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Amygdala and "emotional" modulation of the relative use of multiple memory systems

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Abstract

The basolateral amygdala modulates the cognitive and habit memory processes mediated by the hippocampus and caudate nucleus, respectively. The present experiments used a plus-maze task that can be acquired using either hippocampus-dependent "place" learning or caudate-dependent "response" learning to examine whether peripheral or intra-basolateral amygdala injection of anxiogenic drugs would bias rats towards the use of a particular memory system. In Experiment 1, adult male Long–Evans rats were trained to swim from the same start point to an escape platform located in a consistent goal arm, and received pre-training peripheral injections of the α_2-adrenoceptor antagonists yohimbine (2.5 or 5.0 mg/kg), RS 79948-197 (0.05, 0.1, or 0.2 mg/kg), or vehicle. On a drug-free probe trial from a novel start point administered 24 h following acquisition, vehicle treated rats predominantly displayed hippocampus-dependent place learning, whereas rats previously treated with yohimbine (2.5, 5.0 mg/kg) or RS 79948-197 (0.1 mg/kg) predominantly displayed caudate-dependent response learning. In Experiment 2, rats receiving pre-training intra-basolateral amygdala infusions of RS 79948-197 (0.1 μg/0.5 μl) also predominantly displayed response learning on a drug-free probe trial. The findings indicate (1) peripheral injections of anxiogenic drugs can influence the relative use of multiple memory systems in a manner that favors caudate-dependent habit learning over hippocampus-dependent cognitive learning, and (2) intra-basolateral amygdala infusion of anxiogenic drugs is *sufficient* to produce this modulatory influence of emotional state on the use of multiple memory systems.

Keywords: Amygdala; Caudate nucleus; Striatum; Hippocampus; Emotion; Anxiety; Yohimbine; RS 79948; Memory

1. Introduction

Mammalian memory processes are organized in multiple brain systems that differ in terms of the type of memory they mediate. In rats, double dissociations of the mnemonic functions of the hippocampus and caudate nucleus in "cognitive" and stimulus–response "habit" learning tasks have been demonstrated in studies employing reversible and irreversible lesion techniques (e.g., Kesner, Bolland, & Dakis, 1993; McDonald & White, 1993; Packard, Hirsh, & White, 1989; Packard & McGaugh, 1992), as well as post-training intracerebral drug treatments (e.g., Packard, Cahill, & McGaugh, 1994; Packard & Teather, 1997, 1998; Packard & White, 1991). In addition, evidence indicates that in some learning situations, the basolateral amygdala modulates memory processes occurring in *other* brain structures (for reviews, see McGaugh, 2002; Packard, Cahill, Williams, & McGaugh, 1995). This idea has recently been examined in the context of the multiple memory systems hypothesis of memory organization. Both the hippocampal system and caudate nucleus receive amygdala projections (originating in basolateral/lateral amygdala nuclei; e.g., Kita & Kitai, 1990; Krettek & Price, 1978; Pitkanen, Pikkarainen, Nurminen,

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& Ylinen, 2000), and post-training intra-basolateral amygdala infusion of the indirect catecholamine agonist D-amphetamine enhances both hippocampus-dependent and caudate-dependent memory (Packard et al., 1994; Packard & Teather, 1998).

Previous lesion and drug studies indicate that in many learning situations the hippocampus and caudate nucleus can act independently to acquire cognitive and habit tasks, respectively. However, the intact brain ultimately functions in an integrated fashion to process various types of information, and it is therefore likely that relatively independent memory systems may also interact (for reviews, see Packard & Knowlton, 2002; Poldrack & Packard, 2003).

Previous studies identifying a memory modulatory effect of the basolateral amygdala on hippocampal and caudate memory processes have used learning tasks that *require* the use of one of the two memory systems (i.e., hippocampus-dependent cognitive learning, or caudate-dependent habit learning; Packard et al., 1994; Packard & Teather, 1998). However, in some learning situations both the hippocampal and caudate nucleus memory systems may provide an adequate solution. For example, rats trained in a plus-maze to obtain food from a consistently baited maze arm (west) by starting from the same start arm (south), can acquire this task with a hippocampus-dependent "place" memory (i.e., approach the goal box based on knowledge of the spatial location of the reinforcer), or a caudate-dependent habit or "response" memory (i.e., turn left at the choice point and approach the goal box). When administered a probe trial in which they are allowed to approach the baited arm from a novel start position (north), neural inactivation of the caudate nucleus results in place learning behavior, and inactivation of the hippocampus results in response learning behavior (Packard & McGaugh, 1996). Therefore, an important question concerns the potential influence that the basolateral amygdala may exert on these two memory systems in tasks that can be *simultaneously* acquired by the hippocampus and caudate nucleus. In considering this question, it is of interest to note that in addition to mnemonic functions, the amygdala has also been historically implicated in the neurobiology of mammalian emotion (e.g., Kluver & Bucy, 1939; Weiskrantz, 1956). In rats, the basolateral amygdala regulates drug-induced anxiety (Nagy, Zambo, & Decsi, 1979; Sanders & Shekhar, 1991, 1995; Scheel-Kruger & Petersen, 1982), and the memory modulatory role of the basolateral amygdala is closely tied to the function of various stress hormones (e.g., Gold & Van Buskirk, 1975; McGaugh & Roozendaal, 2002).

