EMOTIONAL MODULATION OF HIPPOCAMPUS-DEPENDENT SPATIAL LEARNING

A Thesis

by

AUDREA ELIZABETH ELLIOTT

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2005

Major Subject: Psychology

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ABSTRACT

Emotional Modulation of Hippocampus-Dependent Spatial Learning.

(August 2005)

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Previous research has indicated that the amygdala exerts a modulatory influence on multiple memory systems. Evidence also indicates that emotional state can influence the use of multiple memory systems and that this effect is mediated by the amygdala. Anxiogenic drugs administered during acquisition in a task that can be acquired either through hippocampus-dependent "place" learning or caudate dependent "response" learning, resulted in the predominant use of response learning. It is not known whether inducing anxiety at other behavioral time points will also influence the relative use of multiple memory systems. In experiment 1, male Long-Evans rats were trained to swim from the same start point to an escape platform constantly located in a goal arm. Prior to memory retrieval rats were administered either alpha- two adrenoceptor antagonist RS 79948-197, peripherally (0.03, 0.01, 0.3 mg/kg) or into the basolateral amygdala (0.1 μg), or saline vehicle. Rats treated with RS 79948-197 prior to memory retrieval exhibited caudate-dependent response learning.

Previous studies examining the effects of RS 77948-197 on memory were conducted with rats trained in an anxiogenic state and subsequently probed in a drug free state. Experiment 2 examined whether state dependency may account for those results. Animals received peripheral (0.1 mg/kg) or intra-amygdala (0.1 μg) administration of RS

79948-197, prior to *both* acquisition and memory retrieval. Rats treated with RS 79948-197 predominantly exhibited response learning.

Finally, experiments 3 and 4 examined whether the use of response learning produced by RS79948-197 was due to the impairing effect on hippocampus-dependent memory. Rats that were administered peripheral (0.03 mg/kg) or intra-amygdala (0.1 μg) injections of RS 79948-197 displayed impaired acquisition of the single solution place task relative to control animals. This indicates that place learning was impaired. Over, all the present findings indicate 1) peripheral and intra-amygdala anxiogenic drug administration results in the use of habit memory at both acquisition *and* retrieval, 2) state dependency does not play a role in the influence of RS 799948-197 on memory system use, 3) the use of response learning produced by peripheral and intra-amygdala injections of RS 79948-197 may result from an impairing effect of hippocampus-dependent memory.

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INTRODUCTION

Evidence for Multiple Memory Systems

An extensive body of evidence indicates that learning in mammals occurs through multiple memory systems (O'Keefe & Nadel, 1978; Packard, Hirsch, & White, 1989; Squire, 1992; Mishkin & Perti, 1984; Eichenbaum & Cohen, 2001). Early interest in the idea of separate memory systems stemmed from examination of a patient who had received a temporal lobe removal to treat epilepsy (Milner, 1965; Scoville & Milner, 1957; Squire, 1992). As a result of this surgery severe anterograde amnesia for hippocampus dependent explicit memories was displayed, while other stimulus-response types of memories were left intact. Studies of learning and memory in rats have confirmed the important role of the hippocampus in establishing the relationships between stimuli that make up representations of space or context (Morris, Garrud, Rawlins, & O'Keefe, 1982; O'Keefe & Nadel, 1978; Hirsh, 1974).

Studies in several mammalian species have shown that if the hippocampal system is damaged then the organism is impaired in "cognitive" or "relational" tasks. These "cognitive" tasks are ones that the animal must learn a spatial relationship among multiple stimuli and this relationship can be used to guide goal- directed behavior. In contrast, stimulus-response learning is normal following hippocampal damage (Packard et al 1989, Packard, 1999; Schroeder, Wingard, & Packard; 2002). Following decades

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This thesis follows the style of *Neurobiology of Learning and Memory.*

of the study of hippocampus dependent learning, researchers started to focus on identifying neural structures that mediate stimulus-response learning (Packard et al., 1989; Packard & McGaugh, 1992). Numerous studies indicate that damage to the caudate nucleus impairs stimulus-response learning, but "cognitive" memory is unaffected. Evidence has shown that basal ganglia has receives input from almost every region of the cerebral cortex and theses pathways are topographically organized (McGeorge & Faull, 1989; Veening, Cornelissen, & Lieven, 1980). The mammalian dorsal striatum (caudate nucleus and putamen) is the area that is often studied in multiple memory systems. This area receives projections from the sensory and motor regions of the cerebral cortex (McGeorge & Faull, 1989), which would indicate a role in stimulus-response learning. The stimulus-response function is based on cortical projections that are also topographically organized (Viaud & White, 1989). Irreversible and reversible lesion techniques (e.g. McDonald & White, 1994; Packard, Hirsh & White, 1989; Packard & McGaugh, 1992), along with intracerebral post-training drug treatments (e.g. Packard & Teather, 1997; Packard & White, 1991; Schroeder, et al., 2002) have been used to demonstrate double dissociations between the mnemonic functions of the hippocampus & the caudate nucleus.

Rats with lesions of the fimbria-fornix (one of the major efferent and afferent pathways of the hippocampus), caudate nucleus, or control animals were trained in one of two versions of the eight arm radial maze task. The win-shift version consists of each of the eight arms being baited once (Olton, & Samuleson, 1976). Records were kept of the arms entered and entry to a previously visited arm was marked as an error. This task is

considered to be a cognitive task because the animals must remember which arms they had previously visited. Animals with fimbria-fornix lesions had impaired acquisition compared to control animals, whereas animals with caudate lesions acquired the task normally (Packard et al., 1989). The win-stay version of this task consists of four arms that are cued with a light. Each arm was baited twice in a trial and after the food was removed for the second time the light was extinguished. Records were kept of the arms entered and visits to unlit arms were scored as errors. This task is considered to be a stimulus-response task because animals must learn to approach a light for food reward. Animals with fimbria-fornix lesions displayed enhanced acquisition in the win-stay task compared to control animals. Animals with caudate nucleus lesion showed impaired acquisition compared to control animals.

Further evidence of multiple memory systems was obtained when rats were trained in one of two water maze tasks (Packard & McGaugh, 1992). In both tasks, two rubber balls with unique visual patterns were used as platforms or cues. One ball was connected to a stable platform, while the other one was connected to an unstable platform. In the spatial version of this task, the stable platform was located in the same spatial location for all trials, but the balls changed from trial to trial. Lesions of the fimbria-fornix, but not the caudate nucleus impaired acquisition compared to saline controls (Packard & McGaugh, 1992). In the stimulus-response version of the task, the stable platform was moved around the water maze, but the ball attached was always the same. Lesions of the caudate nucleus, but not the fimbria-fornix impaired acquisition compared to saline controls (Packard & McGaugh, 1992). These studies provide further evidence of the existence of multiple memory systems.

Another example of a task that has been used to dissociate the hippocampal and caudate memory function is the *dual-solution* plus maze task. This task is capable of being learned through both hippocampus and the caudate (Packard & McGaugh, 1996; Packard & Wingard, 2004). In the appetitive version of this task, animals start from the same start point (e.g. north) and are trained to go to the same goal arm (e.g. west). Early in training, when probed from a novel start point (e.g. south), intact rats predominantly went to the spatial location or "place" that food reward had been located during training. Rats were then given additional training trials and again received a probe trial. After extended training, intact rats predominantly made the body turn response that was reinforced during training. Neural inactivation of the hippocampus during the probe trials blocked place learning, while inactivation of the caudate nucleus during the probe trials blocked response learning (Packard & McGaugh, 1996).

A further example is provided by two additional tasks that have been used to demonstrate a dissociation between these two memory systems in the water maze. The two tasks force the animal to use either "place" or "response" learning (Schroeder, Wingard, & Packard; 2002). In the place task animals are trained from two start points, 180 degrees from each other (e.g. north & south). Rats must then swim to a hidden platform that is located in a constant goal arm of the maze (e.g. west). In the response task rats are also trained from two start points, 180 degrees from each other, but rats must always make the same body turn when leaving the start arm (e.g. turn right) to locate a hidden platform. These tasks can be referred to as *single-solution* tasks, since evidence indicates they can only be acquired through use of one of the two memory systems. Post-

training reversible inactivation of the hippocampus *attenuated* acquisition of the place task, and *enhanced* acquisition of the response task (Schroeder et al., 2002).

