

Research report

Amygdaloid D₁ dopamine receptor involvement in Pavlovian fear conditioning

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Abstract

The amygdala has long been implicated in conditioned fear. The mesencephalic dopaminergic system provides a rich innervation to the amygdala [J.H. Fallon, P. Ciofi, Distribution of monoamines within the amygdala, in: J.P. Aggleton (Ed.), *The Amygdala: Neurobiological Aspects of Emotion, Memory and Mental Dysfunction*, Wiley, New York, 1992, pp. 97–114; L.J. Freedman, M.D. Cassell, Distribution of dopaminergic fibers in the central division of the extended amygdala of the rat. *Brain Research* 633 (1994) 243–252; E. Asan, The catecholaminergic innervation of the rat amygdala. *Advances in Anatomy Embryology and Cell Biology* 142 (1996) 1–107]. Specific activation of the mesoamygdaloid dopaminergic system has been reported to occur in response to conditioned fear-arousing stimuli [M.L. Coco, C.M. Kuhn, T.D. Ely, C.D. Kilts, Selective activation of mesoamygdaloid dopamine neurons by conditioned stress: attenuation by diazepam. *Brain Research* 590 (1992) 39–47] suggesting that dopamine release in the amygdala may contribute to the acquisition and/or expression of conditioned fear. Using a 2 × 2 factorial design, Experiment 1A investigated the effects of bilateral intra-amygdaloid infusions of the selective D₁ receptor antagonist, SCH 23390 (2.0 μg 0.5 μl⁻¹ side⁻¹), on the acquisition and expression of Pavlovian conditioned fear measured by freezing to acoustic and background contextual stimuli. Infusions of SCH 23390 prior to acquisition training, prior to retention testing or prior to both significantly *attenuated* conditioned freezing during retention testing. Experiment 1B investigated the dose-dependent effects of pre-training infusions of SCH 23390 (0.5, 1.0 and 2.0 μg) on conditioned fear. Pre-training infusions of SCH 23390 dose-dependently attenuated conditioned freezing during retention testing. Experiment 2A investigated the effects of bilateral infusions of the selective D₁ receptor agonist, SKF 82958 (2.0 μg 0.5 μl⁻¹ side⁻¹) on the acquisition and expression of conditioned fear. Infusions of SKF 82958 prior to training *facilitated* conditioned freezing during retention testing. Experiment 2B investigated the dose-dependent effects of pre-training infusions of SKF 82958 (1.0, 2.0 and 4.0 μg) on conditioned fear. Pre-training infusions of SKF 82958 dose-dependently facilitated conditioned freezing during retention testing. In conclusion, these results suggest that dopamine transmission within the amygdala contributes to the acquisition and expression of Pavlovian fear conditioning. © 1999 Elsevier Science B.V. All rights reserved.

Keywords: SCH 23390; SKF 82958; Freezing; Mesoamygdaloid dopaminergic system; Stress

1. Introduction

Recent research suggests that the mesencephalic dopaminergic system is activated by stressful events. For example, dopamine-containing neurons in the ventral tegmental area (VTA) demonstrate enhanced firing rates in response to presentations of conditioned fear-arousing stimuli [18,37]. Furthermore, immobilization stress selectively increases Fos protein in VTA dopamine-containing neurons [10] and footshock stress increases dopamine

metabolism in the VTA [11]. The amygdala has been identified as a site of dopamine release in response to stressful events since footshock and conditioned fear-arousing stimuli increase dopamine metabolism in this structure [7,21].

The extant literature strongly suggests that the amygdala is a critical component of the neural circuitry essential for conditioned fear since various manipulations of the amygdala disrupt the acquisition and/or expression of conditioned fear [9,22,23,25,26]. Furthermore, neurons within the amygdala demonstrate associative responses to presentations of conditioned fear-arousing stimuli [30,32]. Finally, the extensive descending projections of the amyg-

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dala, particularly, the projections of the amygdaloid central nucleus, to a variety of brainstem areas offer an anatomical substrate for the expression of several responses indicative of fear [9,22,33].