The question of how emotional state and the memory modulatory influence of the amygdala may affect the *relative* use of multiple memory systems has not been investigated. The present experiments examined whether induction of a drug-induced anxiogenic state can influence the use of multiple memory systems. A water plus-maze task was used in which rats were trained to swim from the same start arm (south) to an escape platform that was located in a consistent goal arm (west). Rats can acquire plus-maze behavior using hippocampus-dependent "place" learning (i.e., approach the goal arm based on knowledge of spatial location of the platform), or caudate-dependent "response" learning (i.e., turn left at the choice point and approach the platform). In Experiment 1, rats received pre-training peripheral injections of the α_2-adrenoceptor antagonists yohimbine or RS 79948-197, at doses previously shown to induce anxiogenesis in rats (e.g., Guy & Gardner, 1985; Handley & Mithani, 1984; White & Birkle, 2001). Following acquisition, the use of hippocampus-dependent place learning or caudate-dependent response learning was examined on a drug-free probe trial. In Experiment 2, the effects of pre-training intra-basolateral amygdala infusions of RS 79948-197 on the relative use of multiple memory systems was examined in order to determine whether intra-amygdala infusion of an anxiogenic drug is sufficient to mediate the influence of emotional arousal on the use of multiple memory systems.

2. Materials and methods

2.1. Subjects

Subjects were 109 male Charles River Long–Evans rats (weighing 275–325 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 7 a.m.). All experiments were conducted during the light phase of the cycle.

2.2. Apparatus

Animals were trained in a black circular water maze (1.83 m diameter, 0.58 m in height; 25 °C 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 surrounded by a square-shaped array of dark curtains hanging form the ceiling to the floor. Extra-maze visual cues consisting of various geometric shapes were placed on the curtains. None of these cues were placed in a spatially congruent/proximal position with the ends of the plus-maze arms. During training, an invisible black Plexiglas escape platform (11×14×19 cm) was consistently located at the end of one arm of the maze (west), 1 cm below water level. The arm opposite the start-arm was blocked off by an additional piece of Plexiglas, such that rats were trained with the maze in a "T" configuration. Swim behavior was observed via an overhead video camera and TV

monitor (HVS Systems) by an experimenter standing outside the curtains.

2.3. Surgery and histology

Prior to surgery rats were anesthetized with an intraperitoneal injection of a ketamine (100 mg/kg)–xylazine (50 mg/kg) cocktail. Rats were implanted with bilateral guide cannulae in the basolateral amygdala using standard stereotaxic surgery techniques. Guide cannulae (23 gauge, 15 mm length) were anchored to the skull with jeweler's screws and dental acrylic. Stereotaxic coordinates for basolateral amygdala were anterior–posterior (AP) = −2.2 mm from bregma, medial–lateral (ML) = ±4.7 mm, and dorsal–ventral (DV) = −7.0 mm. These coordinates were selected based on our previous research (e.g., Hsu, Schroeder, & Packard, 2002; Packard & Teather, 1998). Animals were given six days of post-operative recovery prior to behavioral testing.

Following behavioral testing, rats were deeply anesthetized with a 1-ml injection of ketamine (100 mg/kg)–xylazine (50 mg/kg) cocktail 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 40 μm and every third 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 (1986). Rats with inaccurate cannula placements ($n = 5$) were excluded from the statistical analysis. As illustrated in Fig. 1, the injection needle tips were located in the basolateral amygdala ranging from 1.80 to 3.80 mm AP from bregma.

2.4. Drugs/injection procedures

Yohimbine hydrochloride (Tocris Chemicals, 2.5 and 5.0 mg/kg), an α_2-adrenoceptor antagonist, was dissolved in a vehicle solution consisting of 45 g of 2-hydroxypropyl-β-cyclodextrin (HBC) per 100 ml of physiological saline. HBC is a commonly used non-toxic carrier that enhances the solubility of drugs without altering their bioeffects (e.g., Pitha & Pitha, 1985). RS 79948-197 hydrochloride (Tocris Chemicals, 0.05, 0.1, and 0.2 mg/kg), a recently developed, highly selective α_2-adrenoceptor antagonist, was dissolved in physiological saline. The peripheral doses were selected based on previous evidence of their anxiogenic properties in rats (Guy & Gardner, 1985; Handley & Mithani, 1984; 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 and 0.2 μg) or vehicle saline were administered via guide cannulae using 30-gauge injection needles connected by polyethylene tubing to 10-μl Hamilton microsyringes. Infusions were delivered over 52 s with an electronically controlled syringe pump (Sage Instruments, Boston), and the injection needles were left in place an additional 60 s post-infusion to allow for diffusion of solution away from injection tip.