Amygdala Modulation of Multiple Memory Systems

In addition to the hippocampus and the caudate nucleus, the amygdala has also been implicated as a component of multiple memory systems (McDonald & White, 1993). There is significant evidence that the basolateral amygdala has a variety of roles in learning and memory processes. Evidence has shown that the amygdala plays a role in both stimulus-reward learning (e.g. Cador et al, 1989; Everitt et al, 1991; Hiroi & White, 1991; Hsu, Schroeder & Packard, 2002; McDonald & White, 1993) and stimulus- affect memory of Pavlovian fear conditioning (Davis, 1992; Fendt & Fanselow, 1999; Helmstetter, 1992; LeDoux, 1992, 1998). In addition, several studies have indicated that the basolateral amygdala has a modulatory influence on memory processes occurring in *other* brain structures (McGaugh, 2002; Packard, Cahill, Williams, & McGaugh, 1995; Packard, Cahill, & McGaugh, 1994; Packard & Teather, 1998; Packard & Wingard, 2004). There are several pathways that originate in the basolateral/ lateral amygdala nuclei and project to both the hippocampus and the caudate nucleus (Kita & Kitai, 1990; Krettek & Price, 1978; Pitkanen et al., 2000; or see review Pitkanen, 2000.). There is evidence that the amygdala modulates memory in both hippocampus-dependent and caudate-dependent tasks (Packard et al., 1994; Packard & Teather, 1998). Rats were trained in either a hippocampus-dependent or caudate-dependent version of the Morris water maze task. The hippocampal task consists of learning to swim to a hidden platform located in the same spatial location, while the caudate-dependent version has a moving

visible platform onto which rats may escape the water. *D*-amphetamine was infused into the amygdala post-training and enhanced memory in both tasks (Packard et al, 1994). In a second experiment *d*-amphetamine was again infused into the amygdala post-training. Twenty-four hours later, lidocaine was then infused into the amygdala prior to a probe trial to determine if the enhancing effect of *d*-amphetamine was stored in the amygdala. The enhancing effect of *d*-amphetamine was still observed, indicating that to express the facilitated effects of memory modulation on the hippocampus and the caudate the amygdala did not need to be activated during the time of memory retrieval (Packard, et al., 1994). Additional studies have indicated that intra-hippocampus infusions of lidocaine prior to the probe trial blocked the enhancing effects of the post-training *d*-amphetamine infusions in the spatial water maze task (Packard & Teather, 1998). Also intra-caudate infusions of lidocaine prior to the probe trial blocked the facilitation of intra-amygdala infusions of *d*-amphetamine on the moving visible platform task (Packard & Teather, 1998). These findings suggest that the amygdala can modulate both hippocampus and caudate dependent learning and the modulatory effect is stored in each respective structure.

Amygdala, Emotion, & Memory

The amygdala has been implicated in the modulatory effect that emotional arousal can exert on memory (e.g. Cahill and McGaugh, 1998; Kim, Lee, Han, & Packard, 2001). For example, rats receiving a pre-training stress regimen (restraint stress and uncontrollable tail shock) were impaired in the *retention* of a hippocampus-dependent spatial water maze task (Kim et al, 2001). However, amygdala lesions blocked the

impairing effects of stress on hippocampus-dependent spatial memory (Kim et al, 2001). This study also looked at how amygdala lesions affect LTP, one putative mechanism of learning in the hippocampus. Stressed animals exhibited impaired LTP compared to unstressed rats. However, the amygdala lesions again blocked the impairing effects of stress on hippocampal LTP. These results suggest that the amygdala is necessary to mediate the effects of stress on hippocampal LTP.

Emotional Modulation of the Relative use of Multiple Memory Systems

Recent studies have begun to examine the influence of emotional state on memory within the multiple memory systems framework. One study looked at how stress would affect behavior in a dual solution water maze task (Kim et al., 2001). The platform was visible and located in the same location for all trials. This procedure allows the task to be acquired through both hippocampus-dependent spatial memory and through caudate dependent habit memory. Twenty-four hours after training, the visible platform was moved to a different spatial location and rats were placed back into the maze. The stressed animals more often swam immediately to the cued platform, displaying the caudate dependent strategy, while the unstressed animals first swam to the old spatial location, thereby displaying the hippocampus-dependent strategy (Kim et al., 2001).

An additional study examined whether induction of an emotional state via injection of anxiogenic drugs during acquisition can affect the *relative* use of multiple memory systems (Packard & Wingard, 2004). Rats were administered peripheral *pre-training* injections of the anxiogenic α-$_2$ adrenoceptor antagonists yohimbine or RS 79948-197 prior to training in the dual solution plus maze task. Research has shown that these drugs

bind to presynaptic noradrenergic autoreceptors to prevent the termination of norepinephrine release, therefore causing an increase in synaptic norepinephrine (Hume et al., 1996). Studies have shown that rats that were administered α_2 antagonists showed an increase in anxiety according to a variety of measures including increased startle response, heart rate, blood pressure and locomotion; decrease in social interaction and decreased time spent in the open-arms of an elevated plus maze (Guy and Gardner, 1985; Handley and Mithani, 1984; White and Birkle, 2001). On a drug free probe trial rats previously administered yohimbine or RS 79948-197 predominantly displayed "response" learning, while rats that were injected with vehicle displayed "place" learning. Importantly, intra-amygdala infusions of RS 79948-197 also resulted in the predominant use of response learning (Packard & Wingard, 2004), suggesting that the amygdala may mediate the modulatory effect of anxiogenic drugs on the use of multiple memory systems. Taken together these studies suggest that high levels of emotional arousal may favor the caudate dependent strategy in a task that can be learned by both memory systems. Furthermore, this preference of the caudate-dependent strategy appears to be mediated by the amygdala.

Summary

The evidence reviewed in the previous sections indicates that multiple memory systems exist and that each system mediates a separate type of memory with the hippocampus mediating "cognitive" or "relational" learning, whereas the caudate nucleus mediates "habit" or "response" learning. In addition the amygdala exerts a modulatory influence on multiple memory systems. Finally, emotional state can influence the

relative use of multiple memory systems and the amygdala may mediate this influence. However, several questions remained unanswered.

First, previous studies of the effects of anxiogenic drugs on multiple memory systems have only examined the acquisition phase of learning. It is not known how inducing anxiety prior to retrieval will influence the relative use of multiple memory systems. Therefore experiments were conducted with peripheral injections and intra-amygdala infusions prior to memory retrieval in the dual solution task. Second, previous studies examining the effects of RS 77948-197 on memory were conducted with rats trained in an anxiogenic state and subsequently probed in a drug free state (Packard & Wingard, 2004). This raises the possibility that "state dependency" may have influenced the behavior observed on the probe trials. The state dependency hypothesis proposes that acquired information is best retrieved if the organism is in the same physiological state during both initial acquisition and retrieval (Overton, 1964; Shulz et al, 2000). In order to test for the possible effects of state dependency, experiments were conducted with anxiogenic drugs being administered prior to both training and retrieval. Third, previous research indicates that peripheral and intra-amygdala anxiogenic drug treatments result in the predominant use of caudate-dependent learning. The mechanism underlying this effect is not fully understood. One possibility is that the use of response learning produced by administration of anxiogenic drugs reflects a *direct* enhancement of caudate-dependent response learning. Alternatively, it may reflect a drug-induced impairment in hippocampus-dependent place learning, thereby leading the animal to use a response learning strategy. The impairment in spatial memory produced by a pre-training stress regimen in a single solution hippocampus-dependent task is consistent with the latter

hypothesis (Kim et al., 2001). Therefore, experiments were conducted in which peripheral and intra-amygdala injections of anxiogenic drugs were given in a single solution hippocampus dependent water maze task. Overall, the goal of these experiments is to increase the understanding of the effects of emotional arousal and amygdala modulation of multiple memory use.

METHODS

Subject

Subjects were 140 male Long-Evans rats (weighing 250-450 g). They were individually housed in a climate-controlled vivarium with ad libitum access to food and water. The animals were on a 12:12-h light: dark cycle (lights on at 8 a.m.). All experiments were conducted during the light phase of the cycle.

Apparatus

Animals were trained in a black circular water maze (1.83 m diameter, 0.58 m in height; 25 degrees Celsius water-temperature) into which a clear Plexiglas plus-maze (43 cm height, arm-width of 25 cm, and arm-length of 60 cm) was inserted. The maze was filled to a water-level of 20 cm. The maze was located in the center of a tiled room. Extra-maze visual cues consisting of various geometric shapes were placed on the walls. None of these cues were placed in a spatially congruent/proximal position with the ends of the plus-maze arms. During training, an invisible clear Plexiglas escape platform (11x14x19 cm) was consistently located at the end of one arm of the maze (west), 1cm below water level. The arm opposite the start-arm was blocked off by an additional piece of Plexiglas, such that the rats were trained with the maze in a "T" configuration.

Surgery and Histology

Rats were anesthetized with an intraperitoneal injection of sodium pentobarbital (60 mg/kg) and implanted with bilateral guide cannula in the basolateral amygdala using

standard sterotaxic techniques. Guide cannulae (23 gauge, 15 mm length) were anchored to the skull with jeweler's screws and dental acrylic. Sterotaxic coordinates for the basolateral amygdala were anterior-posterior (AP) = -2.2 mm from bregma, medial-lateral (ML) = +4.7mm, and dorsal-ventral (DV) =-7.00 mm. These coordinates were chosen based on our previous research (e.g., Packard & Wingard, 2004). Animals were given 7-10 days of post-operative recovery prior to behavioral testing.

Following behavioral testing, rats were deeply anesthetized with a 1 ml-injection of sodium pentobarbital (60 mg/kg) and perfused with 0.9% saline, followed by 10% formal-saline solution. The brains were removed and stored in 10% formal-saline solution before slicing with a cryostat. Brains were sectioned at 20 μm and every second slice was collected and stained with cresyl violet. Slides were examined for cannula placements and infusion needle tip location using the atlas of Paxinos and Watson (1998). Rats with blocked or inaccurate cannula placements or evidence of gliosis (n=7) were excluded from the statistical analysis. As illustrated in Figure A-1, the injection needle tips were located in the basolateral amygdala ranging from -1.80 mm to -2.80 mm AP from bregma.

