg/kg LY235959, 5.6 mg/kg morphine produced a selective effect on acquisition. In addition, when this dose of morphine was tested with the higher of the two LY235959 doses, 3.0 mg/kg, both performance and acquisition were impaired.

The group data showing the effects of the combinations of the doses that were common to all three rats reveal that two doses of morphine and LY235959 that had no effect alone were able to disrupt acquisition and performance when given in combination. In addition, upon looking at the individual subject data, one can see that the effects of other doses of the two drugs seem to be potentiated when given in combination as well.

DISCUSSION

The second experiment was an attempt to assess the effects of morphine and LY235959, alone and in combination, using a relatively novel technique. In an attempt to study a form of non-spatial learning in rats, an olfactory discrimination procedure was used. In this procedure rats were required to discriminate between two scents in order to gain access to a food reward. Within a session there were two discriminations. There was a performance discrimination where the stimuli remained the same, and maintained their S+/S- designations. There was also a reversal discrimination that was analogous to the acquisition component in the methodology of the first experiment. This allowed a differentiation between drug effects on olfactory learning, and nonspecific performance effects.

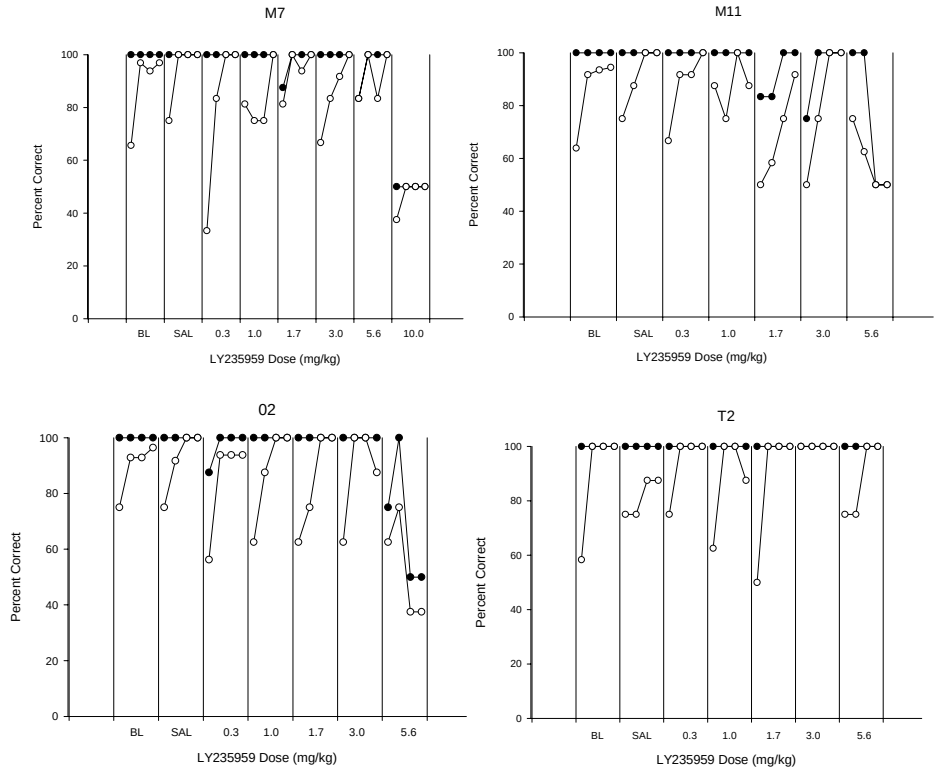

Figure 17. Individual subject mean percent correct plotted as a function of LY235959 dose on performance (closed circles) and repeated reversal learning/acquisition (open circles.

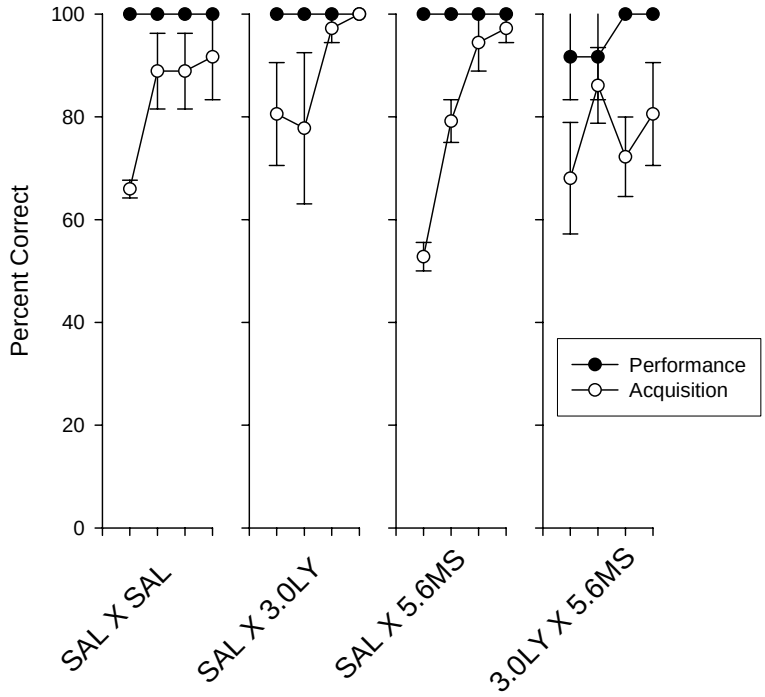

Figure 18. Mean percent correct on performance (closed circles) and repeated reversal learning/acquisition (open circles) for selected doses of morphine and LY235959 given in combination.