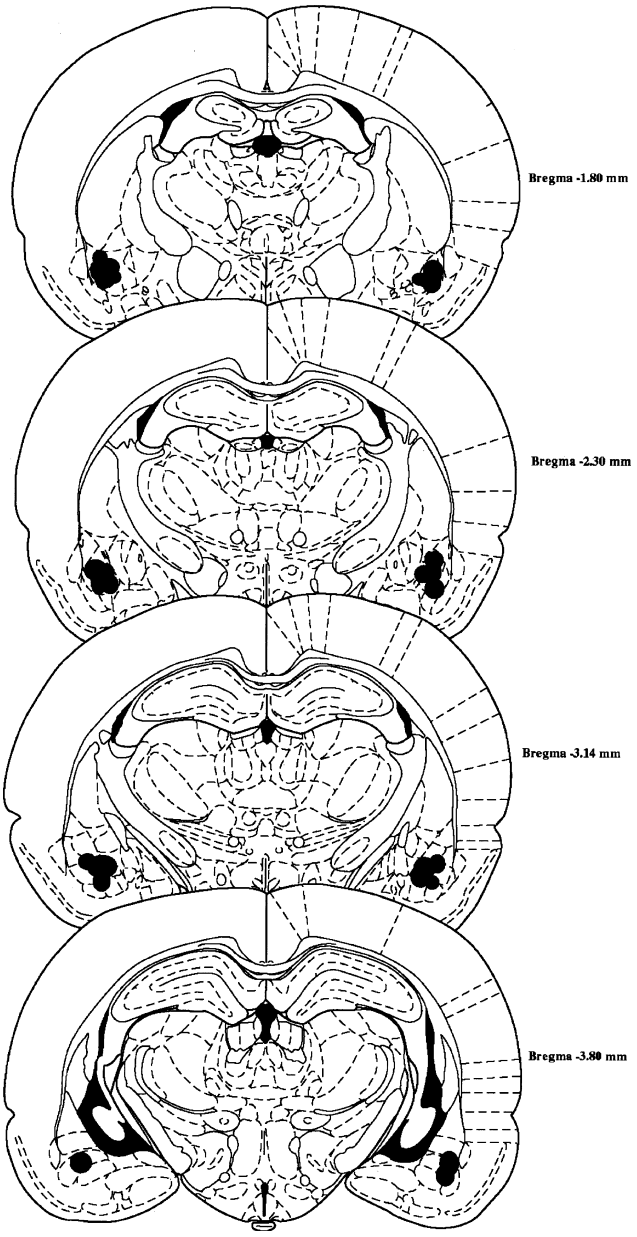

Fig. 1. Location of bilateral injection needle tips in the basolateral amygdala (shown with overlap). Infusion needles were located in the basolateral nucleus ranging from −1.80 to −3.80 mm AP from bregma. Adapted from the rat brain atlas of Paxinos and Watson (1997).

2.5. Behavioral procedures

The water plus-maze behavioral procedures were modified from our previous research using the appetitive version of the plus-maze task (Packard, 1999; Packard & McGaugh, 1996). Rats were trained in the water

plus-maze task for 2 consecutive days. Each training session consisted of six trials, separated by an inter-trial interval of 30 s. During training rats were placed in the start arm of the maze (south), 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 contained 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 10 s before being returned to their cages. Rats that failed to find the escape platform within 60 s were manually guided to it. Prior to training (30 min prior for peripheral injections, 2 min prior for intra-amygdala injections), rats received their assigned drug treatments; (peripheral yohimbine, 2.5 mg/kg, $n=10$, 5.0 mg/kg, $n=11$; peripheral RS 79948-197, 0.05 mg/kg, $n=11$, 0.1 mg/kg, $n=13$, 0.2 mg/kg, $n=12$; intra-amygdala RS 79948-197, 0.1 μg, $n=10$, 0.2 μg, $n=9$) or vehicle injections (peripheral vehicle $n=13$; intra-amygdala vehicle, $n=15$).

On day 3 rats were tested on a probe trial in order to determine their relative use of "place" and "response" learning. No drugs were administered prior to the probe trial. On this trial rats were placed into the start box 180° opposite that used during training (i.e., end of the north arm) and were allowed to make an entry into either the west or east maze arm. A Plexiglas shield blocked the south arm during the probe trial. Rats entering the west arm on the probe trial were designated place learners (i.e., rats going to the place where the escape platform was located during training), and rats entering the east arm on the probe trial were designated response learners (i.e., rats making the same body turn response that had been reinforced during training).

3. Results

3.1. Effects of peripheral yohimbine on plus-maze behavior

The effect of peripheral injections of yohimbine on initial acquisition of plus-maze behavior during the 2 days of training is illustrated in Fig. 2. A two-way one-repeated measures ANOVA comparing vehicle-treated and yohimbine groups on percentage of correct responses for trials 2–12 revealed no significant group effect ($F_{2,31}=1.86$, n.s.), or group×trial interaction ($F_{20,310}=.385$, n.s.). A significant trial effect ($F_{10,310}=3.78$, $p<.01$) indicated that all groups improved over training. These findings indicate that yohimbine did not affect the rate of acquisition of plus-maze behavior relative to vehicle-injected controls. Therefore, any differences between groups in subsequent probe-trial behavior cannot be due to a differential rate of task acquisition.