It should be noted that even though the tip of the injection needles were located in the basolateral amygdala, the possibility of the injection defusing into other nuclei (e.g. central nucleus) cannot be completely ruled out. However, evidence has consistently indicated that the basolateral amygdala and not the central amygdala modulates memory (Roozendaal & McGaugh, 1996, 1997). Studies have shown that lesions of the basolateral but not central nucleus block the memory modulatory effect of the amygdala on the hippocampus (Roozendaal & McGaugh, 1996). Infusions of noradrenergic drugs

into the basolateral nucleus, but not the central nucleus of the amygdala impaired memory of hippocampus-dependent water maze task (Roozendaal & McGaugh, 1997). There are also direct projections originating in the basolateral/ lateral amygdala nuclei to both the hippocampus (Kita & Kitai, 1990; Krettek & Price, 1978; Pitkanen et al., 2000; or see review Pitkanen, 2000.) Evidence also indicates that the basolateral nucleus and not the central nucleus mediated the influence of the amygdala on hippocampal long-term potentiation (LTP), one putative mechanism of memory formation. Electrical stimulation of the basolateral nucleus produced LTP in the hippocampus (Akirav & Richter-Levin, 2002). Taken together these studies support the idea that the basolateral nucleus is critical in the memory modulatory effects exerted by

Drugs/Injection Procedures

RS 79948-197 hydrochloride (Tocris Chemicals, 0.03, 0.1 and 0.3 mg/kg), a recently developed, highly selective α-$_2$ adrenoceptor antagonist, was dissolved in physiological saline (Hume et al., 1996; Milligan et al., 1997). The peripheral doses were selected based on previous evidence of their anxiogenic properties in rats in acoustic startle reflex test (White & Birkle, 2001). Peripheral injections were administered into the intraperitoneal cavity in a volume of 1 ml/kg. Intra-basolateral amygdala infusions of RS 79948-197 (0.1 µg/ 0.5 µl), or vehicle saline, was administered via guide cannulae using 30-gauge injection needles connected by polyethylene tubing to 10-µl Hamilton microsyringes. Infusions were delivered over 52s with an electronically controlled syringe pump (Sage Instruments, Boston), and the injection needles were left in place an additional 60s post-infusion to allow for diffusion of solution away from injection tip.

Experiment 1

This experiment examined whether peripheral injection of RS 79948-197 or intra-amygdala infusions of RS 79948-197 prior to memory retrieval influences the relative use of place and response learning.

Behavioral Procedures

Training procedures were identical to those previously used in our lab (Packard & Wingard, 2004). Rats were trained in the water plus-maze task for two consecutive days. Each training session consisted of six trials, separated by an inter-trial interval of 30s. During training rats were placed in the start arm of the maze (north), and were allowed to swim to the escape platform, which was consistently located in one arm of the maze (west). Entry into the maze arm that contains the escape platform (west arm) was scored as a correct response during the training trials, and entry into the maze arm that did not contain the escape platform (east arm) was scored as an incorrect response. Upon climbing onto the platform, rats remained on it for 10s before being returned to a time out box for thirty seconds. Rats that failed to find the escape platform within 60s were manually guided to it. The number of incorrect arms visits was recorded by the experimenter. An incorrect choice was defined as swimming a full body length into the arm not containing the hidden platform.

On day three rats were tested on a probe trial in order to determine their relative use of ‘‘place’’ and ‘‘response’’ learning. In this trial rats were placed into a start box 180 degrees opposite that used during training (i.e., end of the south arm) and were allowed to make an entry into either the west or east maze arm. A Plexiglas shield blocked the north

arm during the probe trial. Rats entering the arm were the platform had been located in during training were designated place learners, and rats entering the arm that was associated with the body turn response they made during training were designated response learners. Rats received no pharmacological treatment during acquisition. Rats were injected peripherally thirty minutes *prior* to the probe trial with either RS 79948-197 (0.003, 0.1 or 0.3 mg/kg) or saline vehicle. Intra-basolateral amygdala infusions of RS 79948-197 (0.1 μg/side) or saline were administered immediately prior to the probe trial.

Experiment 2

These studies examined the possible role of state dependency on memory system use following peripheral injections or intra-amygdala infusions of RS 79948-197.

Behavioral Procedures

The behavioral procedures were the same as in experiment one, however, rats were injected both prior to training *and* the probe trial with either peripheral RS 79948-197 (0.1 mg/kg) or vehicle. A second set of cannulated animals received intra-amygdala injections of RS 9948-197 (0.1 μg) or saline

Experiment 3

This experiment examined the effect of post-training peripheral injections of RS 79948-197 on the acquisition of the single solution place water maze task. This

experiment tested if anxiogenic drugs would impair consolidation of hippocampus-dependent memory.

Behavioral Procedures

The behavioral procedures were similar to those that previously described (Schroeder, Wingard & Packard, 2002). Rats were trained in the water plus-maze task for five consecutive days. Each training session consisted of six trials, separated by an inter-trial interval of 30s. Rats were allowed to swim to the escape platform, which was consistently located in one arm of the maze (west). Start arm sequence occurred as follows: NSSNNS on odd days; and SNNSSN on even days. Upon climbing onto the platform, rats remained on it for 10s before being returned to a box for the inter-trial interval. Rats that failed to find the escape platform within 60s were manually guided to it. The number of incorrect arms visits was recorded by the experimenter. An incorrect choice was defined as swimming a full body length into the arm not containing the hidden platform. Rats received peripheral injections of RS 79948-197 (0.3 mg/kg) immediately post-training on days one, two, and three.

Experiment 4

This experiment examined the effect of pre-training intra-amygdala infusions of RS 79948-197, on the acquisition of the single solution place water maze task. This experiment tested if anxiogenic drugs infused directly into the amygdala would impair hippocampus-dependent learning.

Behavioral Procedures

Training procedures were the same as experiment three. Animals received pre-training intra-amygdala infusions of RS 79948-197 (0.1 μg) or saline.

RESULTS

Effects of Peripheral Injections or Intra-Amygdala Infusions of RS-79948-197 Prior to Memory Retrieval

The acquisition of the dual solution plus maze task during two days of the initial drug free training is illustrated in Figure A-2. A two-way one-repeated ANOVA comparing vehicle-treated and RS 79948-197 groups on percent correct responses for trials 1-12 revealed no significant group effect($F_{(3,42)} = 0.032$, n.s.) or group X trial interaction ($F_{(11, 42)} = 0.996$, n.s.). However, a significant trial effect was found ($F_{(33, 42)} = 7.532$, $p < 0.01$). These findings indicate that all the animals acquired the task at the same rate, and that any differences observed during the probe-trial could not be due to group differences in the rate of acquisition.

The effects of peripheral injections of RS 79948-197 prior to the probe trial on day three are shown in figure A-3. In order to examine the use of "place" or "response" learning in control and drug-treated rats, a chi-squared analysis was computed on the probe choice behavior. Rats that approached the spatial location where the platform had been located during training were designated "place" learners. Rats that executed the same egocentric turn response that had been reinforced during training were designated "response" learners. The chi-squared analysis showed that vehicle-treated rats tended to display no preference of learning strategy on the probe trial (5 place, 5 response rats; $\chi^2 = 0$, n.s.). In contrast, rats that received RS 79948-197 at 0.1 mg/kg showed significant response learning on the probe trial (11 response rats, 2 place rats; $\chi^2 = 6.23$, $p < 0.05$. RS 79948-197-treated rats (0.3mg/kg and 0.03) displayed a strong trend towards the use of

response learning on the probe trial (9 response rats, 4 place rats; $\chi^2 = 1.923$, n.s.) and (8 response, 2 place; $\chi^2 = 3.6$, n.s.). These findings indicate that in a plus-maze task in which both place and response learning provide an adequate solution, peripheral RS 79948-197 injected prior to retrieval results in the predominant use of response learning.

The acquisition of the dual solution plus maze task during two days of the initial drug free training is illustrated in Figure A-4. A two-way one-repeated ANOVA comparing vehicle-treated and RS 79948-197 groups on percent correct responses for trials 1-12 revealed a non-significant group effect($F_{(1, 20)} = 0.254$, n.s.) and group x trial interaction ($F_{(11, 27)} = 1.446$, n.s.). A significant trial effect indicated that all groups improved over training ($F_{(11, 27)} = 2.062$, $p < 0.05$). These findings indicate that all the animals acquired the task at the same rate, and that any differences observed during the probe-trial could not be due to group differences in the rate of acquisition.

Effects of Peripheral Injections or Intra-Amygdala Infusions of RS-79948-197on Memory System Use: Role for State Dependency

The effects of intra-amygdala infusions of RS 79948-197 injected prior to the probe trial on day three are shown in Figure A-5. The chi-squared analysis showed that vehicle-treated rats tended to display no preference of learning strategy on the probe trial (5 place rats, 5 response rats; $\chi^2 = 0$, n.s.). In contrast, RS 79948-197-treated rats (0.1 μg) displayed a strong trend towards the use of response learning on the probe trial (9 response rats, 3 place rats; $\chi^2 = 3.00$, n.s.). These findings indicate that in a plus-maze task in which both place and response learning provide an adequate solution, intra-

amygdala infusions of RS 79948-197 injected prior to retrieval may influence the type of information used in performing the task

The effect of peripheral injections of RS 79948-197 on initial acquisition of plus-maze behavior during the 2 days of training is illustrated in Figure A-6. A two-way one-repeated measures ANOVA comparing vehicle treated and RS 79948-197 (0.1 mg/kg) groups on percentage of correct responses for training trials 1–12 revealed a non-significant group effect ($F_{(1, 23)}$ =0.503. n.s.) and a non-significant group trial interaction ($F_{(11,23)}$ =1.4, n.s.). A significant trial effect ($F_{(11, 23)}$ =7.007, $p<.01$) indicated that all groups improved over training. These findings indicate that RS 79948-197 did not affect the rate of acquisition of plus- maze behavior relative to vehicle-injected controls, and thus any differences between groups in probe-trial behavior cannot be due to a differential rate of task acquisition.