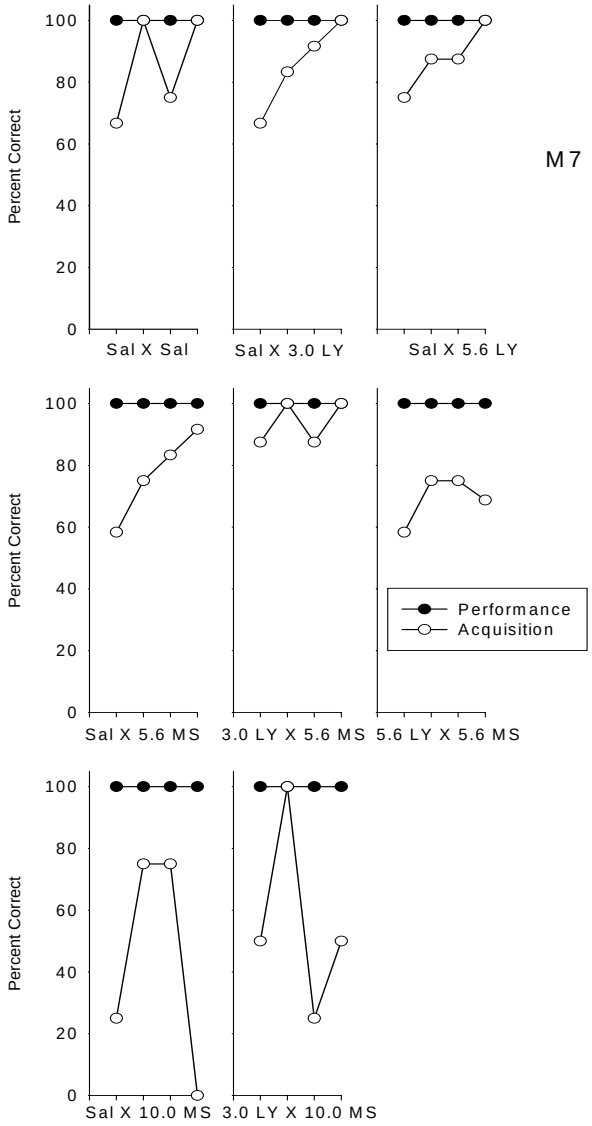

Figure 19. Mean percent correct for Rat M7 on performance (closed circles) and repeated reversal learning/acquisition (open circles) for selected doses of morphine and LY235959 given in combination.

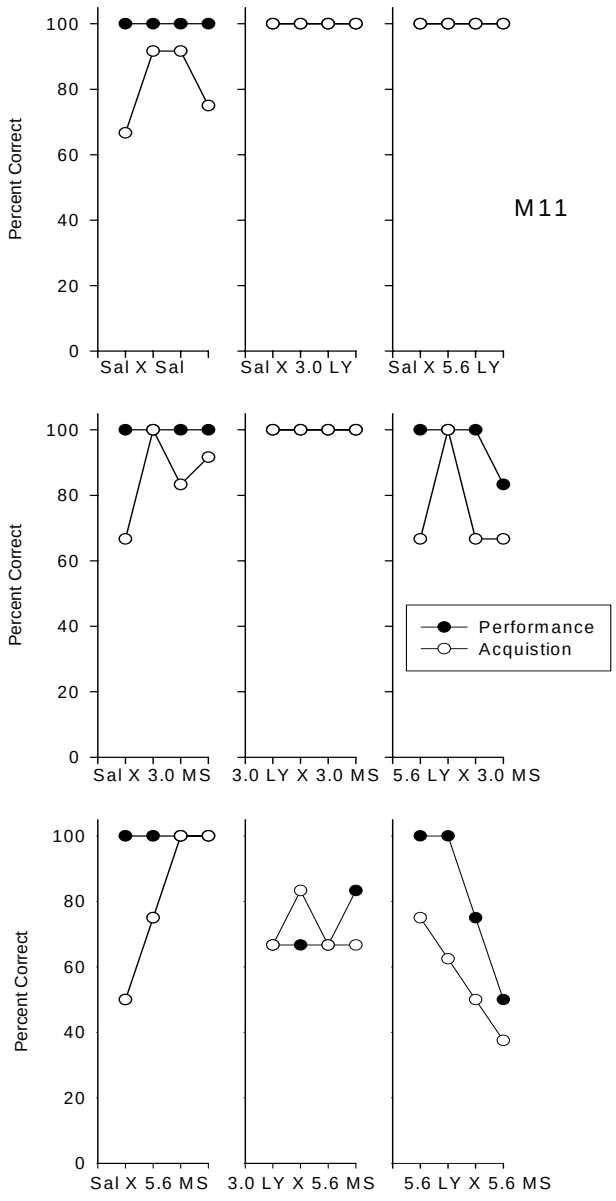

Figure 20. Mean percent correct for Rat M11 on performance (closed circles) and repeated reversal learning/acquisition (open circles) for selected doses of morphine and LY235959 given in combination.

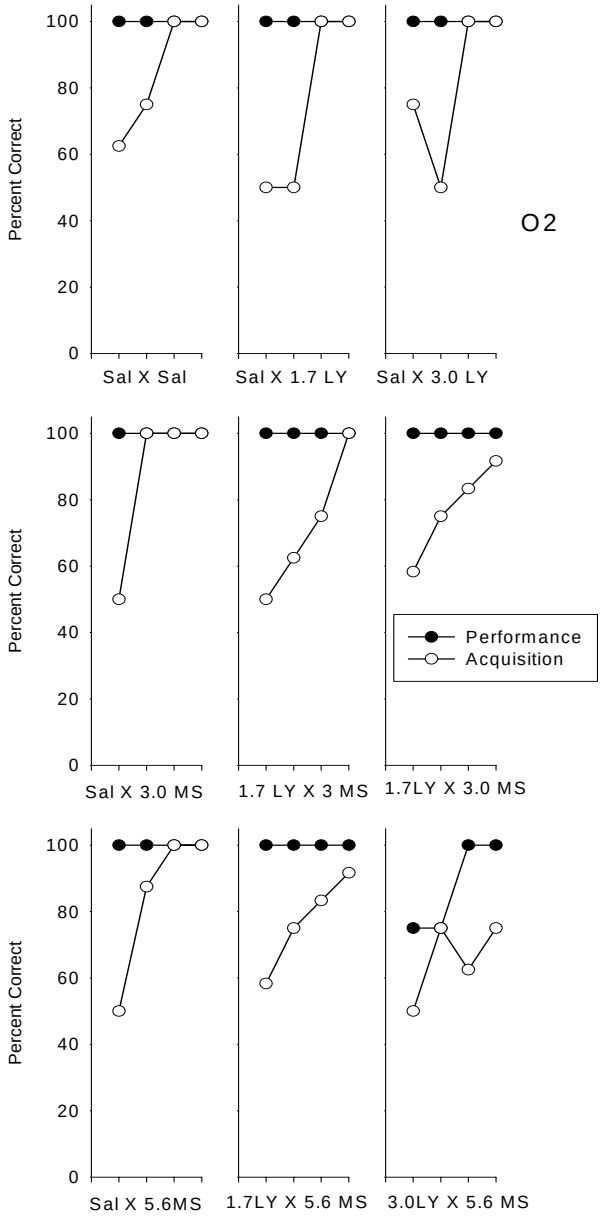

Figure 21. Mean percent correct for Rat O2 on performance (closed circles) and repeated reversal learning/acquisition (open circles) for selected doses of morphine and LY235959 given in combination.