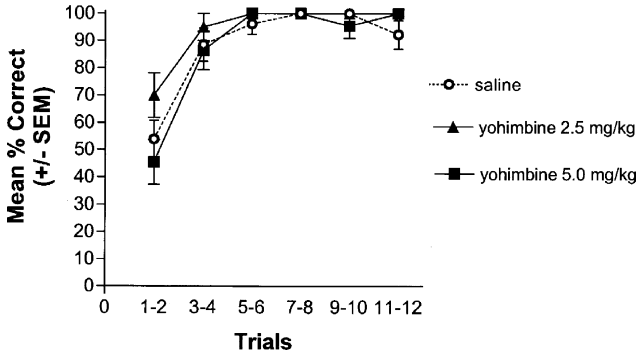

Fig. 2. Effects of pre-training peripheral injections of yohimbine on acquisition of water plus-maze behavior over two days of training (six trials per day). Saline and yohimbine treated rats showed rapid acquisition of the task, reaching asymptote performance on day 1 of training.

The effect of peripheral yohimbine on the relative use of "place" and "response" learning on the day 3 drug-free probe trial is shown in Fig. 3. To examine the use of place or response learning in control and drug-treated rats, χ^2 analyses were computed on the probe trial choice behavior. Rats that entered the west arm, (i.e., approached the spatial location that the platform had been located during training), were designated "place" learners. Rats that entered the east arm, (i.e., executed the same left body turn response that had been reinforced during training), were designated "response" learners. The χ^2 analyses revealed that vehicle-treated rats tended to display place learning on the probe trial (9 place rats, 4 response rats), although the analyses did not reach significance ($\chi^2=1.92$, n.s.). In contrast, peripheral yohimbine-treated rats displayed a highly significant use of response learning on the probe trial at both the 2.5 mg/kg dose (9 response rats, 1 place rat; $\chi^2=6.4$,

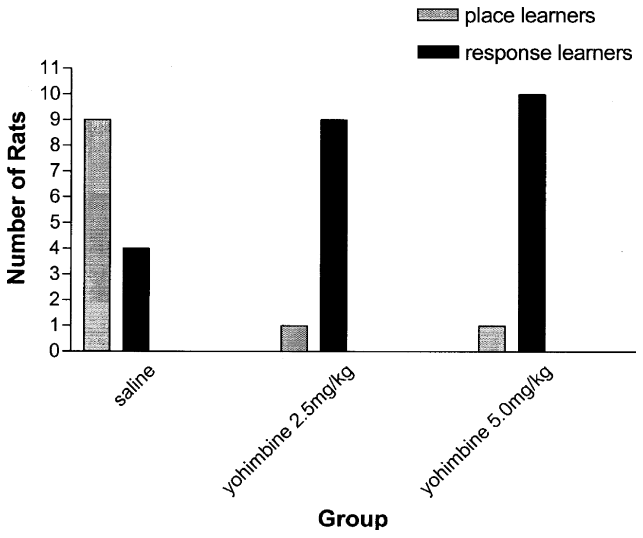

Fig. 3. Number of previously treated peripheral saline or yohimbine rats classified as place or response learners on the day 3 drug-free probe trial.

$p < .05$), and the 5.0 mg/kg dose (10 response rats, 1 place rat; $\chi^2 = 7.36$, $p < .05$). These findings indicate that in a plus-maze task in which both place and response learning provide an adequate solution, yohimbine influenced the type of information used in acquiring the task. Specifically, the drug-free probe trial behavior indicated that yohimbine treatment during training favored the relative use of response learning over place learning.

3.2. Effects of peripheral RS 79948-197 on plus-maze behavior

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 Fig. 4. A two-way one-repeated measures ANOVA comparing vehicle-treated and RS 79948-197 groups on percentage of correct responses for training trials 2–12 revealed no significant group effect ($F_{3,45} = 1.02$, n.s.), or group×trial interaction ($F_{30,450} = .55$, n.s.). A significant trial effect ($F_{10,450} = 3.62$, $p < .01$) 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 subsequent 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 drug-free probe trial is shown in Fig. 5. As described above, χ^2 analyses computed on the probe trial choice behavior revealed that vehicle-treated rats tended to display place learning on the probe trial (9 place rats, 4 response rats; $\chi^2 = 1.92$, n.s.). In contrast, RS 79948-197-treated rats (0.1 mg/kg) displayed a highly significant use of response learning on the probe trial (11 response rats, 2 place rats; $\chi^2 = 6.23$, $p < .05$). These findings indicate that in a plus-maze task in which both place and response learning provide an adequate solution, peripheral RS 79948-197 influenced the type of information used in acquiring the task. Specifically, the drug-free probe trial behavior indicated that RS 79948-197 treatment (0.1 mg/kg) during training favored the relative use of response learning over place learning. At doses of 0.05 and 0.2 mg/kg, RS 79948-197 treated rats displayed a tendency towards the predominant use of response learning, although the analyses did not reach significance (8 response rats, 3 place rats $\chi^2 = 2.27$, n.s., and 8 response rats, 4 place rats, $\chi^2 = 1.33$, n.s, respectively).

