Research report

Intra-amygdala infusions of scopolamine impair performance on a conditioned place preference task but not a spatial radial maze task

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Abstract

Lesions of the amygdala impair performance on a conditioned place preference (CPP) but not a spatial radial maze task. The role of cholinergic receptors within the amygdala in performance of these tasks was evaluated using intra-amygdala injections of the muscarinic receptor antagonist, scopolamine. Food deprived rats were trained on a CPP task, which consisted of four training trials on two arms of a radial eight-arm maze. One arm was consistently paired with a large amount of food (14 g) while the other arm was never baited. Prior to the fourth trial, rats received bilateral intra-amygdala infusions of the muscarinic receptor antagonist, scopolamine (SCOP; 5 μg/0.5 μl) or vehicle. On a retention test 24 h later, unoperated and vehicle-infused rats, but not SCOP-treated rats, spent significantly more time in the paired arm than chance (50%). Therefore, the scopolamine treatment appeared to block learning and/or memory on trial 4. The same rats were then trained on a radial maze task on the same apparatus, in which rats had access to all eight arms but only four were baited with food (1 pellet). Rats were trained until they reached criterion and then infusions were given prior to testing. SCOP treatment did not affect performance on the radial maze task. Thus, intact cholinergic mechanisms in the amygdala are necessary for learning or memory on a CPP task with a high reward component but not performance on a spatial radial maze task with a lower reward component. © 1998 Elsevier Science B.V. All rights reserved.

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1. Introduction

Considerable evidence suggests that the formation of different types of memory involves participation of different neural regions [14,22,26,38]. These findings imply that memory processing in the brain may be mediated by parallel, and possibly somewhat independent, systems. Most of the evidence for multiple memory systems is derived from tests of subjects with brain lesions. For example, the types of memory tasks that are affected with lesions of the hippocampus appear to include elements of spatial, contextual, allocentric, or relational learning [5,11,23,36]. Hippocampal lesions do not, however, appear to interfere severely with memory formation or storage for response, stimulus-response or egocentric learning [14,22,25]. In contrast, striatal lesions impair acquisition and retention of these latter classes of memory, but do not interfere greatly with acquisition and retention of hippocampal-dependent tasks [14,22,25].

Another class of memories appears to be dependent on the integrity of the amygdala. Tasks that are sensitive to amygdala lesions usually have high emotional content, as seen most often in aversive conditioning tasks. For example, lesions of the amygdala affect

memory for avoidance training [12] and fear conditioning [4,17]. However, an intact amygdala is not required for good performance on other tasks that are impaired by hippocampal or striatal lesions [22,35,38].

While most tests of amygdala function in learning and memory have involved training with aversive stimuli, there is also evidence that the amygdala is important for learning tasks that use appetitive motivation when the reinforcement is of high affective value [8,16,27]. Conditioned place preference (CPP), a behavioral task often used to measure the reinforcing properties of drugs [34], has been used to measure memory or learning of simple stimulus-reward associations. When a large reward is associated with a place, control animals acquire a preference for that place while animals with amygdala lesions do not show a preference [8,39]. Given these findings, McDonald and White (1993) proposed that a neural system that includes the amygdala is recruited in the acquisition of a CPP due to the affective or emotional nature of the large reward. This amygdala-dependent task together with win-shift (hippocampus-dependent) and win-stay (striatum-dependent) tasks were the basis for a triple dissociation of memory systems described by McDonald and White (1993) [22].

These and other neural dissociations [14,26,38] provide substantial evidence for multiple, independent neural systems of memory. However, little is known about the involvement of particular neurotransmitter systems within a given neuroanatomical region. Although systemically administered drugs appear to enhance or impair learning and memory for many types of tasks, drugs injected directly into a specific brain site appear to affect learning and memory for a restricted set of tasks [7]. Thus, as with lesions, it appears that task dissociations may be evident with drug injections into multiple, relatively independent, memory systems [7].