Under control conditions in the reversal discrimination, rats were able to show rapid within-session learning. This was illustrated by near-chance accuracy in the first bin of trials, and then a dramatic improvement in the subsequent trials. By the second half of reversal trials near perfect accuracy was common. In the performance discrimination rats were able to maintain consistently high levels of accuracy from the beginning of the session.

Morphine produced dose dependent disruptions of accuracy in both components. The lowest dose of morphine (1.0 mg/kg) did not seem to have an effect in either component. Rats maintained their accuracy in the performance component, and exhibited learning curves similar to that of control conditions in the acquisition component. At the highest dose (17.0 mg/kg) there was clearly no evidence of learning within the session. In addition performance was severely disrupted, with accuracy not reaching above-chance levels.

Importantly, morphine selectively affected acquisition at the 10.0 mg/kg dose, and other doses seemed to have this sort of effect in certain rats. Even the lowest dose (1.0 mg/kg) produced a selective effect in one rat, M7 (although this effect was not present in some of the higher doses tested in this rat). In addition the 3.0 and 5.6 mg/kg doses produced selective effects on acquisition in four of the seven rats tested respectively.

The second phase of Experiment 2 studied the effects of the competitive NMDA antagonist LY235959 on olfactory learning. Visual inspection of the group data would suggest that LY235959 affected behavior in this procedure in a dose-dependent manner, although this effect was not sufficiently robust to produce statistical significance. The individual subject data reveals that, indeed, the drug did impair accuracy in most animals.

Unlike morphine, LY235959 did not consistently produce any selective effects on acquisition. There were doses that selectively impaired learning in individual rats, but these occurrences are rare and inconsistent to say the least.

The low dose of 0.3 mg/kg produced a clearly selective effect in one rat (M11), an arguably selective effect in one rat (M7), a non selective effect in one rat (02), and no effect in another (T2). The 1.0 mg/kg dose could be said to have produced the most compelling argument for a selective effect, although it would not be a strong argument. Learning was clearly impaired with rat M7, and it could be argued that it is impaired in rats M11 and T2 although these two rats did achieve high levels of accuracy in two of the three final bins of trials. The next three doses in the dose response curve (1.7, 3.0, and 5.6 mg/kg) inconsistently produced either no effect on both components, or a nonselective effect of both components. Finally the high dose (5.6 mg/kg) produced impairments of accuracy on the performance discrimination and the reversal discrimination in all but one rat. T2 was unaffected by 5.6 mg/kg LY235959.

The data from the first two phases of experiment 2 fall in line with the effects seen in Experiment 1 as well as previous research from this lab studying learning in rats. Unlike other work using non-human primates and pigeons, morphine selectively affected acquisition. On the other hand, the NMDA antagonist only affected acquisition at doses that also disrupted performance.

The final phase of this experiment assessed the effects of combinations of selected doses of morphine and LY235959. Three rats were selected for this phase of the study. The doses were selected on the basis of the individual dose response curves for each drug in addition to the effects of the doses of the drugs in combination with saline. The aim

was to select the highest dose of each drug that had no effect and the lowest dose that produced an acquisition effect.

After using the above method of selecting doses for this phase of the study, there was one dose of morphine (5.6 mg/kg) and a dose of LY235959 (3.0 mg/kg) that was common to all three rats. Surprisingly, while there was evidence of within session learning, when given the saline/saline combination, the level of accuracy reached on the reversal discrimination was not at the level that was reached in other phases. High accuracy was maintained across trials for the performance discrimination.
When the LY235959 dose (3.0 mg/kg) was given in combination with saline there was rapid within-session learning, and the rats reached levels of accuracy comparable to that of control conditions in the previous phases of the experiment. This was also the case for the morphine dose (5.6 mg/kg). When the dose of morphine was given in combination with the dose of LY235959 there was a very clear disruption of learning. The rats did show a slight improvement across trials, but showed clear impairments in accuracy, even with respect to the relatively limited acquisition observed in the control conditions of this phase. In addition, there was an obvious disruption of the performance discrimination when this combination was given. When one considers that neither drug dose alone produced effects on learning or performance, it is compelling that giving these doses in combination produced impairments on olfactory reversal learning and performance, suggesting the possibility of potentiation by the combination.

The individual data support the possible potentiation by the common doses, and also by other doses of the drugs. The two doses of LY235959 tested in rat M7, 3.0 and 5.6 mg/kg, had no effect, when given in combination with saline, relative to the

saline/saline combination. It did seem that the low dose of morphine had a small effect on acquisition when given in combination with saline. However, when this dose of morphine was given in combination with the ineffective high dose of LY235959, the rat never achieved the level of accuracy that was reached when either drug dose when given in combination with saline.

Rat M11 also showed no effect of the LY235959 doses (3.0 and 5.6 mg/kg) in combination with saline. In addition, neither morphine dose (3.0 and 5.6 mg/kg) had an effect when given in combination with saline. However, when the low dose of morphine was given in combination with the high dose of LY235959, both reversal discrimination learning and performance were disrupted. In addition, the high dose of morphine produced nonselective disruptions of behavior.

For rat O2, none of the doses of morphine (3.0 and 5.6 mg/kg), or LY235959 (1.7 and 3.0 mg/kg) produced major impairments when given in combination with saline. When the low dose of morphine was given in combination with the two doses of LY235959 there was a selective effect on acquisition. The high dose of morphine also produced selective effects on acquisition when given in combination with the low dose of LY235959. When this high dose of morphine was given in combination with the high dose of LY235959 performance and acquisition were disrupted.

GENERAL DISCUSSION

The present study was a systematic replication of previous work (Galizio, Keith, Mansfield, and Pitts 2003) that attempted to characterize the effects of morphine and the NMDA antagonist LY235959 on learning using a repeated acquisition and performance design. This study aimed to extend those findings in two ways. First, it included an

attempt to evaluate the extent to which the effects of these drugs generalized across learning tasks, and second, the study aimed to extend those findings to include the effects of combinations of morphine and LY235959.

Galizio et al. found that morphine selectively impaired acquisition at doses that did not disrupt performance. In contrast, LY235959 only impaired acquisition at doses that also disrupted performance. These results were in contrast to that of work that studied opiates and NMDA antagonists in different species and other types of learning tasks (Moerschbaecher and Thompson, 1983; Moerschbaecher, Thompson, and Winsauer 1985). This previous work showed selective effects of NMDA antagonists while morphine produced non-selective effects.