The well-documented role of the amygdala in conditioned fear and the substantial dopaminergic innervation of the amygdala [2,11,12] suggest a contribution of the mesoamygdaloid dopaminergic system to the acquisition and/or expression of conditioned fear. Surprisingly, little effort, however, has been devoted to investigating this contribution. Nevertheless, evidence has accumulated indicating that dopamine activation is involved in conditioned fear. For example, Davis [8] has demonstrated that systemic administration of SCH 23390, a D_1 -receptor antagonist, or raclopride, a D_2 -receptor antagonist, attenuates fear-potentiated startle. This finding is consistent with the recent report that VTA lesions block fear-potentiated startle, whereas electrical stimulation of the VTA facilitates acoustic startle [5]. However, the forebrain terminal region(s) where dopamine exerts its effects were not revealed using these techniques. For this reason, we have been investigating the role of the mesoamygdaloid dopaminergic system in conditioned fear using a well-defined model of Pavlovian fear conditioning in the rat: conditioned freezing to an acoustic stimulus predictive of footshock. We found that intra-amygdaloid infusions of a dopaminergic antagonist disrupted conditioned freezing [17]. These observations are consistent with the recently published results of Lamont and Kokkinidis [24], which indicate that the amygdaloid dopaminergic system contributes to the expression of fear in the fear potentiated startle paradigm. The present study was designed to extend our preliminary findings by investigating the dose-dependent effects of intra-amygdaloid infusions of either a dopaminergic agonist or antagonist on the *acquisition* and *expression* of Pavlovian fear conditioning.

2. Materials and method

2.1. Experiment 1A

The purpose of Experiment 1A was to assess the effects of intra-amygdaloid infusions of the D_1 -receptor antagonist, SCH 23390, on the acquisition and expression of conditioned fear. Fear conditioned to both acoustic and background contextual cues was assessed by measuring freezing, a well-established measure of conditioned fear in the rat [4,38]. A D_1 receptor antagonist was chosen because of the existence of post-synaptic D_1 receptors in the amygdala [1,2,20,36].

2.1.1. Animals

Some 48 female Long–Evans rats (*Rattus norvegicus*) weighing 200–250 g were used (Harlan Sprague Dawley,

Indianapolis, IN). The animals were individually housed and maintained on a 12 h light/12 h dark cycle. Food and water were available ad libitum. The behavioral procedures took place during the light portion of the cycle. Handling occurred daily throughout the experiment.

2.1.2. Surgery

Surgeries were performed under sodium pentobarbital (65 mg/kg, i.p.) anesthesia using aseptic conditions. The animals were pretreated with atropine sulfate (0.05 mg/kg, i.p.) to prevent respiratory distress. The skull was leveled so that bregma and lambda were on an equal plane. Twenty-six-gauge guide cannulae (Plastics One, Roanoke, VA) were bilaterally secured to the skull with two jeweler's screws and dental cement. The cannula tips were aimed at the dorsal surface of the amygdala, (i.e., immediately dorsal to the amygdaloid central nucleus) to avoid damage to any amygdaloid nuclei. The following coordinates were used: 2.3 mm posterior to bregma, ± 4.2 mm from the midline and 7.0 mm ventral from skull surface [31]. Stylets (30-gauge, Plastics One), cut flush with the cannula tips, were placed into the guide cannulae. Immediately following surgery, the animals were placed under a heat lamp and observed continuously until locomotion returned; at which time they were returned to their home cages. Buprenorphine (0.01 mg/kg, s.c.) was administered over a 36 h period as a post-operative analgesic.

2.1.3. Apparatus

Behavioral training and testing was conducted in one of four identical conditioning chambers. Each conditioning chamber, measuring 24 cm in length, 30.5 cm in width and 24 cm in height, was constructed of three stainless steel walls and one Plexiglas front wall (MED Associates, Georgia, VT). The floor in each conditioning chamber consisted of nine stainless steel rods (4.8 mm diameter), spaced 1.5 cm apart. A shock generator and scrambler (MED Associates) were connected to each floor. Each chamber was mounted on individual platforms, which contained linear load cells (MED Associates). The amount of force exerted on the floor of each chamber by an animal was registered by the load cells as an analog signal, which was amplified and analyzed using MED Associates Threshold 2.0 software. Each chamber was illuminated by a 2.2 cm jewel light with a white lens located in the center of one of the stainless steel walls adjacent to a 6 cm speaker, which was connected to an audio-frequency generator (MED Associates). Each chamber was located within a sound attenuating chamber. Behavioral observations were made via a video camera (Panasonic, model WVBP500) attached to a video monitor (Panasonic, model CT2084Y) and recorded with a VCR (Panasonic, model AG-1290P).