The purpose of the present experiment was to test a possible dissociation of the effects of a cholinergic antagonist injected directly into the amygdala on learning of a CPP and a spatial radial maze task. Specifically, the muscarinic antagonist scopolamine was bilaterally infused into the amygdalae of rats prior to training or testing. While the effects of cholinergic drugs on aversive tasks appear to involve the amygdala [3,31], cholinergic drug effects on spatial tasks do not seem to depend on the amygdala [31]. If cholinergic functions within the amygdala are important specifically for memory processes which involve high levels of arousal, with either aversive or appetitive motivators, then intra-amygdala scopolamine infusions would be expected to impair learning in a highly rewarded, appetitive CPP task, but would not be expected to affect a spatial maze task that employed a lower reward.

2. Materials and methods

2.1. Subjects

Adult male Sprague–Dawley rats (Hilltop breeders) were housed individually and maintained on a 12-h light/dark cycle (on at 07.00). All rats were allowed to adjust to their new environment, with ad libidum access to food and water for 1 week following arrival.

2.2. Surgery

All rats undergoing surgery received atropine sulfate (0.4 mg/kg, i.p.) prior to being anesthetized with sodium pentobarbital (50 mg/kg, i.p.). Stainless steel guide cannulae (22 gauge; Plastics One, Roanoke, VA) aimed dorsal to the lateral nucleus of the amygdala were implanted bilaterally. The stereotaxic coordinates were 0.2 mm posterior to bregma, +4.6 mm lateral, and 6.9 mm ventral from dura. The nose bar was set at 5.0 mm above the interaural line according to the atlas of Pellegrino et al. (1979) [28].

2.3. Intra-amygdala injections

Bilateral injections into the amygdala were administered through an inner cannula that extended 1.5 mm below the guide cannula. The 28 gauge inner cannula was attached by a polyethylene tube to a 25 μl Hamilton syringe. The syringe was driven by an infusion pump (Harvard Apparatus 22) with solutions administered for 1 min at a rate of 0.5 μl /min. Prior to removal, the injection cannula was left in place for an additional 1 min to allow diffusion of the drug. Scopolamine was mixed in artificial cerebrospinal fluid (aCSF) at a pH of 7.4. The dose of scopolamine (5 μg/0.5 μl) was chosen on the basis of pilot data showing that it was the highest dose that did not attenuate food consumption during behavioral testing; the interference with eating at higher scopolamine doses may result from blocked salivation [9].

2.4. Apparatus

An eight-arm radial maze, made of wood and painted black, was used for training. The maze was elevated 40 cm from the floor. The center platform was 34 cm in diameter. Each arm was 12 cm wide and 70 cm long. The room contained various extramaze cues and the maze was rotated 45° each day following training to reduce the utility of intramaze cues.

2.5. Behavioral procedures

Three groups of unoperated control rats were food deprived and handled daily (5 min/day). Rats receiving

surgeries were given 5 days to recover before being handled and food deprived. After 1 week of food restriction, rats weighed $85 \pm 2.14\%$ of their free-feeding body weights (mean initial weight $= 330 \pm 9$ g).

2.6. Conditioned place preference (CPP)

The procedures were similar to those described in White and McDonald (1993) [39]. Two arms of an eight-arm radial maze were randomly chosen and assigned to each rat for the duration of the experiment. The other six arms were not accessible from the center platform (Fig. 1a). The two selected arms were not adjacent or directly across from one another and the position of assigned arms was constant relative to the room over days of training and testing. One day prior to training, rats were given a habituation trial and were allowed to explore both of the assigned arms freely for 10 min.