The first study produced data that was similar to previous work with spatial learning done with rats. Morphine was again found to selectively disrupt spatial learning at doses where nonspecific performance effects could be ruled out. In addition, LY235959 only affected acquisition at doses where performance deficits were present as well. These results directly replicated the results obtained by Galizio et al.

The olfactory discrimination procedure extended these findings. The selective effects of morphine on olfactory learning occurred only at relatively high doses and were not as consistent as the effects on spatial learning, but there were clearly doses that produced selective effects. In addition, there is little doubt that LY235959 affected reversal discrimination learning only at doses that also affected the rats' performance of a well-learned discrimination.

These data suggest that the differences in the literature in regard to the effects of morphine and NMDA antagonists might not be related to whether the learning task is

spatial vs. non-spatial or other obvious procedural differences. After all, the procedures used in the present studies differed in numerous ways. The most apparent divergence is the requirement of the animal to use spatial cues to navigate in the swim task versus the requirement of the animal to discriminate two cups of sand using olfactory stimuli. There are obviously further differences between the two procedures as well. In the swim task the animal must swim to a platform hidden below the surface of water, is removed from the experimental arena between trials, and the maintaining event, escape from the pool, involves a negative reinforcement contingency. On the other hand, in the olfactory procedure the animal must dig in sand to make a response, remains in the experimental chamber throughout the session, and the maintaining event, food pellet, involves a positive-reinforcement contingency. Thus, the selective effects of morphine, and relatively non-selective effects of LY235959 on learning, were replicated across a variety of behaviorally relevant conditions.

It is noteworthy that, as a whole, behavior in the swim task seems to be more sensitive to the effects of morphine than behavior in the olfactory discrimination. Morphine produced a performance disruption at the 10.0 mg/kg dose in the swim task. However, performance was not consistently affected until the 17.0 mg/kg dose in the olfactory procedure. This higher sensitivity in the swim task was less apparent with LY235959. LY235959 produced severe performance deficits at the 3.0 mg/kg dose in the swim task, but not until doses of 5.6 mg/kg were reached in the olfactory task. However, the 1.7 mg/kg dose seemed to affect acquisition slightly while leaving performance unaffected in the swim task. In the olfactory task this dose produced only slight effects on performance.

One possible explanation for the different results seen in the present study versus work where morphine has failed to produce non-selective effects on acquisition and NMDA antagonists have selectively affected acquisition is the species being studied. The two experiments conducted here utilized rats, as did the Gerak et al. study that found non-selective effects of ketamine. On the other hand much of the previous work has used non-human primates. Species differences in sensitivity to the effects of the drugs could account for some of the discrepancies in the results.

Another possibility takes us back to the procedures. While one of the goals of the present studies was to examine the extent to which the effects of these drugs might generalize across different types of tasks (spatial vs. non-spatial, etc.), there are still differences between the procedures used here and the operant procedures used in some of the other work. For instance, the water maze obviously requires the rat to move through space to locate the platform. Although the olfactory procedure does not require spatial learning, it does require the rat to move to a specific location in the chamber and dig in sand. Most operant procedures' response requirements involve the pressing of a key, plate, or some other operandum. It may be that the response modality that is required of the subject has an influence on drug effects.

Another issue to consider is the rate of acquisition for a given task. Many of the operant procedures that are used, and that have produced data that are in contrast to those of the present study involve relatively gradual acquisition. On the other hand, the two procedures used in this study produced rapid learning. Often rats reached asymptotic levels of learning after one trial. However, one has to consider the Gerak et al. study again. This study produced data that are similar to the present study: an effect of the

NMDA antagonist ketamine that was not specific to the acquisition component. However, the rats in this study showed very gradual learning. Another thing to consider about the Gerak et al. study, though, is the fact that at no point were the rats in this study as proficient as the rats in the present experiments.

The final phase of the two experiments involved testing morphine and LY235959 in combination in the two tasks. In both procedures, doses of the two drugs that produced no disruption of learning when given in combination with saline, did produce effects when given in combination. In addition, there were doses of each drug that when tested alone did not affect performance, but did so when given in combination. Some of the limitations of the study such as incomplete dose-effect curves and a lack of common dose combinations for all rats in each experiment did not allow for the most in depth drug interaction analyses available (see Tallarida, 2001). However, the fact that ineffective doses produced effects when combined suggests the possibility of potentiation. It seems that the degree of impairment produced by some of these dose combinations was greater than that that which would have been produced if the effects of the drugs simply additive. This apparent potentiation extends the findings of previous work on the effects of LY235959 (Allen et al., 2003) and other, clinically available, NMDA antagonists (Baker et al., 2002; Bulka et al., 2002).

The mechanism of the role that NMDA antagonists have in opiate analgesia, and whether this mechanism is related to the effects that combinations of the drugs have on learning and memory is unknown. In a review of the literature on NMDA antagonist/opiate combinations, Trujillo (2000) attempted to determine whether the effects were due to an inhibition of neural and behavioral plasticity or if other

explanations were viable. Indeed, the effects produced by these combinations do not appear to be due to drug side effects, a blockade of context-dependent tolerance, or state-dependency, and may be the result of the blockade of plasticity. If alternate explanations do not account for the combinations effects on analgesia, and it is neural and behavioral plasticity, it may be the case that the mechanisms of the effects are similar to those of the effects that we have seen on learning.

Comment [llm1]: Reference?

If these mechanisms are similar, it could have implications on the practicality of using NMDA antagonist/morphine combinations clinically in the treatment of pain. The present studies employed acute administration of the combinations, as have other studies that saw potentiation (Allen et al., 2003; Baker et al., 2002; Bulka et al., 2002) suggesting that using acutely administered combinations may produce unwanted cognitive effects. In addition, given that NMDA antagonists have been shown to attenuate the development of opiate tolerance when given chronically, further work on the cognitive effects produced by chronic administration of these combinations is needed.

Whatever the mechanism, NMDA antagonists seem to be capable of potentiating the effects of opiates, particularly morphine, one of our more commonly used analgesics. The results of the present study do no preclude the use of NMDA antagonist/opiate combinations clinically. It only emphasizes that there is much work to be done to extend the literature on the role of these combinations on the effects relevant to pain treatment, in addition to other endpoints such as reward, locomotion, and learning.

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APPENDICES

Appendix A. Morphine and DBL sessions for each rat in experiment 1.