2.1.4. Behavioral procedure

A 2×2 (SCH 23390/VEHICLE \times ACQUISITION/RETENTION SESSION) factorial design was used to

assess the effects of intra-amygdaloid infusions of SCH 23390 on the acquisition and/or expression of conditioned freezing during retention testing using an aversive Pavlovian conditioning paradigm. This design assessed the effects of drug infusions on both the acquisition and expression of conditioned fear and incorporated an assessment of state-dependent learning effects.

Following a 7 day post-operative recovery period, the animals were randomly assigned to one of four drug treatment groups: (1) drug immediately prior to both acquisition training and retention testing; (2) vehicle immediately prior to both acquisition training and retention testing; (3) drug immediately prior to acquisition training and vehicle prior to retention testing or (4) vehicle immediately prior to acquisition training and drug prior to retention testing. This experiment was run in three replications.

2.1.4.1. Acquisition training. For 3 days prior to behavioral testing (i.e., the habituation sessions) and on each of the days of behavioral testing, the animals were transported into a laboratory holding room for 30 min. Immediately following the last of these habituation sessions, the animals were transported into the infusion room located adjacent to the behavioral testing room and were administered a mock infusion to acclimate them to the infusion procedure. After 24 h, the animals were transported back into the infusion room. Two sterile 33-gauge injection cannulae (Plastics One) cut to extend 0.5 mm ventral to the tips of the guide cannulae, were inserted into the guide cannulae. Either SCH 23390 or vehicle (0.5 μ l) was infused into each hemisphere via polyethylene tubing (PE 50) attached to a 10 μ l Hamilton syringe mounted on an infusion pump (kd Scientific, Model 200, Stoelting, Wood Dale, IL). The infusions were made over a 150 s period. Injection cannulae remained in place for 60 s to insure diffusion away from injector tips. Stylets were replaced after infusions.

The walls and floor of each conditioning chamber were cleaned with 5% ammonium hydroxide just prior to placing the animals in the chambers. The animals then received three Pavlovian conditioning trials during which the offset of a tone conditioned stimulus (CS: 2000 Hz, 80 dB, 24 s) was always coincident with a footshock unconditioned stimulus (US: 500 ms, 0.5 mA). The first conditioning trial was administered 128 s after the animals were placed in the chambers. The remaining two trials were presented on a 128 s fixed interval schedule. The animals were then removed and returned to their home cages.

Conditioned fear was assessed by measuring freezing every 4.0 s by an observer who was blind to group assignment (R.J.F.). Freezing was defined as the absence of all movement except that necessary for respiration [38]. Background contextual fear (i.e., context-freezing) was assessed by sampling freezing during the 24 s period immediately prior to the CS presentations (i.e., the PRE-CS period). Freezing during CS presentations was assessed by

sampling freezing during the 24 s CS period. The number of observations during which freezing occurred was converted to percentages of the total sample time.

2.1.4.2. Retention testing. After 24 h following the training session, freezing to CS presentations and background contextual cues was assessed during retention testing. Immediately prior to the test, either SCH 23390 or vehicle was administered depending on group assignment. Following procedures similar to those described above for acquisition, the animals were placed in the conditioning chambers for 128 s and received three CS presentations on a 128 s fixed interval schedule in the absence of footshock.

2.1.4.3. Baseline activity levels. To determine the effects of drug infusions on general activity, levels of activity were monitored for 128 s prior to the first acquisition trial in a subset of 16 animals. The magnitude of the force that each animal applied to the floor of the conditioning chamber was recorded every 100 ms by linear load cells as an analog signal. The amount of force applied to the floor of each chamber is directly proportional to the amount of activity of the animal. Software designed by MED Associates was used to calculate the cumulative sum of these samples for the 128 s period for each animal. Differential effects of drug versus vehicle infusions on activity would be reflected in different mean cumulative scores between the groups.

2.1.5. Drugs and vehicle

The D₁ receptor antagonist, SCH 23390 (Research Biochemical International, Natick, MA) was dissolved in sterile physiological saline (0.9%; 2.0 μ g/0.5 μ l) and administered to each hemisphere. This dose was selected because it has been shown to alter other behaviors for at least 1 h following intracranial infusions [6,27]. Sterile physiological saline (0.9%) was used for vehicle infusions.