For each rat, one of the selected arms was baited with 14 g of wheat puffs. One exposure to the arm paired with food and one exposure to the unpaired arm constituted a trial. Each 30-min exposure was given on consecutive days for a total of 2 days per trial. The order of presentation of paired and unpaired arms varied between rats. A retention test was given 24 h after the last day of training. On the test trial, both arms were open and no food was on the maze. Rats were free to move between the two arms for 20 min. Percent time spent in each arm was recorded. Unoperated control rats were given a retention test after receiving 2, 3, or 4 training trials (days: 5, 7, and 9; $n = 8$ for each group). Rats in drug and vehicle groups received bilateral infusions of the muscarinic antagonist, scopolamine ($n = 8$), or an equal volume of aCSF ($n = 8$), 15 min prior to training on both days of trial 4 (days 7 and

8) and were tested the following day (day 9). Drug infusions were given only prior to trial 4 in order to avoid damage from repeatedly disturbing the target region. Timing of injections was determined based on White and McDonald's (1993) [39] report that rats exhibited a significant preference for the paired arm after receiving no less than four training trials. As explained below, rats with ventral placements in the cortical nuclei were treated separately for both aCSF ($n = 4$) and scopolamine ($n = 4$) conditions.

2.7. Radial maze

Two days after CPP testing, the same rats were then trained on a spatial version of the eight-arm radial maze, using the same apparatus as above. All eight arms of the maze were accessible but only four were baited with food (1 wheat puff per arm). No more than two of the baited arms were directly across from each other or adjacent (Fig. 1b). The four selected arms remained constant relative to the room and to room cues over days of training and testing. Rats were allowed to enter all arms on training trials, and were not removed until all food was consumed or 15 min had elapsed. One training trial was given each day until rats made two errors or fewer across 2 consecutive days. Twenty-four hours later, infusions of scopolamine ($n = 6$) or aCSF ($n = 6$) were given 15 min prior to a retention test. Both working and reference memory components were observed separately in order to investigate the possibility that a dissociation between the two behavioral tasks may be due to a differential involvement of the amygdala in reference vs. working memory [38]. Working memory errors were recorded when rats repeated entries into baited arms and reference memory errors were recorded when rats entered arms that were never baited. Treatment condition was varied from CPP training to the radial maze task; i.e. some rats that were given scopolamine on the CPP task were infused with aCSF for the radial maze task and vice-versa.

2.8. Histology

After testing was completed for both experiments, rats received an overdose (i.p.) of sodium pentobarbital followed by a 0.5 μl injection of ink into each cannula. Intracardial perfusions were performed with 0.9% saline followed by a 10% formalin solution. Brains were removed and placed in a 30% sucrose/formalin solution.

In preparation for sectioning, brains were frozen at $-20°C$ and mounted on a Reicher-Jung cryostat. Forty-micrometer sections were taken beginning at the anterior amygdala, mounted onto slides, dried, and stained with cresyl violet. Fig. 2 illustrates the locations of injection sites and the diffusion of ink in the amygdalae of rats included in the experimental and control
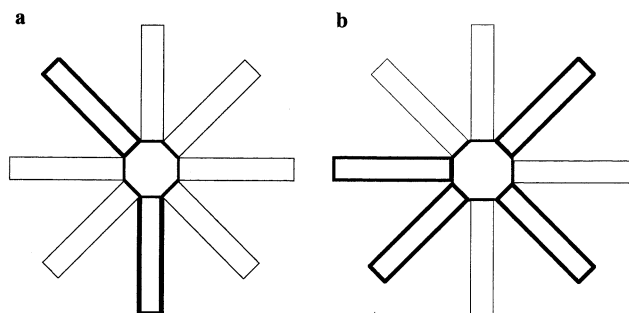