Rat	Date	Drug	Dose
O1	5/6/2003	MS	0.0
O1	5/27/2003	MS	0.0
O1	3/7/2003	MS	1.0
O1	5/23/2003	MS	1.0
O1	3/25/2003	MS	3.0
O1	5/20/2003	MS	3.0
O1	5/30/2003	MS	3.0
O1	6/6/2003	MS	3.0
O1	3/28/2003	MS	5.6
O1	4/4/2003	MS	5.6
O1	5/16/2003	MS	5.6
O1	6/10/2003	MS	5.6
O1	5/2/2003	MS	10.0
O1	6/3/2003	MS	10.0
O1	6/13/2003	MS	10.0
O1	3/6/2003	MS	DBL
O1	3/27/2003	MS	DBL
O1	4/3/2003	MS	DBL
O1	5/1/2003	MS	DBL
O1	5/15/2003	MS	DBL
O1	5/22/2003	MS	DBL
O1	5/29/2003	MS	DBL
O1	6/5/2003	MS	DBL
O1	6/12/2003	MS	DBL
O21	7/23/2002	MS	0.0
O21	8/13/2002	MS	0.0
O21	7/26/2002	MS	1.0
O21	8/27/2002	MS	1.0
O21	9/6/2002	MS	1.0
O21	7/30/2002	MS	3.0
O21	8/16/2002	MS	3.0
O21	8/23/2002	MS	3.0
O21	8/6/2002	MS	5.6
O21	8/30/2002	MS	5.6
O21	9/10/2002	MS	5.6
O21	8/9/2002	MS	10.0
O21	9/3/2002	MS	10.0
O21	7/25/2002	MS	DBL
O21	8/8/2002	MS	DBL
O21	8/15/2002	MS	DBL
O21	8/22/2002	MS	DBL
O21	8/29/2002	MS	DBL
O21	9/5/2002	MS	DBL

O22	9/27/2002	MS	0.0
O22	10/29/2002	MS	0.0
O22	11/12/2002	MS	0.0
O22	9/17/2002	MS	1.0
O22	10/18/2002	MS	1.0
O22	11/1/2002	MS	1.0
O22	9/20/2002	MS	3.0
O22	10/15/2002	MS	3.0
O22	11/5/2002	MS	3.0
O22	9/24/2002	MS	5.6
O22	10/22/2002	MS	5.6
O22	11/8/2002	MS	5.6
O22	10/2/2002	MS	10.0
O22	10/25/2002	MS	10.0
O22	10/4/2002	MS	17.0
O22	9/26/2002	MS	DBL
O22	10/3/2002	MS	DBL
O22	10/10/2002	MS	DBL
O22	10/17/2002	MS	DBL
O22	10/21/2002	MS	DBL
O22	10/31/2002	MS	DBL
O22	11/7/2002	MS	DBL
T1	4/29/2003	MS	0.0
T1	2/27/2003	MS	0.0
T1	5/2/2003	MS	1.0
T1	5/23/2003	MS	1.0
T1	5/20/2003	MS	3.0
T1	5/30/2003	MS	3.0
T1	6/6/2003	MS	3.0
T1	5/6/2003	MS	5.6
T1	6/13/2003	MS	5.6
T1	6/3/2003	MS	10.0
T1	5/1/2003	MS	DBL
T1	5/22/2003	MS	DBL
T1	5/29/2003	MS	DBL
T1	6/5/2003	MS	DBL
T1	6/12/2003	MS	DBL
K5	11/16/2001	MS	0.0
K5	11/30/2001	MS	0.0
K5	1/25/2002	MS	0.0
K5	11/23/2001	MS	1.0
K5	1/1/2002	MS	1.0
K5	1/18/2002	MS	1.0
K5	11/27/2001	MS	3.0
K5	1/4/2002	MS	3.0
K5	1/22/2002	MS	3.0
K5	12/21/2001	MS	5.6
K5	1/8/2002	MS	5.6

K5	1/15/2002	MS	5.6
K5	12/14/2001	MS	10.0
K5	12/18/2001	MS	10.0
K5	12/28/2001	MS	10.0
K5	1/11/2002	MS	17.0
K5	11/20/2001	MS	30.0
K5	10/25/2001	MS	DBL
K5	11/1/2001	MS	DBL
K5	11/8/2001	MS	DBL
K5	11/15/2001	MS	DBL
K5	11/22/2001	MS	DBL
K5	11/29/2001	MS	DBL
K5	12/6/2001	MS	DBL
K5	12/20/2001	MS	DBL
K5	1/3/2002	MS	DBL
K5	1/10/2002	MS	DBL
K5	1/17/2002	MS	DBL
K5	1/24/2002	MS	DBL

Appendix B. LY235959 and DBL sessions for each rat in experiment 1.

Rat	Date	Drug	Dose
T1	5/27/2003	LY	0.0
T1	10/7/2003	LY	0.0
T1	9/9/2003	LY	0.3
T1	9/30/2003	LY	0.3
T1	9/5/2003	LY	1.0
T1	9/23/2003	LY	1.0
T1	9/16/2004	LY	1.7
T1	9/26/2003	LY	1.7
T1	10/3/2003	LY	3.0
T1	10/10/2003	LY	3.0
T1	9/4/2003	LY	DBL
T1	9/25/2003	LY	DBL
T1	10/2/2003	LY	DBL
T1	10/9/2003	LY	DBL
O22	7/23/2002	LY	0.0
O22	8/13/2002	LY	0.0
O22	7/26/2002	LY	0.3
O22	8/6/2002	LY	0.3
O22	8/9/2002	LY	0.3
O22	7/30/2002	LY	1.0
O22	8/20/2002	LY	1.0
O22	8/2/2002	LY	1.7
O22	8/23/2002	LY	1.7
O22	9/6/2002	LY	1.7
O22	8/16/2002	LY	3.0
O22	8/27/2002	LY	3.0
O22	9/3/2002	LY	3.0
O22	9/10/2002	LY	3.0
O22	8/30/2002	LY	5.6
O22	7/25/2002	LY	DBL
O22	8/1/2002	LY	DBL
O22	8/8/2002	LY	DBL
O22	8/15/2002	LY	DBL
O22	8/22/2002	LY	DBL
O22	8/29/2002	LY	DBL
O22	9/5/2002	LY	DBL
R6	2/24/2003	LY	0.0
R6	3/30/2003	LY	0.0
R6	4/13/2003	LY	0.0
R6	3/12/2003	LY	0.3
R6	4/2/2003	LY	0.3
R6	4/6/2003	LY	1.0
R6	4/6/2003	LY	1.0
R6	3/23/2003	LY	1.7