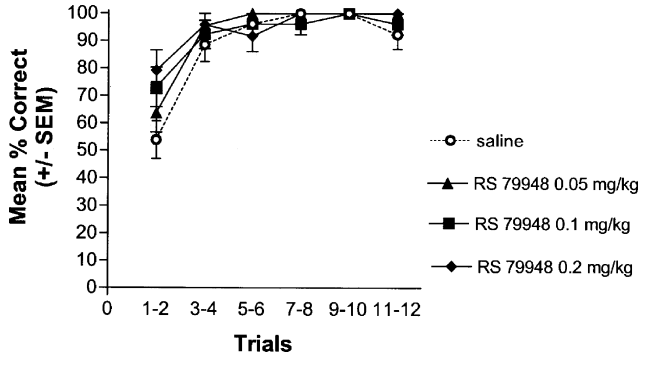

Fig. 4. Effects of pre-training peripheral injections of RS 79948 on acquisition of water plus-maze behavior over two days of training (six trials per day). Saline and RS 79948 treated rats showed rapid acquisition of the task, reaching asymptote performance on day 1 of training.

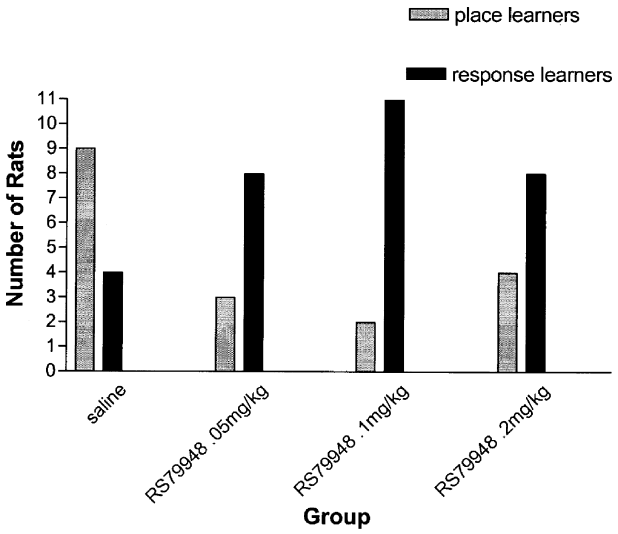

Fig. 5. Number of previously treated peripheral saline or RS 79948 rats classified as place or response learners on the day 3 drug-free probe trial.

3.3. Effects of intra-basolateral amygdala RS 79948-197 on plus-maze behavior

The effect of intra-basolateral amygdala injections of RS 79948-197 on initial acquisition of plus-maze behavior during the 2 days of training is illustrated in Fig. 6. A two-way one-repeated measures ANOVA comparing saline and RS 79948-197 groups on percentage of correct responses for training trials 2–12 revealed a significant group effect ($F_{2,31} = 5.49$, $p < .05$), and a significant group×trial interaction ($F_{20,310} = 2.24$, $p < .05$). However, tests of simple main effects (group within trial ANOVA's) showed that group differences in acquisition were in fact minimal, with a significant difference on trial 3 ($F = 4.68$, $p < .01$), and non-significant statistical "trends" on trial 2 ($F = 2.96$, $p = .07$, n.s.), and trial 4 ($F = 3.14$, $p = .06$, n.s.). Subsequent Scheffe's post hoc tests revealed that rats receiving the 0.2 μg dose of RS 79948-197 were impaired relative to saline-injected controls only on acquisition trial 3 ($F = 2.68$, $p < .05$). A significant trial effect ($F_{10,310} = 4.30$, $p < .01$) indicated that all groups improved with successive training trials, and both RS 79948-197 groups and the saline group

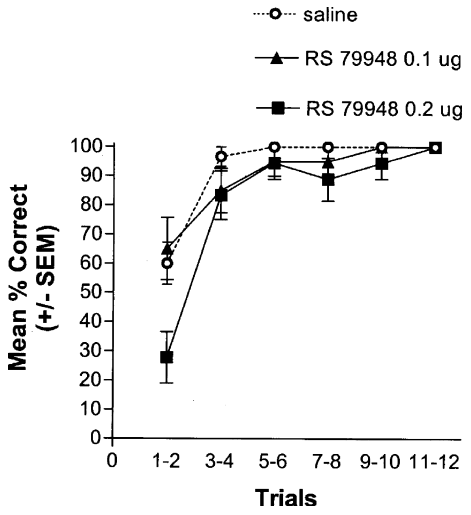

Fig. 6. Effects of pre-training intra-basolateral amygdala injections of RS 79948 on acquisition of water plus-maze behavior over two days of training (six trials per day). Saline and RS 79948 treated rats showed rapid acquisition of the task, reaching asymptote performance on day 1 of training.

displayed 100% correct performance on the last three trials (10, 11, and 12 of training).

The effect of intra-basolateral amygdala RS 79948-197 on the relative use of "place" and "response" learning on the day 3 drug-free probe trial is shown in Fig. 7. The probe trial choice behavior of intra-amygdala saline-treated rats was essentially split between the use of place and response learning (8 place rats, 7 response rats; $\chi^2 = 0.067$, n.s.). In contrast, intra-basolateral amygdala RS 79948-197-treated rats (0.1 μg) displayed a highly significant use of response learning on the probe trial (9 response rats, 1 place rat; $\chi^2 = 6.40$, $p < .05$). This effect was dose-dependent, as rats that had received intra-basolateral amygdala RS 79948-197 at a dose of 0.2 μg displayed similar levels of place and response learning on the probe trial. (5 response rats, 4 place rats $\chi^2 = 0.11$, n.s). These findings indicate that in a plus-maze task in which both place and response learning provide an adequate solution, intra-amygdala RS 79948-197 influenced the type of information used in acquiring the task. Specifically, the drug-free probe trial behavior indicated that RS 79948-197 treatment (0.1 lg) during training favored the relative use of response learning over place learning.