Fig. 1. a. CPP configuration of an eight-arm radial maze. Only the bold arms were accessible, all other arms were blocked off throughout the experiment. Both bold arms were accessible during the habituation and test trials, while only one of the bold arms was open each day during training. b. Spatial configuration of an eight-arm radial maze. All arms were open throughout the experiment, however, only the bold arms were baited. The same four arms were baited each day for a given rat. No more than two arms were adjacent or directly across from one another.
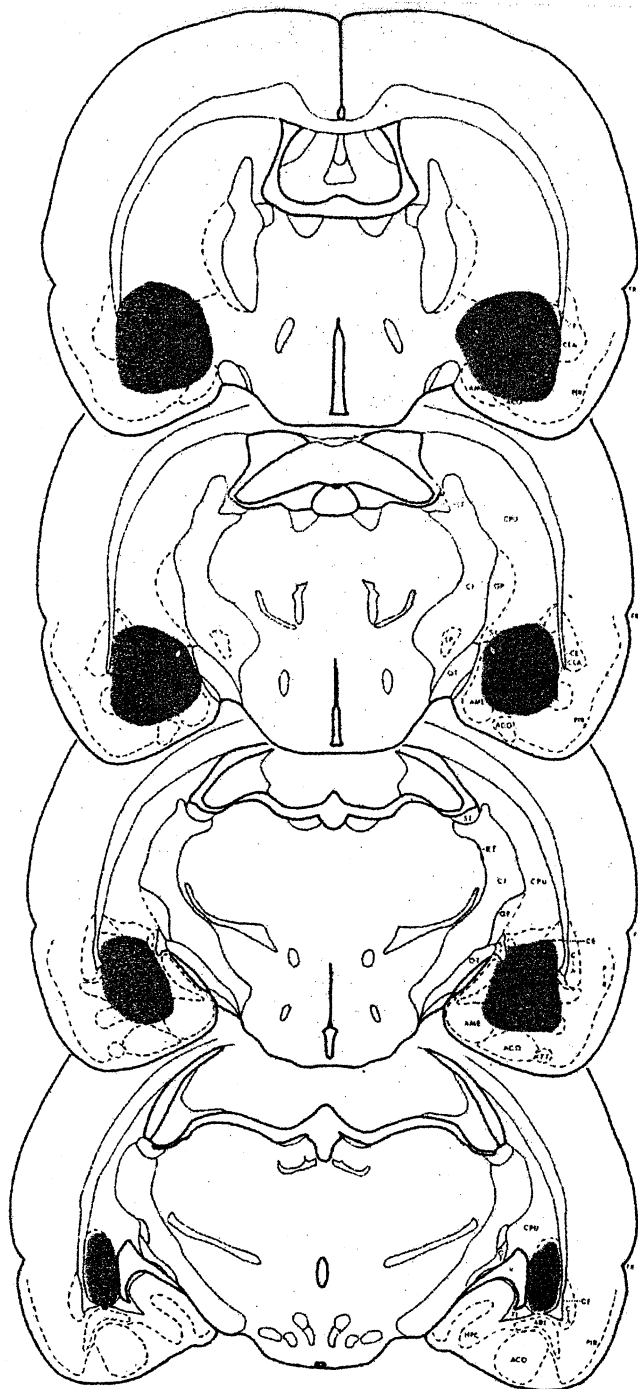
Fig. 2. Location of the injection cannula tips and diffusion of ink in the amygdala for rats included in the analysis of drug vs. control conditions. Rat brain sections were taken from the stereotaxic atlas of Pellegrino et al. (1979) [28].

conditions. Behavioral scores for rats with cannulae outside this area were excluded from the grouped data analyses. Although cannulae were aimed at the lateral nucleus of the amygdala, data obtained from rats with other injection sites within the amygdala were included in the analyses. An exception was that rats with placements in the cortical nuclei were treated separately, as

early data suggested that placements in this region resulted in impaired performance in both aCSF and scopolamine groups. Because these ventral placements passed through more dorsal regions of the amygdala, including the lateral and basolateral nuclei, it is likely that the damage from these cannula placements resulted in the behavioral deficits seen in both control and treatment groups.

2.9. Statistical analyses

Conditioned place preference results were analyzed with one sample, two-tailed t-tests comparing percent time spent in each arm to chance (50%). Between-group comparisons were done for percent time spent on the paired arm with two-sample, two-tailed t-tests. Consumption was compared between controls and drug-treated animals with a two-sample, two-tailed t-test. Correlations between food intake and time spent on the paired arm within groups were also analyzed. Unpaired, two-tailed t-tests were used in the analysis of errors for the two groups on the radial maze test, following infusions.