R6	4/9/2003	LY	1.7
R6	3/26/2003	LY	3.0
R6	3/11/2003	LY	DBL
R6	3/18/2003	LY	DBL
R6	3/25/2003	LY	DBL
R6	4/1/2003	LY	DBL
R6	4/8/2003	LY	DBL
N24	2/5/2002	LY	0.0
N24	2/8/2002	LY	0.0
N24	2/19/2002	LY	0.3
N24	3/19/2002	LY	0.3
N24	2/15/2002	LY	1.0
N24	3/29/2002	LY	1.0
N24	4/5/2002	LY	1.0
N24	2/22/2002	LY	1.7
N24	3/22/2002	LY	1.7
N24	4/9/2002	LY	1.7
N24	2/26/2002	LY	3.0
N24	3/26/2002	LY	3.0
N24	2/7/2002	LY	DBL
N24	2/14/2002	LY	DBL
N24	2/21/2002	LY	DBL
N24	3/21/2002	LY	DBL
N24	3/28/2002	LY	DBL
N24	4/4/2002	LY	DBL
O1	9/24/2002	LY	0.0
O1	10/25/2002	LY	0.0
O1	11/15/2002	LY	0.0
O1	10/1/2002	LY	0.3
O1	10/15/2002	LY	0.3
O1	11/19/2002	LY	0.3
O1	11/22/2002	LY	0.3
O1	10/4/2002	LY	1.0
O1	10/18/2002	LY	1.0
O1	10/8/2002	LY	1.7
O1	10/22/2002	LY	1.7
O1	12/3/2002	LY	1.7
O1	10/11/2002	LY	3.0
O1	10/29/2002	LY	3.0
O1	11/29/2002	LY	3.0
O1	12/6/2002	LY	3.0
O1	11/26/2002	LY	5.6
O1	10/3/2002	LY	DBL
O1	10/10/2002	LY	DBL
O1	10/17/2002	LY	DBL
O1	10/24/2002	LY	DBL
O1	11/14/2002	LY	DBL

O1	11/21/2002	LY	DBL
O1	11/28/2002	LY	DBL
O1	12/5/2002	LY	DBL

Appendix C. Morphine and LY235959 combination and DBL sessions for each rat in experiment 1.

Rat	Date	Drug	Dose
O1	9/19/2003	COMBO	SALXSAL
O1	12/2/2004	COMBO	SALXSAL
O1	10/3/2003	COMBO	1.0LYXSAL
O1	11/14/2003	COMBO	1.0LYXSAL
O1	10/7/2003	COMBO	1.7LYXSAL
O1	11/18/2003	COMBO	1.7LYXSAL
O1	10/14/2003	COMBO	SALX1.0MO
O1	11/25/2003	COMBO	SALX1.0MO
O1	10/17/2003	COMBO	1.0LYX1.0MO
O1	11/21/2003	COMBO	1.0LYX1.0MO
O1	9/26/2003	COMBO	1.7LYX1.0MO
O1	11/7/2003	COMBO	1.7LYX1.0MO
O1	10/21/2003	COMBO	SALX3.0MO
O1	11/11/2003	COMBO	SALX3.0MO
O1	9/23/2003	COMBO	1.0LYX3.0MO
O1	10/28/2003	COMBO	1.7LYX3.0MO
O1	11/4/2003	COMBO	1.7LYX3.0MO
O1	11/28/2003	COMBO	1.7LYX3.0MO
O1	9/18/2003	COMBO	DBL
O1	9/25/2003	COMBO	DBL
O1	10/2/2003	COMBO	DBL
O1	10/16/2003	COMBO	DBL
O1	11/6/2003	COMBO	DBL
O1	11/13/2003	COMBO	DBL
O1	11/20/2003	COMBO	DBL
O1	11/27/2003	COMBO	DBL
T1	10/14/2003	COMBO	SALXSAL
T1	12/2/2003	COMBO	SALXSAL
T1	11/14/2003	COMBO	1.0LYXSAL
T1	12/5/2003	COMBO	1.0LYXSAL
T1	11/18/2003	COMBO	1.7LYXSAL
T1	12/9/2003	COMBO	1.7LYXSAL
T1	2/10/2003	COMBO	1.7LYXSAL
T1	10/24/2003	COMBO	SALX1.0MO
T1	11/25/2003	COMBO	SALX1.0MO
T1	12/30/2003	COMBO	SALX1.0MO
T1	10/17/2003	COMBO	1.0LYX1.0MO
T1	11/21/2003	COMBO	1.0LYX1.0MO
T1	2/3/2003	COMBO	1.0LYX1.0MO
T1	11/4/2003	COMBO	1.7LYX1.0MO
T1	12/23/2003	COMBO	1.7LYX1.0MO
T1	1/30/2003	COMBO	1.7LYX1.0MO

T1	2/6/2003	COMBO	1.7LYX1.0MO
T1	11/11/2003	COMBO	SALX3.0MO
T1	12/12/2003	COMBO	SALX3.0MO
T1	10/28/2003	COMBO	1.0LYX3.0MO
T1	12/19/2003	COMBO	1.0LYX3.0MO
T1	2/13/2003	COMBO	1.0LYX3.0MO
T1	10/21/2003	COMBO	1.7LYX3.0MO
T1	11/28/2003	COMBO	1.7LYX3.0MO
T1	10/16/2003	COMBO	DBL
T1	10/23/2003	COMBO	DBL
T1	11/13/2003	COMBO	DBL
T1	11/20/2003	COMBO	DBL
T1	11/27/2003	COMBO	DBL
T1	12/4/2003	COMBO	DBL
T1	12/11/2003	COMBO	DBL
T1	12/18/2003	COMBO	DBL
T1	1/29/2003	COMBO	DBL
T1	2/5/2003	COMBO	DBL
T1	2/12/2003	COMBO	DBL
R6	5/21/2003	COMBO	SALXSAL
R6	4/20/2003	COMBO	SALX3.0MO
R6	4/23/2003	COMBO	SALX5.6MO
R6	5/14/2003	COMBO	1.0LYX5.6MO
R6	5/11/2003	COMBO	1.7LYX5.6MO
R6	5/7/2003	COMBO	SALX10.0MO
R6	4/30/2003	COMBO	1.0LYX10.0MO
R6	5/18/2003	COMBO	1.7LYX10.0MO
R6	4/22/2003	COMBO	DBL
R6	4/29/2003	COMBO	DBL
R6	5/6/2003	COMBO	DBL
R6	5/13/2003	COMBO	DBL

Appendix D. Morphine and DBL sessions for each rat in experiment 2.