The effect of peripheral RS 79948-197 on the relative use of place and response learning on the day 3 state dependent probe trial is shown in Figure A-7. Chi-square analyses computed on the probe trial choice behavior revealed that vehicle-treated rats tended to display a strong trend towards the use of place learning on the probe trial (10 place rats, 3 response rats; $\chi^2 = 3.769$, n.s.). In contrast, RS 79948-197-treated rats (0.1 mg/kg) displayed a trend towards the use of response learning on the probe trial (9 response rats, 4 place rats; $\chi^2 = 1.923$, n.s.). These findings suggest that RS 79948-197 treatment (0.1 mg/kg) during training and prior to retrieval may favor the relative use of response learning over place learning.

The effect of intra-amygdala infusions of RS 79948-197 on initial acquisition of plus-maze behavior during the 2 days of training is illustrated in Figure A-8. A two-way

one-repeated measures ANOVA comparing vehicle treated and RS 79948-197 (0.1 μg) groups on percentage of correct responses for training trials 1–12 revealed a non-significant group effect ($F_{(11, 14)}$=0.836, n.s.) and a non-significant group trial interaction ($F_{(1, 14)}$= 0.699; n.s.). A significant trial effect ($F_{(11, 14)}$ =3.845, $p<.01$) indicated that all groups improved over training, indicates that RS 79948-197 did not affect the rate of acquisition of plus- maze behavior relative to vehicle-injected controls

The effect of intra-amygdala infusions RS 79948-197 on the relative use of place and response learning on the day 3 state dependent probe trial is shown in Figure A-9. Chi-square analyses computed on the probe trial choice behavior revealed that vehicle-treated rats displayed no preference of learning strategy on the probe trial (4 place rats, 4 response rats; $\chi^2 = 0$, n.s.). In contrast, RS 79948-197-treated rats (0.1 μg) displayed a trend towards the use of response learning on the probe trial (6 response rats, 2 place rats; $\chi^2 = 2.00$, n.s.). These findings suggest that RS 79948-197 treatment (0.1 μg) during training and prior to retrieval may favor the relative use of response learning over place learning.

Effects of Post-Training Peripheral Injections of RS 79948-197on Hippocampus Dependent Memory

Figure A-10 illustrates acquisition of the single solution place water maze task. A one-way ANOVA was used to compute the percentage correct data on training day 1 (before post-training treatment began). This indicated that there was no significant group differences in performance ($F_{(1,12)}$ = .012 , n.s.) prior to post-training injections, therefore any differences in subsequent performance were not the results of differences in

performance on the initial day of training. A two-way one-repeated measure ANOVA (group X trial) computed on the percentage correct data of days 2-5 (i.e., post-injection) revealed a significant main effect for group ($F_{(1, 9)} = 21.783$, $p < 0.01$), non-significant interaction, ($F_{(3, 27)} = 1.656$, n.s.), or trial ($F_{(3, 27)} = 0.249$, n.s.).

Effects of Pre-Training Intra-Amygdala Infusions of RS 79948-197on Hippocampus-Dependent Memory

Figure A-11 illustrates acquisition of the single solution place water maze task. A two-way one-repeated measure ANOVA (group X trial) computed on the percentage correct data of days 1-5 revealed a significant interaction, ($F_{(1,8)} = 5.197$, $p < 0.052$), and a significant main effect of group ($F_{(2, 8)} = 19.924$, $p<0.01$), and non significant trial ($F_{(2, 8)} = 0.160$, n.s). Tests of simple main effects (group within trial) showed that RS 79948-197 (0.1 µg) treated rats were impaired relative to saline controls on training day 2 ($F_{(1, 8)} = 9.901$, $p <0.05$) and day 3 ($F_{(1, 8)} = 6.603$, $p<0.05$). This indicates that RS 79948-197 infused into the basolateral amygdala impairs hippocampus dependent learning.

DISCUSSION AND CONCLUSIONS

Previous research has indicated that memory is organized in multiple memory systems. In addition, it has been found that the amygdala exerts a modulatory influence on these systems. Evidence also indicates that emotional state can influence the relative use of multiple memory systems and this effect is mediated by the amygdala (Packard & Wingard, 2004). The overall goal of the present studies was to increase the understanding of the relationship between emotional state, amygdala function, and the relative use of multiple memory systems. Three specific questions were addressed in the present experiments. First, do anxiogenic drugs influence memory system use when administered at memory retrieval? Second, are the effects of anxiogenic drugs on memory systems due to state dependency? Third, does the relative use of habit memory that is produced by anxiogenic drugs result from an impairment of hippocampus dependent cognitive memory?

4.1 *Do Anxiogenic Drugs Administered at Retrieval Affect the Relative Use of Multiple Memory Systems*

Previous research has shown that RS 79948-197 (0.1 mg/kg) injected peripherally prior to training resulted in a significant use of response learning during a drug free probe trial (Packard& Wingard, 2004). In order to further investigate the effects of anxiety induced at different behavioral time points, rats were given RS 79948-197 prior to memory retrieval. Peripheral administration of RS 79948-197 (0.1 mg/kg) prior to memory retrieval also produced a significant use of response learning. The 0.1 mg/ kg

dose of RS 79948-197 has been shown in other studies to have anxiogenic properties in the acoustic startle reflex test (White& Birkle, 2001). The 0.03 mg /kg and the 0.3 mg/kg doses were chosen to construct a more extensive dose-response curve of peripheral RS 79948-197 in rats. Animals that received RS 79948-197 (0.03 mg /kg and 0.3 mg/ kg) displayed a strong trend toward the use of response learning. These findings indicate that the relative use of memory systems can be modulated by peripheral injections of RS 79948-197 not only during acquisition (Packard & Wingard, 2004), but also at the time of memory retrieval.

A previous study using the dual solution water maze task has shown intra-basolateral amygdala infusions of RS 79948-197 prior to training results in a significant use of response learning (Packard & Wingard, 2004). In the present research, rats that were administered intra-amygdala infusions of RS 79948-197 (0.1 μg) prior to memory retrieval *also* exhibited a strong trend towards the use of response learning. These findings indicate that infusions of an anxiogenic drug into the basolateral amygdala prior to memory retrieval is sufficient to mimic the effect of peripheral drug injections.

Other findings indicate that stress induced prior to *retrieval* impairs hippocampus-dependent spatial memory in a single solution task (deQuervain, Roozendaal & McGaugh, 1998, de Quervain et al., 2003; Roozendaal, 2002, 2003; and Roozendaal, deQuervain, Schelling & McGaugh, 2004). In one experiment rats were trained in the Morris water maze to find a submerged platform in a fixed location. Rats that were exposed to foot shock prior to retrieval showed a significant impairment of memory for the location of the platform (de Quervain et al., 1998). This impairment was time dependent for thirty minutes after foot shock, but not at two minutes or four hours later.

The impairment also correlated with the timing of peak plasma level of corticosterone. Corticosterone and other glucocorticoids are released shortly after a stressful experience and have a variety of effects on memory. In addition rats that were administered peripheral injections of stress inducing doses of corticosterone thirty minutes prior to the probe trial showed a similar impairment of memory (de Quervain et al., 1998). Comparable effects of glucocorticoids on memory retrieval have also been demonstrated in human subjects (de Quervain, Roozendaal, Nitsch, McGaugh & Hock, 2000). Healthy human subjects viewed a series of unrelated nouns for four seconds on a computer screen for both an immediate and delayed retention test. Twenty-four hours later subjects were administered an oral dose of 25 mg of mannite (a placebo) or 25 mg of cortisone. Cortisone significantly impaired free-recall of previously learned words.

Evidence also suggested that the amygdala is needed for glucocorticoids to modulate memory consolidation in other brain structures (Roozendaal et al., 1999). A recent study indicates that the basolateral amygdala is involved in the effects of glucocorticoids on hippocampus-dependent learning (Roozendaal et al., 2002.). In this study the specific glucocorticoid receptor agonist RU 28362, dose-dependently impaired memory of a spatial water maze task, when it was infused into the hippocampus prior to retrieval. Lesions of the basolateral amygdala blocked the memory impairment. These findings indicate that the basolateral amygdala mediates the impairing effects of stress hormones of retrieval of hippocampus dependent memory. The current findings indicate that emotional arousal accompanying drug induced anxiety also may impair hippocampus dependent memory. In the dual task solution task, such an impairment may lead the animal to rely on caudate-dependent response learning.

Are the Effects of Anxiogenic Drugs on the Relative Use of Memory Systems Due to State Dependency

The "state dependency" hypothesis proposes that acquired information is best retrieved if the organism is in the same physiological state during both initial acquisition and retrieval (Overton, 1964 and Shulz et al, 2000). In the original demonstration of this phenomenon, rats were trained to escape from shock in a T-maze either after receiving injections of sodium pentobarbital 25 mg/kg or no injections. When placed back into the task in the opposite state, animals did not display the escape response. Furthermore, they showed no savings when retrained in a task that had been previously learned in an alternate state (Overton, 1964).