3. Results

3.1. CPP

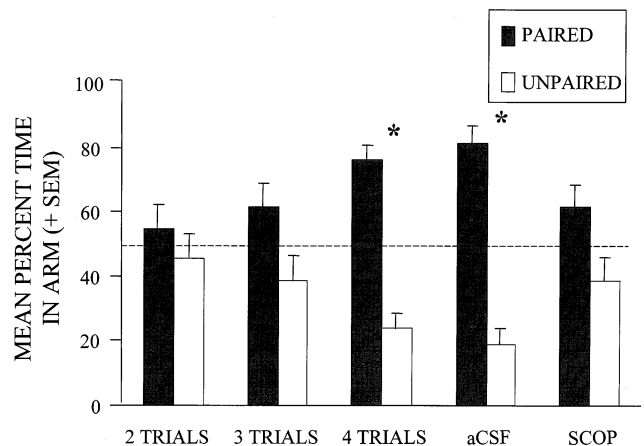Rats treated with scopolamine prior to trial 4 did not show a significant preference for the arm that was

Fig. 3. Effects of intra-amygdala scopolamine infusions on the CPP task. Mean percent time spent in the paired arm is represented by dark bars while mean percent time spent in the unpaired arm is represented by light bars. Note that dark and light bars equal 100% for each group. After receiving four training trials, unoperated and aCSF rats, but not scopolamine-treated rats, spent significantly more time than chance in the paired versus unpaired arm. Notice that rats that received scopolamine on trial 4 appeared to spend an amount of time in the paired arm similar to that seen in unoperated controls after only three training trials. * $P < 0.05$ versus 50% (chance)
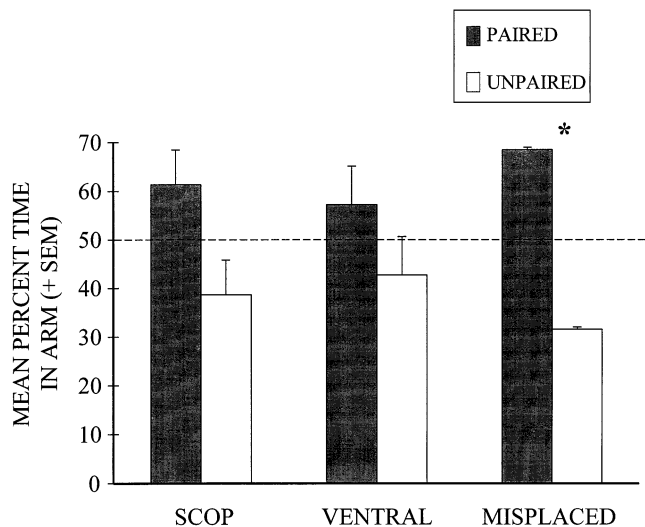
Fig. 4. Effects of misplaced infusions of scopolamine. Rats receiving scopolamine infusions ventral to the amygdala were impaired on the CPP task. However, scopolamine administered rostral or dorsal to the amygdala apparently did not interfere with the acquisition or expression of a preference. $P < 0.05$ versus 50% (chance).

3.2. Radial maze

Findings for the spatial radial maze task are displayed in Fig. 5. A comparison of mean number of errors recorded during the test day on the radial maze task for both aCSF and scopolamine-treated rats revealed that there were no significant differences between the aCSF and scopolamine-treated rats (mean errors $\pm$ SEM $= 1.67 \pm 0.98$ and 1.67 ± 0.75, respectively, $t(5) = 0$). Type of errors made on the test day (working vs reference) did not differ between the two groups. Both groups made a total of six working memory errors and one reference memory error.