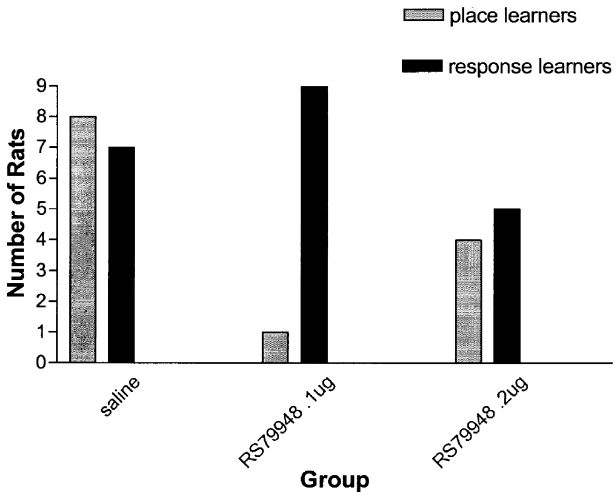

Fig. 7. Number of previously treated intra-basolateral amygdala saline or RS 79948 rats classified as place or response learners on the day 3 drug-free probe trial.

4. Discussion

The findings indicate that pre-training peripheral or intra-basolateral amygdala injections of α_2-adrenoceptor antagonists yohimbine or RS 79948-197 influence the relative use of multiple memory systems. Specifically, in a water plus-maze task that can be acquired using either hippocampus-dependent "place" learning or caudate-dependent "response" learning, rats that had been administered α_2-adrenoceptor antagonists prior to training acquired the task at a normal rate. However, on a drug-free probe trial, these rats demonstrated a robust use of response learning relative to saline-treated controls.

4.1. Peripheral injection of anxiogenic drugs and the relative use of multiple memory systems

The peripheral doses of yohimbine (2.5 and 5.0 mg/kg) that resulted in the use of caudate-dependent response learning also produce anxiogenic responses in rats tested in social interaction (Guy & Gardner, 1985), and maze exploration (Handley & Mithani, 1984) models of anxiety. The activity of yohimbine as an antagonist at presynaptic α_2-adrenoceptor sites, and concomitant increases in noradrenergic function, has also been related to anxiogenesis in humans (e.g., Charney, Heninger, & Redmond, 1983). However, it should be noted that other findings suggest that the anxiogenic properties of yohimbine may not be entirely due to an action on noradrenergic systems, and suggest a potential role for dopaminergic (Johnston & File, 1989) and GABAergic sites (Pellow, Chopin, & File, 1985). Therefore, in addition to yohimbine, the present study also examined the effects of RS 79948-197, a recently developed and highly selective α_2-adrenoceptor antagonist (Hume et al., 1996; Milligan et al., 1997). Peripheral injections of RS 79948-197 at a dose of 0.1 mg/kg also resulted in a significant use of response learning on the drug-free probe trial. Moreover, this dose of RS 79948-197 has anxiogenic properties in rats tested in an acoustic startle reflex test (White & Birkle, 2001). Taken together, the findings suggest a possible relationship between emotional state during training and the relative use of multiple memory systems. Specifically, they support the

general hypothesis that increasing levels of emotional arousal, at least to a particular threshold, may selectively impair "cognitive" memory function, and thereby favor the use of "habit" memory systems (e.g., Arnsten, 1998; Packard & Cahill, 2001).

4.2. Basolateral amygdala and modulation of multiple memory systems

Findings of numerous studies indicate a role for the basolateral amygdala in learning and memory processes. One such role involves amygdala mediation of stimulus-affect memory, as revealed by studies of acquisition and retrieval of Pavlovian fear conditioning (e.g., Davis, 1992; Fendt & Fanselow, 1999; Helmstetter, 1992; LeDoux, 1992, 1998), and stimulus-reward learning (e.g., Cador, Robbins, & Everitt, 1989; Everitt, Morris, O'Brien, & Robbins, 1991; Hiroi & White, 1991; Hsu et al., 2002; McDonald & White, 1993). In addition, in some learning situations, the role of the amygdala in memory involves modulation of memory processes occurring in *other* brain structures (e.g., McGaugh, 2002; Packard et al., 1994; Packard & Teather, 1998). For example, both the hippocampal system and caudate nucleus receive amygdala projections (originating in basolateral/lateral amygdala nuclei; e.g., Kita & Kitai, 1990; Krettek & Price, 1978; Pitkanen et al., 2000), and post-training intra-basolateral amygdala infusion of the indirect catecholamine agonist D-amphetamine enhances memory in both a hippocampus-dependent (stationary hidden platform) and a caudate-dependent (moving visible platform) water maze task (Packard et al., 1994; Packard & Teather, 1998). Each of these former water maze tasks *requires* the use of one of the two memory systems for acquisition, and therefore might be considered "single-solution" tasks. That is, in the hidden platform water maze task in which rats learn to approach an escape platform from multiple start points around the maze perimeter, there is no readily available "S–R habit" solution. Likewise, in the visible platform task in which the platform is moved among spatial locations on each trial, there is no readily available "cognitive" spatial solution.