Rat	Date	Drug	Dose
M7	6/25/2002	MS	0.0
M7	7/5/2002	MS	0.0
M7	7/23/2002	MS	0.0
M7	6/14/2002	MS	1.0
M7	6/28/2002	MS	1.0
M7	8/27/2002	MS	1.0
M7	8/30/2002	MS	1.0
M7	6/21/2002	MS	3.0
M7	7/19/2002	MS	3.0
M7	8/16/2002	MS	3.0
M7	6/18/2002	MS	5.6
M7	7/26/2002	MS	5.6
M7	8/9/2002	MS	5.6
M7	7/2/2002	MS	10.0
M7	7/16/2002	MS	10.0
M7	8/20/2002	MS	10.0
M7	7/12/2002	MS	17.0
M7	7/30/2002	MS	17.0
M7	6/13/2002	MS	DBL
M7	6/20/2002	MS	DBL
M7	6/27/2002	MS	DBL
M7	7/4/2002	MS	DBL
M7	7/11/2002	MS	DBL
M7	7/18/2002	MS	DBL
M7	7/25/2002	MS	DBL
M7	8/8/2002	MS	DBL
M7	8/15/2002	MS	DBL
M7	8/29/2002	MS	DBL
M11	10/4/2002	MS	0.0
M11	11/26/2002	MS	0.0
M11	11/1/2002	MS	1.0
M11	11/19/2002	MS	1.0
M11	10/15/2002	MS	3.0
M11	11/5/2002	MS	3.0
M11	11/29/2002	MS	3.0
M11	12/17/2002	MS	3.0
M11	10/22/2002	MS	5.6
M11	11/8/2002	MS	5.6
M11	11/15/2002	MS	5.6
M11	12/6/2002	MS	5.6
M11	12/13/2002	MS	5.6
M11	10/25/2002	MS	10.0
M11	11/12/2002	MS	10.0

M11	12/3/2002	MS	10.0
M11	12/20/2002	MS	10.0
M11	11/22/2002	MS	17.0
M11	10/3/2002	MS	DBL
M11	10/10/2002	MS	DBL
M11	10/24/2002	MS	DBL
M11	10/31/2002	MS	DBL
M11	11/7/2002	MS	DBL
M11	11/14/2002	MS	DBL
M11	11/21/2002	MS	DBL
M11	11/28/2002	MS	DBL
M11	12/5/2002	MS	DBL
M11	12/12/2002	MS	DBL
M11	12/19/2002	MS	DBL
O2	9/12/2003	MS	0.0
O2	10/14/2003	MS	0.0
O2	9/16/2003	MS	1.0
O2	10/10/2003	MS	1.0
O2	9/23/2003	MS	3.0
O2	10/7/2003	MS	3.0
O2	9/26/2003	MS	5.6
O2	10/3/2003	MS	5.6
O2	9/30/2003	MS	10.0
O2	10/2/2003	MS	10.0
O2	10/17/2003	MS	17.0
O2	9/11/2003	MS	DBL
O2	9/25/2003	MS	DBL
O2	10/2/2003	MS	DBL
O2	10/9/2003	MS	DBL
O2	10/16/2003	MS	DBL
N22	3/12/2002	MS	0.0
N22	3/15/2002	MS	0.0
N22	3/19/2002	MS	1.0
N22		MS	1.0
N22	3/22/2002	MS	3.0
N22	4/16/2002	MS	3.0
N22	3/26/2002	MS	5.6
N22		MS	5.6
N22	3/29/2002	MS	10.0
N22	4/2/2002	MS	10.0
N22		MS	17.0
N22	3/21/2002	MS	DBL
N22	3/28/2002	MS	DBL
N22	4/4/2002	MS	DBL

Appendix E. LY235959 and DBL sessions for each rat in experiment 2.

Rat	Date	Drug	Dose (mg/kg)
M7	8/14/2002	LY	0.0
M7	9/10/2002	LY	0.0
M7	9/13/2002	LY	0.3
M7	10/15/2002	LY	0.3
M7	10/29/2002	LY	0.3
M7	9/17/2002	LY	1.0
M7	10/18/2002	LY	1.0
M7	11/12/2002	LY	1.0
M7	12/13/2002	LY	1.0
M7	10/8/2002	LY	1.7
M7	10/22/2002	LY	1.7
M7	12/3/2002	LY	1.7
M7	12/17/2002	LY	1.7
M7	10/4/2002	LY	3.0
M7	10/11/2002	LY	3.0
M7	12/2/2002	LY	3.0
M7	10/25/2002	LY	5.6
M7	11/5/2002	LY	5.6
M7	12/24/2002	LY	5.6
M7	11/8/2002	LY	10.0
M7	11/26/2002	LY	10.0
M7	9/12/2002	LY	DBL
M7	10/3/2002	LY	DBL
M7	10/10/2002	LY	DBL
M7	10/17/2002	LY	DBL
M7	10/24/2002	LY	DBL
M7	11/7/2002	LY	DBL
M7	12/12/2002	LY	DBL
M7	12/19/2002	LY	DBL
M11	7/23/2002	LY	0.0
M11	8/13/2002	LY	0.0
M11	7/26/2002	LY	0.3
M11	8/6/2002	LY	0.3
M11	8/9/2002	LY	0.3
M11	7/30/2002	LY	1.0
M11	8/30/2002	LY	1.0
M11	8/2/2002	LY	1.7
M11	8/23/2002	LY	1.7
M11	9/6/2002	LY	1.7
M11	8/16/2002	LY	3.0
M11	9/10/2002	LY	3.0
M11	9/13/2002	LY	5.6
M11	9/20/2002	LY	5.6