4. Discussion

Intra-amygdala infusions of scopolamine impaired performance on a conditioned place preference task but did not impair performance on a spatial task with lower reward on the same maze. Rats given infusions of scopolamine prior to trial 4, unlike controls, failed to show a significant preference for the paired arm on the CPP task. In fact, mean percent time spent in the paired arm was similar to that of unoperated control rats who received only three training trials. This suggests that the scopolamine treatment blocked learning and/or memory on trial 4. Performance on the spatial radial maze task was not affected by intra-amygdala infusions of the same dose of scopolamine that impaired performance on CPP. Therefore, muscarinic receptors within the amygdala do appear to be involved in learning or memory for a conditioned, or 'stimulus reward (White and McDonald, 1993, [39])' type task, but are not necessary for good performance on a spatial-type radial maze task on the same maze.

previously paired with food (mean $\% \pm$ SEM $= 61.375 \pm 7.15$, $t(7) = 1.59$, $P > 0.1$), as shown in Fig. 3. Rats receiving aCSF, like unoperated controls, did display a significant preference for the paired arm after receiving four training trials (aCSF: 81.375 ± 5.22, $t(7) = 6.01$, $P < 0.001$; Unoperated controls: 76.13 ± 4.80, $t(7) = 5.44$, $P < 0.001$). Furthermore, there were no significant differences between unoperated controls after receiving only three training trials and rats infused with scopolamine prior to training trial 4 ($t(14) = 0.01$, $P > 0.99$). Between-group comparisons revealed a significant difference in time spent in the paired arm between aCSF and scopolamine-treated rats after receiving four training trials ($t(7) = 3.34$, $P < 0.02$).

Fig. 4 shows that scopolamine-injected rats with placements outside of but not ventral to the amygdala did show a significant preference for the paired arm (68.5 ± 0.50, $t(1) = 37.00$, $P < 0.02$). In contrast, when cannulae went through the amygdala and infusions were ventral to the lateral and basolateral nuclei, mean percent time spent in the paired arm was not significantly different from chance whether rats were treated with scopolamine or aCSF (57.25 ± 7.91, $t(7) = 0.92$, $P > 0.3$).

Amount of food consumed on the day of drug injection was significantly lower for rats receiving scopolamine than for those receiving aCSF ($t(7) = 5.03$, $P < 0.001$). However, the correlation between time spent on the paired arm and amount of food consumed on the drug day within the scopolamine group was not significant ($r = 0.34$, $P > 0.4$).
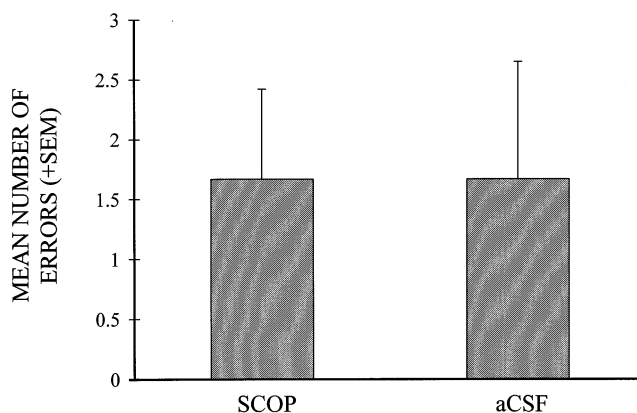

Fig. 5. Effects of intra-amygdala scopolamine infusions on the spatial radial maze task. Bars represent mean number of errors on the spatial radial maze task for rats receiving aCSF and scopolamine infusions. Notice that the mean is the same for both groups.

The results of the present study are consistent with the lesion data reported by McDonald and White (1993) [22] and White and McDonald (1993) [39]. The dissociation seen with scopolamine administration, like lesions, suggests that an important difference exists between the two behavioral tasks used. This difference might be the magnitude of reward. Food-deprived rats were reinforced on the CPP task with 14 g of wheat puffs on one arm, but were reinforced with only one wheat puff on each arm for the radial maze task. Thus, the high level of arousal elicited by the CPP task may be what is necessary for amygdala involvement in learning and/or memory, while the less arousing, spatial, radial maze task is handled by a separate neural system, such as the hippocampus [22,27,39]. Supporting this notion is evidence that responses to increases or decreases in magnitude of reward are disrupted with amygdala lesions in monkeys and rats [6,10,13,32,33]. The amygdala's involvement in the learning of affective information has also been demonstrated in humans. Patients with damage to the amygdaloid region exhibit deficits in memories with emotional content when compared with normal subjects [2], and in some cases demonstrate better memory for neutral material than for emotional material [1,20].