Studies have shown similar results in humans in situational contexts. Deep-sea divers were given lists of words that they learned on the boat or 20 feet below the surface of the water. Recall of the words was better when in the same context (Godden & Baddeley, 1975). Other studies have shown that retrieval of autobiographical memories may be affected by mood (Bower, 1981). Subjects placed in a happy or sad mood were better able to recall events that originally were experienced in the same mood (Bower, 1981).

To examine whether the use of response learning produced by anxiogenic drugs (Packard & Wingard, 2004) was due to state dependency, animals in the current studies received drug treatment *both* at training and at retrieval. The results suggest that rats administered RS 79948-197 (1 mg/kg) prior to both training and retrieval displayed a trend towards the use of response learning. This pattern of results is identical to those of

rats receiving RS 79948-197 only prior to training (Packard & Wingard, 2004), or retrieval (experiment 1). This suggests that the effect of RS 79948-197 on the relative use of multiple memory systems is not due to state dependency.

Are the Effects of Anxiogenic Drugs on Memory System Use the Result of an Impairment of the Hippocampus

Previous research has indicated that peripheral and intra-amygdala anxiogenic drug treatments resulted in the predominant use of caudate-dependent learning in the dual solution task. One possible mechanism underlying this effect is that anxiogenic drugs may impair hippocampal place learning, thereby leading the animal to use a caudate dependent response strategy. In order to investigate this hypothesis rats were trained in a single solution hippocampus-dependent place learning task. In this task, rats are required to swim to the same spatial location from varying start points. However, left and right body turn responses are equally reinforced. Therefore, response learning does not provide an adequate solution. Rats that received peripheral injections of RS 79948-197 were impaired in the acquisition of this place task relative to controls. This finding suggests that the use of response learning produced by anxiogenic drugs in the dual solution task may result from an impairment of hippocampus-dependent memory.

Findings from other studies using stress to induce emotional arousal, suggests a role for the amygdala in modulating hippocampus dependent memory (Kim et al, 2001; Roozendaal, Portillo-Marquez & McGaugh, 1996; Roozendaal et al, 1999). For example, rats that were stressed prior to training in a hippocampus-dependent hidden platform water maze task (Kim et al. 2001). Stressed animals displayed a memory impairment for

the location of the hidden platform 24 hours later during a probe trial, but amygdala lesions blocked the effects of stress on memory (Kim et al, 2001). A second study was conducted in a dual solution water maze task. Stressed and unstressed rats were trained to swim to a visible platform in a fixed spatial location. Twenty-four hours later the visible platform was moved to a new spatial location. Control animals first swam to the old platform location, indicating the use of a hippocampus-dependent "place" strategy. In contrast, stressed animals more preferentially swam immediately to the visible platform in the new spatial location, exhibiting the use of a caudate-dependent "stimulus-response" strategy (Kim et al, 2001). In the present studies intra-amygdala infusions of RS 79948-197 impaired acquisition of a single solution place learning task. These findings indicate that drug infusion into the basolateral amygdala is sufficient to impair hippocampus-dependent learning.

Amygdala, Emotion, and Modulation of Hippocampal Memory: Possible Mechanisms

The current research suggests that the use of response learning produced by intra-amygdala infusions of anxiogenic drugs may be due to the impairment of hippocampus-dependent memory. However, these experiments do not reveal the mechanism(s) underlying amygdala modulation of hippocampal memory. There is evidence that noradrenergic function plays a vital role in memory modulation of other brain structures by the basolateral amygdala (for reviews see, Ferry, Roozendaal & McGaugh, 1999; Ferry & McGaugh, 2000; McGaugh, 2002). This is particularly relevant because RS 79948-197, a noradrenergic alpha-2 antagonist, causes an increase of norepinephrine in the synaptic cleft. The direct projection from the basolateral/ lateral amygdala nuclei to

the hippocampus is glutamatergic in nature (Petrovich, Canteras, & Swanson, 2001), and evidence indicates a role for glutamate in hippocampus dependent memory (e.g. Packard, 1999; Szapiro et al, 2000). One possibility is that RS 79948-197 infused into the basolateral amygdala decreases glutamatergic output to the hippocampus, thereby impairing hippocampus-dependent memory. Alternatively, a supranormal activation of glutamatergic output to the hippocampus by RS 79948-197 could also conceivably impair hippocampus-dependent memory. Microdialysis experiments examining hippocampal glutamate release in response to intra-amygdala infusions of RS 79948-197 could potentially differentiate these two possibilities.

Although anxiety and stress likely do not share precisely the same physiological underpinnings, a consideration of the role of the basolateral amygdala in mediating the effects of stress on hippocampus-dependent learning maybe useful in understanding the effects of general emotional arousal on memory system use. For example, long-term potentiation LTP is one putative mechanism of learning and memory (Bliss & Limø, 1973; Bliss & Collingridge, 1993; Izquierdo, 1993), and evidence indicates that there are several shared attributes between low levels of stress and hippocampal LTP. For example, both hippocampal LTP and low levels of stress increase α-amino-3 hydroxy-5-methylisoxazole- 4-propionate (AMPA) receptors in the hippocampus (Tocco, Shors, Baudry & Thompson, 1991; Tocco, Maren, Shors, Baudry & Thompson, 1992). Additional research has shown that both hippocampal LTP and the memory impairing effects of stress involve N-methyl-D-aspartate (NMDA) receptors (Brun, Ytterbo, Morris, Moser & Moser, 2001; Kim, Foy & Thompson, 1996, Morris, Anderson, Lynch & Baudry, 1986). Although, mild stress and mild LTP also do not produce amnesia during

a retention test for a recently learned task, but if the hippocampus is saturated by LTP or the organism is presented with a considerable amount of stress then amnesia is produced (Barnes et al., 1994; Moser, Krobert, Moser & Morris, 1998; Diamond, et al., 1992). A recent study has examined the role of the amygdala in mediating the effects of stress on hippocampal LTP (Kim et al., 2001). Rats were exposed to a regimen of restraint stress and uncontrollable tail shock, and immediately after stress exposure, brain slices were prepared and LTP was measured. Animals that had received stress showed impairments of LTP, but brain slices of animals with lesions to the amygdala exhibited normal LTP (Kim et al., 2001). This suggests that emotional arousal may influence hippocampal function by impairing LTP, and that the amygdala may mediate this effect. Evidence from a broad range of studies indicates that a multitude of other stressors such as exposure to unfamiliar environments (Diamond et al., 1990), exposure to a predator (Mesches et al., 1999), and elevated levels of corticosterone (Filipini, Gijsbers, Birmingham & Dubrovsky, 1991; Diamond et al., 1992; Alfarez, Wiegert, Joels & Krugers, 2002), also impair LTP induction. Further research is necessary to examine whether other forms of emotional arousal such as anxiety also impair hippocampal memory processes via an influence on LTP.

Conclusions

The current findings indicate that an anxiogenic state induced prior to retrieval results in the use of response learning in a situation where either "cognitive" or "habit" learning can be utilized. Moreover, the predominant use of response learning in a dual solution task is not a result of state dependency. Evidence also indicates that the same

anxiogenic state that resulted in a significant use of habit learning in a dual solution task impaired cognitive memory in a single solution hippocampus-dependent task. These findings suggest that an anxiogenic state may produce the use of response learning by impairing hippocampus-dependent memory. The results also suggest that anxiogenic drug action in the basolateral amygdala is sufficient to produce the use of response learning when given prior to retrieval in the dual solution task, and also to impair hippocampus-dependent memory in the single solution task.

The amygdala has been identified as playing a role in several human psychopathologies involving emotional dysfunction, such as general anxiety disorders and post-traumatic stress disorder (for reviews, see Davis & Walen, 2001; Lang, Davis & Ohman, 2000). To the extent that these psychopathologies involve anxiety or stress induced habitual behaviors (e.g. stress induced relapse in drug addiction), further understanding of the role of the amygdala in modulating memory system use may have important clinical implications (e.g. improved treatments for anxiety disorders and post-traumatic stress disorder).