M11	7/25/2002	LY	DBL
M11	8/1/2002	LY	DBL
M11	8/8/2002	LY	DBL
M11	8/15/2002	LY	DBL
M11	8/22/2002	LY	DBL
M11	8/29/2002	LY	DBL
M11	9/5/2002	LY	DBL
M11	9/12/2002	LY	DBL
M11	9/19/2002	LY	DBL
O2	9/27/2002	LY	0.0
O2	11/15/2002	LY	0.0
O2	12/6/2002	LY	0.0
O2	10/1/2002	LY	0.3
O2	10/15/2002	LY	0.3
O2	11/19/2002	LY	0.3
O2	11/22/2002	LY	0.3
O2	10/4/2002	LY	1.0
O2	12/3/2002	LY	1.0
O2	10/8/2002	LY	1.7
O2	10/22/2002	LY	1.7
O2	10/11/2002	LY	3.0
O2	10/29/2002	LY	3.0
O2	10/25/2002	LY	5.6
O2	11/26/2002	LY	5.6
O2	9/26/2002	LY	DBL
O2	10/3/2002	LY	DBL
O2	10/10/2002	LY	DBL
O2	10/24/2002	LY	DBL
O2	11/14/2002	LY	DBL
O2	11/21/2002	LY	DBL
O2	12/5/2002	LY	DBL
T2	7/11/2003	LY	0.0
T2	8/26/2003	LY	0.0
T2	7/18/2003	LY	0.3
T2	9/9/2003	LY	0.3
T2	7/22/2003	LY	1.0
T2	8/5/2003	LY	1.0
T2	7/25/2003	LY	1.7
T2	7/15/2003	LY	3.0
T2	7/29/2003	LY	5.6
T2	7/10/2003	LY	DBL
T2	7/17/2003	LY	DBL
T2	7/24/2003	LY	DBL
T2	7/31/2003	LY	DBL
T2	8/14/2003	LY	DBL
T2	8/21/2003	LY	DBL
T2	9/4/2003	LY	DBL

Appendix F. Morphine and LY combination and DBL sessions for each rat in experiment 2.

Rat	Date	Drug	Dose
M7	3/11/2003	COMBO	SALXSAL
M7	3/18/2003	COMBO	SALXSAL
M7	3/7/2003	COMBO	5.6LYX3.0MO
M7	3/4/2003	COMBO	SALX5.6MO
M7	3/25/2003	COMBO	SALX5.6MO
M7	4/18/2003	COMBO	SALX5.6MO
M7	2/4/2003	COMBO	3.0LYX5.6MO
M7	4/4/2003	COMBO	3.0LYX5.6MO
M7	2/14/2003	COMBO	5.6LYX5.6MO
M7	3/21/2003	COMBO	5.6LYX5.6MO
M7	4/11/2003	COMBO	5.6LYX5.6MO
M7	4/22/2003	COMBO	5.6LYX5.6MO
M7	2/28/2003	COMBO	SALX10.0MO
M7	2/7/2003	COMBO	3.0LYX10.0MO
M7	3/28/2003	COMBO	5.6LYXSAL
M7	4/15/2003	COMBO	5.6LYXSAL
M7	2/6/2003	COMBO	DBL
M7	2/13/2003	COMBO	DBL
M7	2/27/2003	COMBO	DBL
M7	3/6/2003	COMBO	DBL
M7	3/20/2003	COMBO	DBL
M7	3/27/2003	COMBO	DBL
M7	4/3/2003	COMBO	DBL
M7	4/10/2003	COMBO	DBL
M7	4/17/2003	COMBO	DBL
O2	10/24/2003	COMBO	SALXSAL
O2	12/2/2003	COMBO	SALXSAL
O2	11/7/2003	COMBO	1.7LYX1.0MO
O2	11/11/2003	COMBO	SALX3.0MO
O2	10/28/2003	COMBO	1.0LYX3.0MO
O2	12/19/2003	COMBO	1.0LYX3.0MO
O2	11/4/2003	COMBO	1.7LYX3.0MO
O2	12/16/2003	COMBO	1.7LYX3.0MO
O2	12/12/2003	COMBO	3.0LYX3.0MO
O2	1/13/2004	COMBO	3.0LYX3.0MO
O2	11/25/2003	COMBO	SALX5.6MO
O2	12/30/2003	COMBO	SALX5.6MO
O2	11/28/2003	COMBO	1.7LYX5.6MO
O2	12/23/2003	COMBO	1.7LYX5.6MO
O2	1/30/2003	COMBO	1.7LYX5.6MO
O2	12/5/2003	COMBO	3.0LYX5.6MO
O2	1/9/2003	COMBO	3.0LYX5.6MO

O2	11/14/2003	COMBO	1.0LYXSAL
O2	11/18/2003	COMBO	1.7LYXSAL
O2	12/9/2003	COMBO	3.0LYXSAL
O2	10/23/2003	COMBO	DBL
O2	11/6/2003	COMBO	DBL
O2	11/13/2003	COMBO	DBL
O2	11/20/2003	COMBO	DBL
O2	11/27/2003	COMBO	DBL
O2	12/4/2003	COMBO	DBL
O2	12/11/2003	COMBO	DBL
O2	12/18/2003	COMBO	DBL
O2	1/8/2004	COMBO	DBL
O2	1/29/2003	COMBO	DBL
M11	1/10/2003	COMBO	SALXSAL
M11	1/14/2003	COMBO	SALXSAL
M11	3/18/2003	COMBO	SALXSAL
M11	3/25/2003	COMBO	SALX3.0MO
M11	4/1/2003	COMBO	SALX3.0MO
M11	4/29/2003	COMBO	SALX3.0MO
M11	4/8/2003	COMBO	3.0LYX3.0MO
M11	3/11/2003	COMBO	5.6LYX3.0MO
M11	4/15/2003	COMBO	5.6LYX3.0MO
M11	4/25/2003	COMBO	5.6LYX3.0MO
M11	3/4/2003	COMBO	SALX5.6MO
M11	3/28/2003	COMBO	SALX5.6MO
M11	2/4/2003	COMBO	3.0LYX5.6MO
M11	3/21/2003	COMBO	3.0LYX5.6MO
M11	4/4/2003	COMBO	3.0LYX5.6MO
M11	2/14/2003	COMBO	5.6LYX5.6MO
M11	4/11/2003	COMBO	5.6LYX5.6MO
M11	2/28/2003	COMBO	SALX10.0MO
M11	2/11/2003	COMBO	3.0LYX10.0MO
M11	5/2/2003	COMBO	3.0LYXSAL
M11	3/7/2003	COMBO	5.6LYXSAL
M11	1/9/2003	COMBO	DBL
M11	2/13/2003	COMBO	DBL
M11	2/27/2003	COMBO	DBL
M11	3/6/2003	COMBO	DBL
M11	3/20/2003	COMBO	DBL
M11	3/27/2003	COMBO	DBL
M11	4/3/2003	COMBO	DBL
M11	4/10/2003	COMBO	DBL
M11	4/24/2003	COMBO	DBL
M11	5/1/2003	COMBO	DBL