In contrast, some learning situations might be considered "dual-solution" tasks, as the types of information acquired by both the hippocampus *and* caudate nucleus may each provide an adequate solution. For example, rats trained in a plus-maze to obtain food from a consistently baited maze arm (west) by starting from the same start arm (south), can acquire this task using hippocampus-dependent "place" memory (i.e., approach the goal box based on knowledge of the spatial location of the reinforcer), or a caudate-dependent habit or "response" memory (i.e., turn left at the choice point and approach the goal box). When administered a probe trial in which they are allowed to approach the baited arm from a novel start position (north), neural inactivation of the caudate nucleus results in place learning behavior, and inactivation of the hippocampus results in response learning behavior (Packard & McGaugh, 1996). Similarly, in the water plus-maze task used here, approach behavior to the escape platform can be acquired by employing either hippocampus-dependent place learning (Schroeder, Wingard, & Packard, 2002) or caudate-dependent response learning. The present findings indicate that in this type of dual-solution task, pre-training intra-basolateral amygdala infusion of the α_2-adrenoceptor antagonist RS 79948-197 resulted in a robust use of response learning on a drug-free probe trial. Thus, intra-basolateral amygdala infusion of RS 79948-197 mimicked the effect of peripheral administration of the drug, suggesting that blockade of α_2 adrenoceptors in this brain region is *sufficient* to bias rats towards the use of response learning relative to place learning.

The present findings also indicate a possible difference between the modulatory influence of the amygdala on behavior in single-solution and dual-solution tasks. Specifically, in our previous work using single-solution hidden platform and visible platform water maze tasks, intra-amygdala infusions of amphetamine enhanced both hippocampus-dependent and caudate-dependent learning (Packard et al., 1994; Packard & Teather, 1998). In contrast, in the current dual-solution task, intra-amygdala infusions of RS 77849 had a positive effect on the use of caudate-dependent learning (the possibility that this effect may have involved either an enhancing effect on caudate memory or an impairing effect on hippocampal memory is discussed below). It is not entirely clear why the modulatory influence of the amygdala may be different for single-solution and dual-solution tasks. It should be noted that different drug treatments (i.e., amphetamine versus RS 77948-197) and injection regimens (post-training versus pre-training infusions) were used in the previous and current studies, respectively. Perhaps in single-solution tasks, a high level of *relevant* task-related activity in the hippocampus or caudate nucleus selects for an enhancing modulatory influence of the amygdala. Future studies investigating the effects of intra-amygdala infusions of amphetamine on memory in dual-solution tasks, as well as examination of a more extensive dose–response curve for the effect of RS 79948-197 should be useful in clarifying this issue.

It should be noted that although the injection needle tips in the present study were located in the basolateral amygdala, the possibility of injection spread to other amygdala nuclei (e.g., central nucleus) could not be completely ruled out. However, converging evidence is consistent with the hypothesis that RS 79948-197 influenced multiple memory system use via an action originating in the basolateral amygdala. First, direct projections to the hippocampal system and caudate nucleus arise in the basolateral amygdala (e.g., Kita & Kitai, 1990; Krettek & Price, 1978; Pitkanen et al., 2000). Second, noradrenergic

drugs modulate memory when infused into the basolateral, but not central nucleus of the amygdala, and lesions of the basolateral, but not central nucleus block the memory modulatory influence of the amygdala on hippocampus-dependent memory (Roozendaal & McGaugh, 1996, 1997). Finally, the basolateral, but not central nucleus mediates the influence of the amygdala on hippocampal long-term potentiation, a candidate physiological mechanism of memory formation (Akirav & Richter-Levin, 2002; Ikegaya, Saito, & Abe, 1994).

The findings indicating that peripheral injections of doses of yohimbine and RS 79948-197 that possess anxiogenic properties produced a significant use of caudate-dependent response learning, suggest a relationship between emotional state and the relative use of different memory systems. Specifically, the results suggest that anxiety may influence the relative use of multiple memory systems. Consistent with this suggestion, we have recently observed that a pre-training stress regimen also biases rats towards the use of caudate-dependent habit learning in a water maze task in which both hippocampal and caudate based solutions are available (Kim, Lee, Han, & Packard, 2001). Other findings indicate that acute stress can enhance acquisition of classically conditioned responding (e.g., Shors, 2001). Of course, it is also possible that the anxiogenic properties of the peripherally injected drugs, and their influence on the use of cognitive and habit memory systems represent separate functions. The findings of experiment 2, in which RS 79948-197 was infused directly into the basolateral amygdala addressed this question more directly, as this brain site has been previously implicated in drug-induced anxiety (e.g., Nagy et al., 1979; Sanders & Shekhar, 1991, 1995; Scheel-Kruger & Petersen, 1982). In addition, in vivo microdialysis data indicate that anxiety-provoking immobilization stress (Tanaka et al., 1991) and aversive footshock stimulation (Galvez, Mesches, & McGaugh, 1996) increase norepinephrine release in the basolateral amygdala, an effect that would also presumably be produced in the present study by blockade of presynaptic α_2 adrenoceptors. Further research examining the effect of intra-amygdala RS 79948-197 in animal models of anxiety, and correlation of dose–response relationships between measures of anxiety and memory modulation following direct intra-amygdala drug infusion are necessary to strengthen the hypothesized relationship between emotional state and the modulatory influence of amygdala function on multiple memory system use.