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APPENDIX

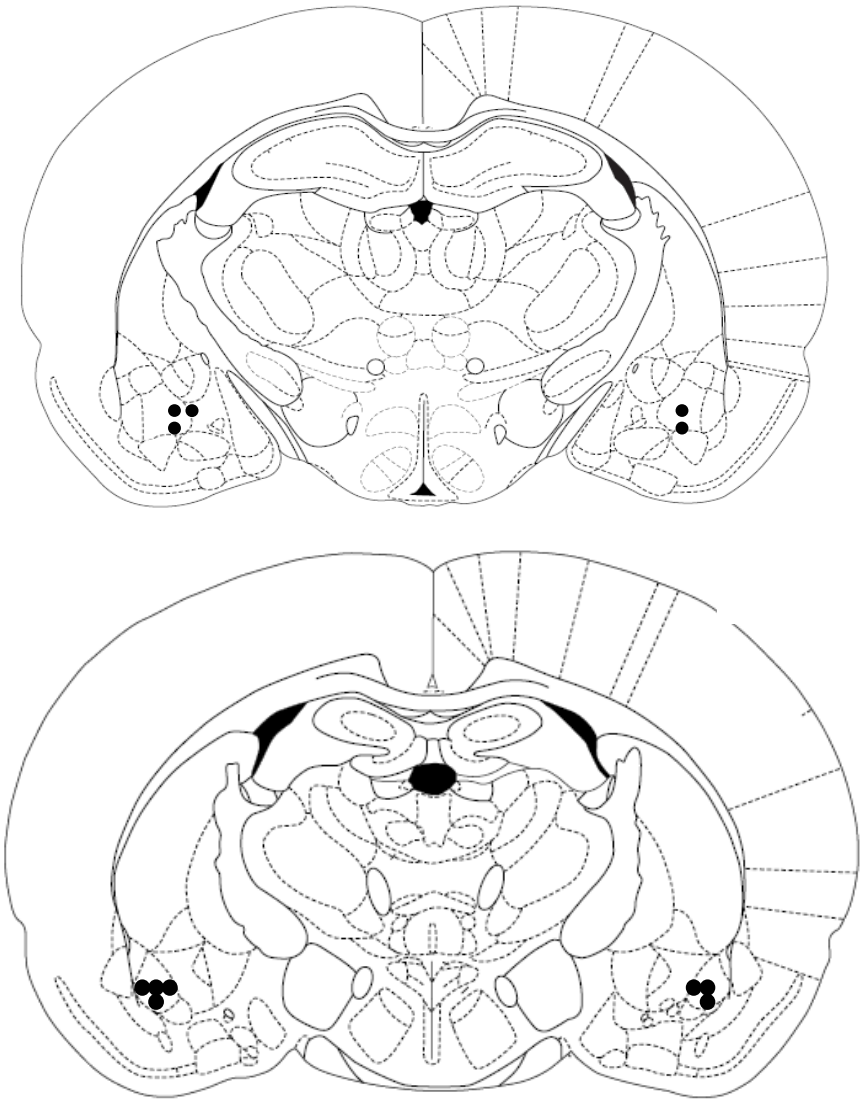

Figure A-1. Location of bilateral injection needle tips in the basolateral amygdala (shown with overlap). Infusions needles were located in the basolateral nucleus ranging from -1.80 to - 2.80 mm AP from bregma. Adapted from the rat brain atlas of Paxinos and Watson (1998).

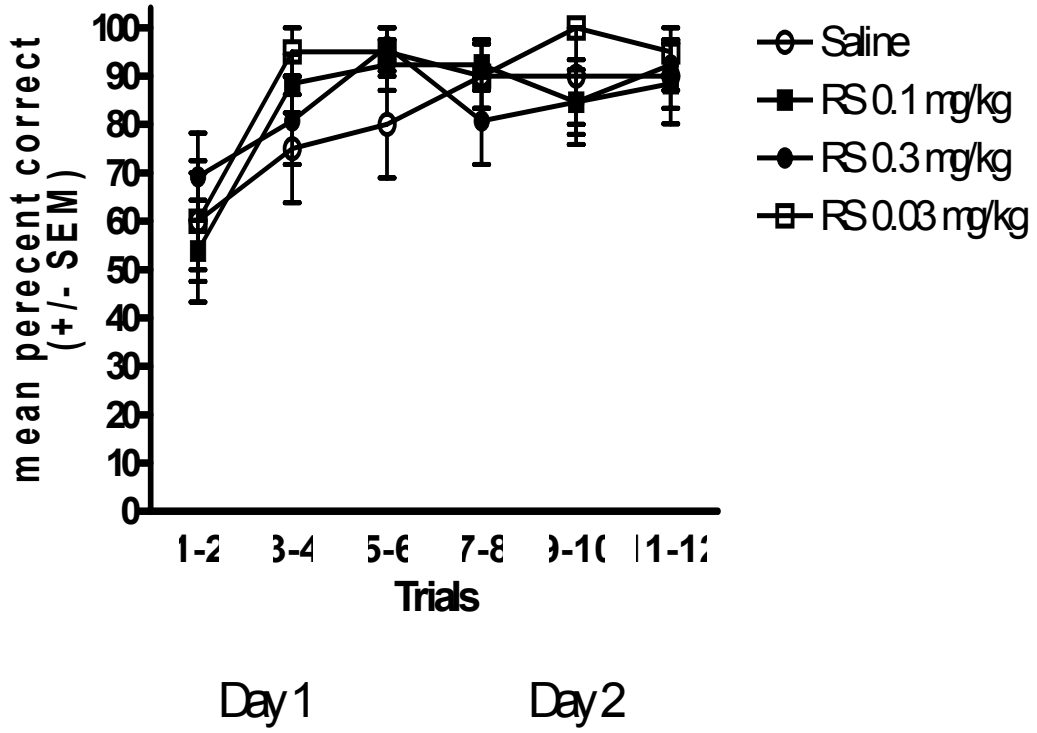

Figure A-2. Acquisition of the dual solution plus maze task during two days of drug free training.

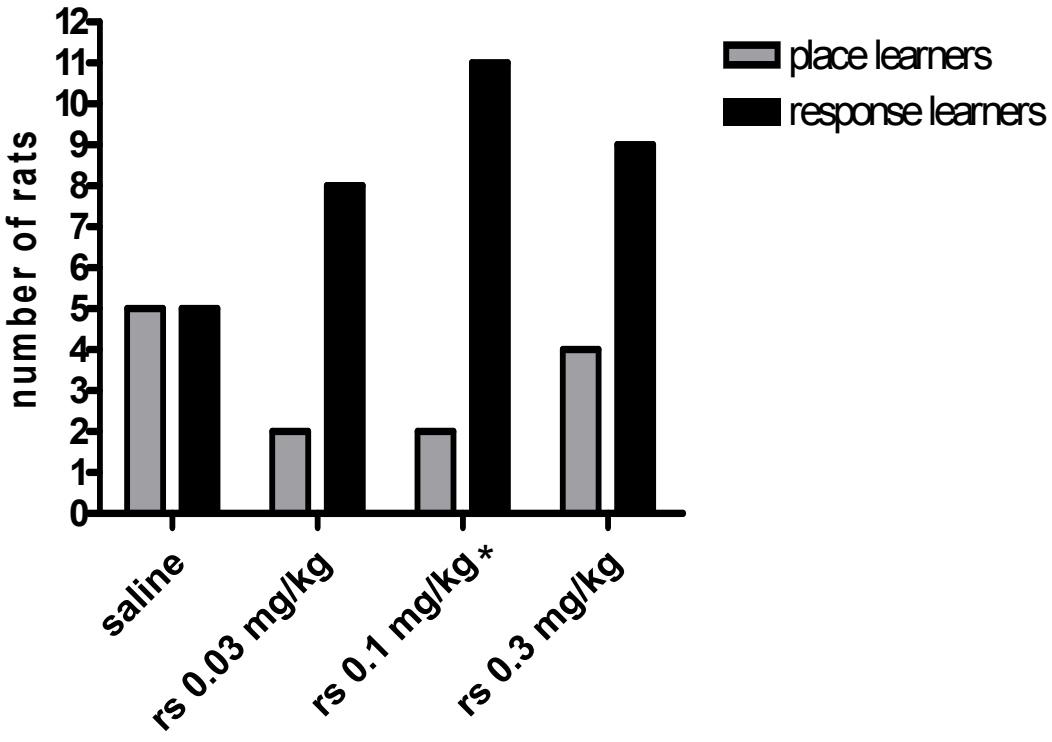

Figure A-3. Number of rats treated peripherally with saline or RS 79948-197 prior to retrieval classified as place or response learners on the day three probe trial. The asterisk indicates significance.

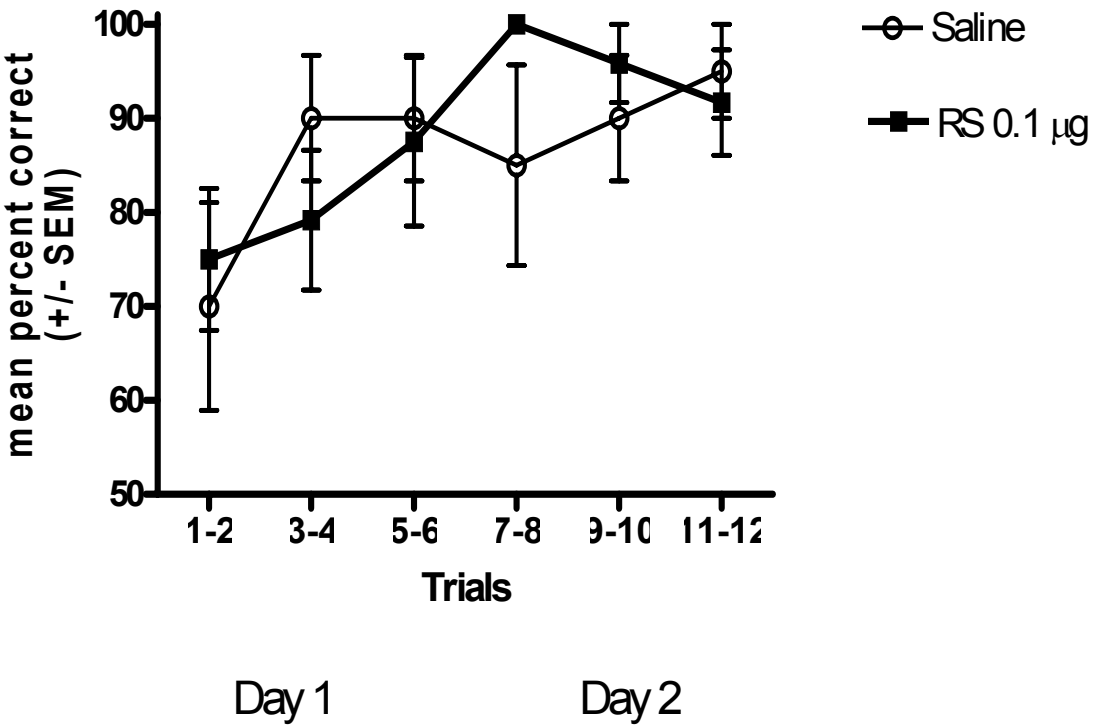

Figure A- 4. Acquisition of the dual solution plus maze task during two days of drug free training.