Most variables remained constant across behavioral paradigms, including the room and room cues, animals, the maze used for training and testing, and motor and perceptual requirements. Thus, the effects of scopolamine treatment on general performance and arousal should have been exhibited in both tasks. Drug infusions were done on the test day for the radial maze task, requiring that rats recall the location of food from previous trials as well as where they have been within the trial. No impairment was observed in scopolamine-treated rats on performance of the spatial radial maze task, indicating that intra-amygdala scopolamine infusions do not affect general performance or learning of such spatial material. While we believe the dissociation across tasks reflects differences in the type of learning across tasks, it is possible that differences in the extent of learning also contributed to the dissociation.

Cannulae were aimed at the lateral nucleus of the amygdala because of the high density of M2 muscarinic receptors in the lateral and basolateral nuclei [21], and because the lateral nucleus has been implicated in learning of simple stimulus-reward associations [8]. However, rats with cannulae directed toward other nuclei of the amygdala, with the exception of cortical nuclei, were included in the analysis. Observations of ink infusions suggest that diffusion occurred throughout the various nuclei but was confined to the amygdala. Although the rate of diffusion of the ink may not be comparable to that of scopolamine, autoradiography studies indicate that 1-μl injections of 2-amino-5-phosphonopetanoic acid (AP5) or cycloheximide were either confined to the amygdala [37] or covered the entire amygdala and spread up the cannula slightly, via the internal capsule to the caudate-putamen region [15]. Thus, the 0.5 μl volume used in the present study likely resulted in diffusion contained within the amygdala, although localization to individual nuclei within the amygdala was not evident.

Rats with cannula tip placements ventral to the amygdala showed deficits whether they were treated with aCSF or scopolamine. Mean percent time spent in the paired arm was not significantly greater than chance for this group, which was divided evenly between aCSF and scopolamine treated rats. The impairment may be attributed to cannulae going through the amygdala, therefore damaging nuclei located dorsal to the placement. Scopolamine infusions outside of the amygdala (dorsal, rostral, or caudal) did not significantly affect performance of the CPP task. Therefore, it appears that the impairment seen with intra-amygdala infusions of scopolamine was the result of cholinergic blockade within the amygdala selectively.

The amount of food consumed on the day of the drug injection was significantly lower for rats receiving scopolamine than for those receiving aCSF, however scopolamine-infused rats appeared to be motivated by food as eating was never ceased over the 30-min trial. The correlation between time spent on the paired arm and amount of food consumed on the drug day within the scopolamine group was not significant. Furthermore, rats receiving scopolamine infusions outside the amygdala consumed comparable amounts of food to rats receiving intra-amygdala scopolamine, however they did exhibit a significant preference for the paired arm. Thus, reduced consumption was most likely not the primary cause of drug-induced impairment.

Both working memory and reference memory components were included in the radial maze task in order to investigate the possibility that McDonald and White's dissociation of the amygdala and hippocampal systems is based on the differential involvement of the two neuroanatomical regions in working vs. reference types of memory [38]. There were no significant differences in type of errors between scopolamine and aCSF treated rats on the day of drug infusions. Therefore, these results do not support the notion that hippocampal/amygdala dissociations are based on selective participation in working vs. reference memory. However, these findings do not rule out such a possibility as a dissociation of working and reference memory may be evident with increased task difficulty [24].

In summary, the findings presented here show that a dissociation of impairments on a memory task can be seen with drug injections into a neural region as well as with lesions of that neural region. Others have reported that neural dissociations of memory systems may be accomplished when a drug is injected into different

neural regions [18,19,29,30]. Thus far, the present findings in combination with others suggest that such dissociations may depend more on neuroanatomy than neuropharmacology. However, with additional pharmacological approaches it may be possible to tease apart a differential involvement of neurotransmitter systems within a given region in different classes of memory.

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