4.3. Possible mechanisms of intra-amygdala RS 79948-197 on multiple memory system use

The ability of intra-amygdala infusions of RS 79948-197 to influence the type of learning used in a dual-solution plus-maze task could conceivably be due to an *enhancing* effect on caudate-dependent response learning. According to this hypothesis, the increase in norepinephrine release produced by blockade of presynaptic α_2 adrenoceptors in the amygdala (Starke, Gothert, & Kilbinger, 1989; Wamsley, Alburges, Hunt, & Bylund, 1992) would influence the activity of amygdalo-striatal pathways in a manner that would enhance mechanisms of synaptic plasticity underlying learning within the caudate nucleus. Amygdalo-striatal pathways are glutamatergic in nature, and intra-caudate infusions of glutamatergic agents modulate memory (Packard & Teather, 1997; Packard, Vecchioli, Schroeder, & Gasbarri, 2001), including response learning in an appetitive version of the plus-maze (Packard, 1999). Therefore, it is possible that noradrenergic activation of glutamatergic amygdalo-striatal projections could enhance caudate-dependent habit learning. Extensive evidence indicates a role for noradrenergic function in the basolateral amygdala in memory modulation (for reviews, see Ferry & McGaugh, 2000; McGaugh, 2002), however, it is currently unknown whether norepinephrine release in the basolateral amygdala activates amygdalo-striatal efferents.

Alternatively, intra-amygdala infusions of RS 79948-197 may have produced an *impairing* effect on hippocampus-dependent place learning, resulting in the ultimate use of caudate-dependent response learning. According to this hypothesis, the level of noradrenergic activation of amygdalo-hippocampal pathways produced by RS 79948-197 would impair mechanisms of synaptic plasticity underlying learning within the hippocampus. Several recent findings indicate that the basolateral amygdala can exert a facilitatory influence on hippocampal plasticity (i.e., long-term potentiation or LTP, Akirav & Richter-Levin, 1999, 2002; Ikegaya et al., 1994, 1995). This facilitatory influence in part involves activation of β-adrenergic receptors within the basolateral amygdala, as it is blocked by administration of propranolol (Ikegaya, Nakanishi, Saito, & Abe, 1997). However, recent evidence indicates that stimulation of the basolateral amygdala can have a biphasic effect, such that spaced activation can suppress induction of hippocampal LTP (Akirav & Richter-Levin, 1999). Moreover, this suppression is also mediated in part by norepinephrine, as depletion of this transmitter prevents this inhibitory effect on hippocampal synaptic plasticity (Akirav & Richter-Levin, 2002). Therefore, it is conceivable that a level of noradrenergic activation produced by intra-basolateral infusion of RS 79948-197 could impair hippocampal synaptic plasticity, thereby resulting in the predominant use of caudate-dependent response learning in the dual-solution plus-maze task used here. Ultimately, full dose–responses evaluations for the effects of intra-amygdala RS 779948-197 would be useful for determining whether the bias towards response learning produced by the drug in the present study involves an enhancing effect on striatal memory, or an impairing effect on hippocampal processing.

As both the hippocampus and caudate nucleus receive *direct* anatomical projections from the basolateral amygdala, it seems parsimonious to suggest that such projections likely mediate the functional influence of the basolateral amygdala on the two memory systems (in the present situation influencing the type of information used by either enhancing caudate memory processes, or impairing hippocampal memory processes). However, it should also be noted that the basolateral amygdala projects to other brain regions that influence behavioral and autonomic responses to stressors, including for example the frontal cortex (e.g., Arnsten, 1998). Further research is necessary to determine whether the basolateral amygdala influences the relative use of multiple memory systems via direct projections to the hippocampal system and/or caudate nucleus, or via projections to other brain regions that subsequently "select" for the use of hippocampus or caudate nucleus memory systems.

Finally, the role of the amygdala in mediating the link between emotion, memory, and human psychopathology has received considerable empirical and theoretical attention (for reviews, see Davis & Whalen, 2001; Lang, Davis, & Ohman, 2000). The present findings raise the speculation that the influence of the amygdala on the use of multiple memory systems may provide a neurobiological mechanism by which emotional experiences influence various psychopathologies. Further investigation of amygdala modulation of cognitive and habit memory systems may aid understanding of anxiety disorders in which emotional events are recalled in a maladaptive manner (e.g., post-traumatic stress disorder), or in which anxiety impairs cognitive control and may unmask maladaptive behaviors mediated by the caudate "habit" memory system (e.g., obsessive compulsive disorder, or stress-induced relapse in drug addiction).

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