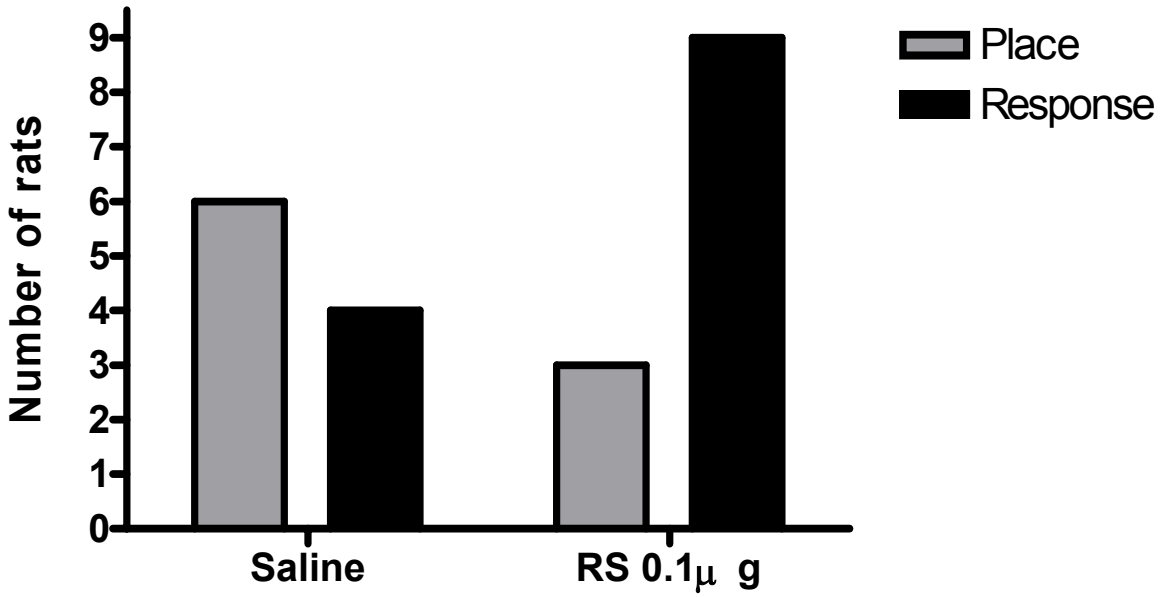

Figure A-5. Number of rats given intra-amygdala infusions of saline or RS 79948-197 prior to retrieval classified as place or response learners on the day three probe trial.

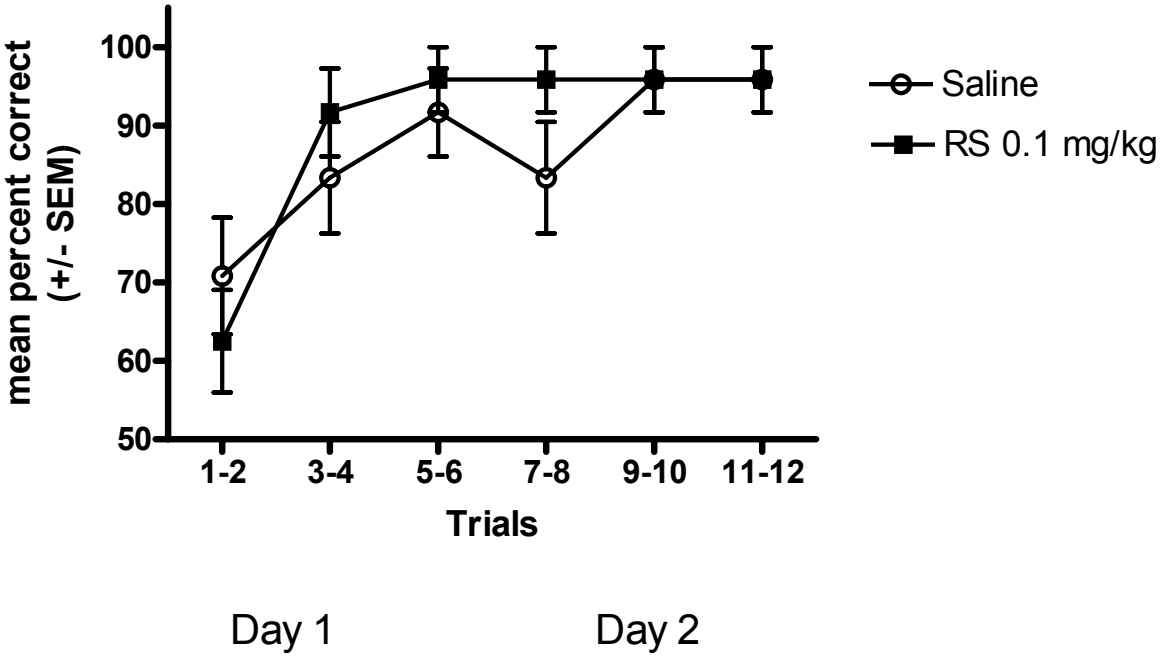

Figure A-6. The effects of pre-training peripheral injections of RS 79948-197 and saline on acquisition of water.

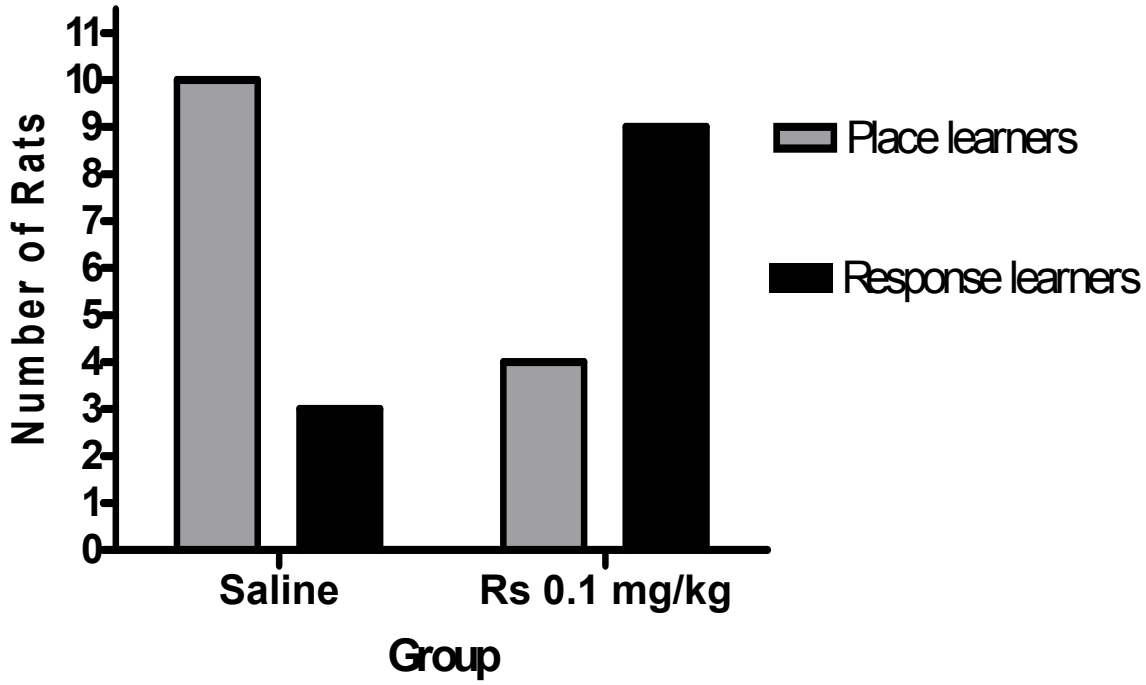

Figure A-7. Number of rats treated with peripheral saline or RS 79948-197 prior to training and retrieval classified as place or response learners on the day three probe trial.

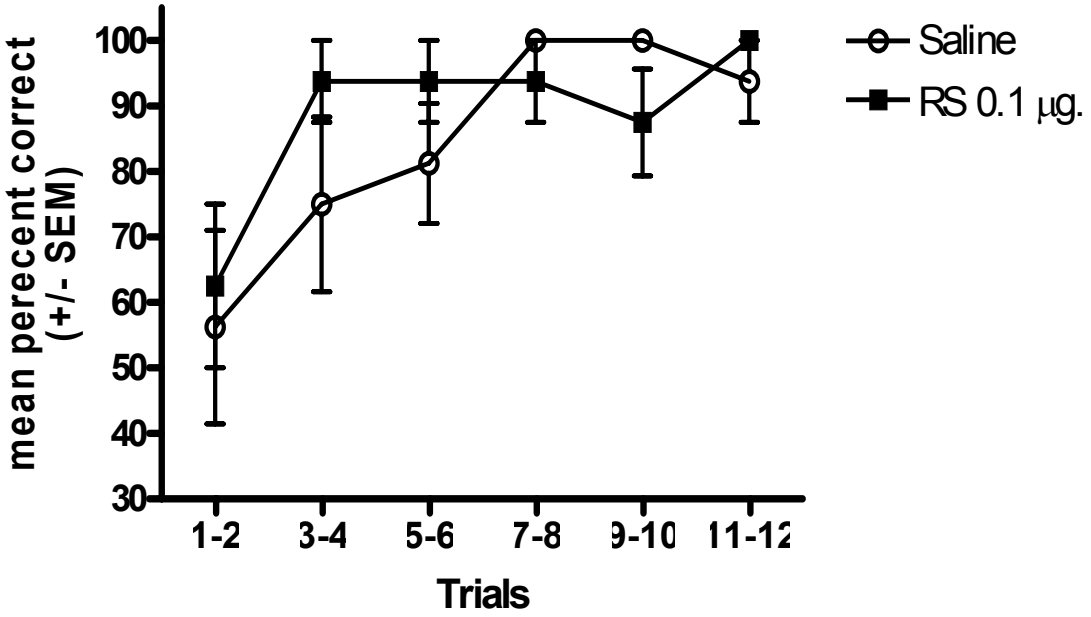

Figure A-8. The effects of pre-training intra-amygdala infusions of RS 79948-197 and saline on acquisition of water plus-maze behavior over two days of training (six trials per day).

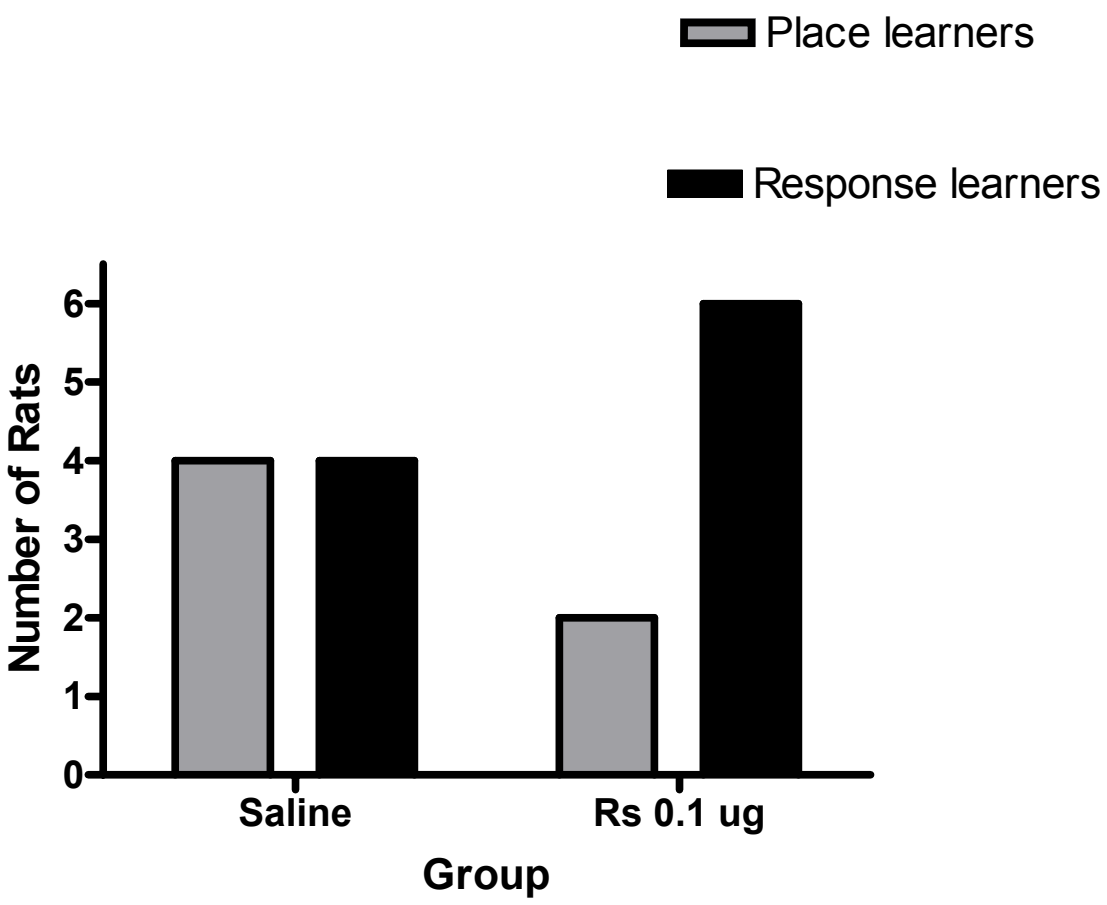

Figure A-9. Number of rats treated with intra-amygdala saline or RS 79948-197 prior to training and retrieval classified as place or response learners on the day three probe trial.

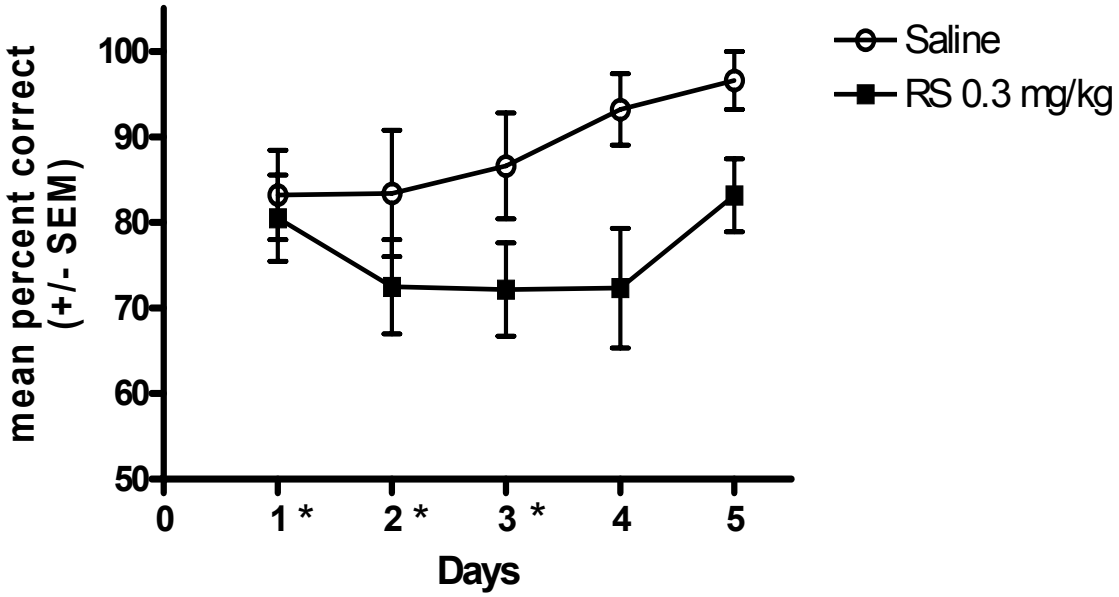

Figure A-10. Effects of post-training peripheral injections of saline or RS79948-197 (administered on training days1-3; asterisks), on acquisition of the single solution place water maze task.

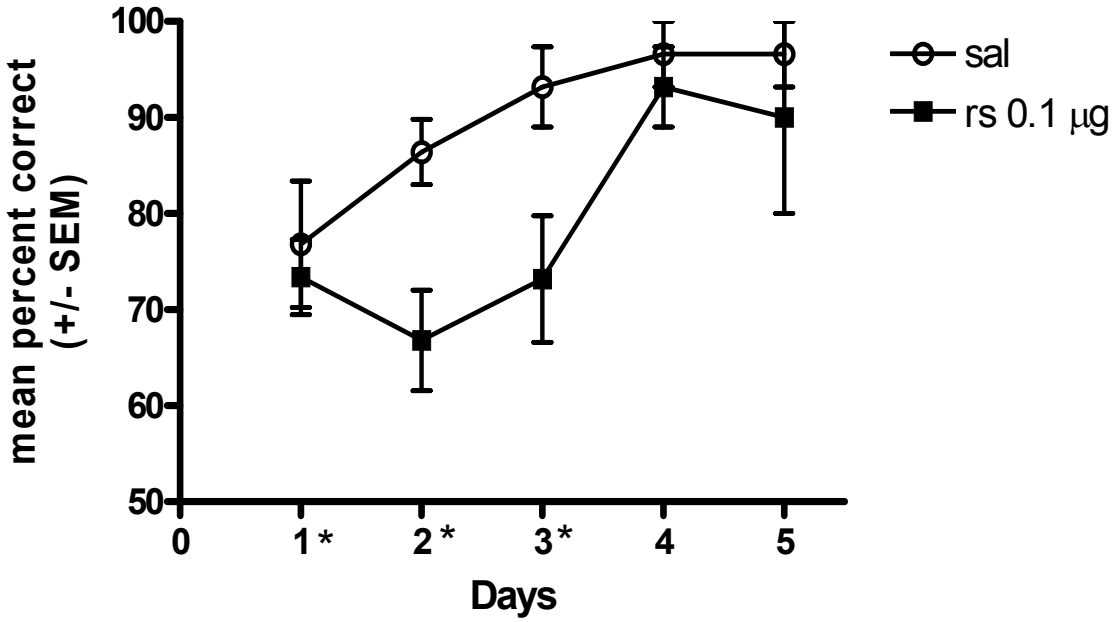

Figure A-11. Effects of pre-training intra-amygdala infusions of saline or RS79948-197 (administered on training days1-3; asterisks), on acquisition of the single solution place water maze task.

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