2.1.6. Statistical analyses

Conditioned freezing during the acquisition training session was assessed with two 2×3 (SCH 23390/SALINE \times TRIALS) repeated measures analyses of variance (ANOVA), one for freezing during CS presentations and one for freezing during PRE-CS periods. Conditioned freezing during the retention test was assessed with two, one-way ANOVAs of drug group (SCH–SCH, SCH–SALINE, SALINE–SCH, SALINE–SALINE) collapsed across trials, one for freezing during CS presentations and one for freezing during PRE-CS periods. Differences between groups were examined using Tukey's Honestly Significant Difference post-hoc comparisons. The alpha level was set at $p < 0.05$.

2.1.7. Histological verification

Following the retention test, the animals were given an overdose of sodium pentobarbital (90 mg/kg) and per-

fused with saline (0.9%) followed by formalin–saline (10%). Brains were removed and fixed in a formalin–sucrose solution (15%) for at least 24 h. Brains were frozen, sliced into 75 μm sections and stained with thionin. An observer blind to drug assignment (R.J.F.) determined the location of cannulae tips using the rat brain atlas of Paxinos and Watson [31] as a guide.

3. Results

3.1. Experiment 1A

3.1.1. Histology

Cannulae placements were considered accurately located if both guide cannula tips were microscopically verified to be no more than 0.7 mm dorsal to the surface of the amygdala in the region of the amygdaloid central nucleus and did not produce damage to any amygdaloid nuclei. All placements meeting these criteria were located between 2.12–3.0 mm posterior to bregma. Fig. 1 depicts all the accurate placements from Experiment 1A. A total of 17 of the 48 animals were not included in the statistical analyses because of inaccurate cannula placements, equipment failure or illness.

3.1.2. Acquisition training

3.1.2.1. CS-freezing. As depicted in Fig. 2A, the animals that received SCH 23390 or SALINE prior to training demonstrated a significant increase in freezing to the CS presentations during acquisition training. An ANOVA revealed a significant main effect of Trials $F(2,58) = 19.62$, but no significant main effect of Group $F(1,29) = 2.05$ or Group \times Trials interaction $F(2,58) = 1.75$. These results indicate that both groups demonstrated a similar increase in freezing to the CS across trials during acquisition training.

3.1.2.2. Context-freezing. The animals that received SCH 23390 or SALINE prior to training demonstrated a significant increase in freezing to the context during acquisition training (Table 1). An ANOVA revealed a significant main effect of Trials $F(2,58) = 13.72$, but no significant main effect of Group $F(1,29) < 1.0$ or Group \times Trials interaction $F(2,58) = 1.44$. These results indicate that both groups demonstrated a similar increase in freezing to the context across trials during acquisition training.

3.1.2.3. Specificity of freezing. An additional analysis was calculated to determine if the freezing behavior observed during CS presentations, at least in part, represented freezing specific to the CS rather than simply freezing to background contextual cues in this paradigm. Freezing during the PRE-CS periods (i.e., background contextual freezing) was compared to freezing during the CS presentations in the SALINE control group. Given that very little

PRE-CS- or CS-freezing behavior was observed during the first two acquisition trials, this comparison was only calculated on the third trial data. A *t*-test for related measures did not reveal a significant increase in freezing during the CS presentation compared to freezing during the PRE-CS period for Trial 3 $t(15) = 0.40$. This suggests that freezing during acquisition training was not elicited by the CS but reflected a more general fear associated with the contextual cues of the conditioning chamber.

3.1.3. Retention testing

3.1.3.1. CS-freezing. As depicted in Fig. 2B, the animals in the SALINE–SALINE group demonstrated significantly more freezing to the CS presentations during the retention test than the animals that received SCH 23390 infusions at any time (i.e., SCH–SCH, SCH–SALINE, SALINE–SCH). An ANOVA revealed a significant main effect of Group $F(3,27) = 17.72$. Further post-hoc comparisons indicated that the SALINE–SALINE group froze significantly more than any of the SCH 23390 groups (all p 's < 0.05). However, the groups that received SCH 23390 at any time were not significantly different from one another (all p 's > 0.05).

3.1.3.2. Context-freezing. The animals in the SALINE–SALINE group demonstrated significantly more freezing to the context during the retention test than the animals that received SCH 23390 infusions at any time (Table 2). An ANOVA revealed a significant main effect of Group $F(3,27) = 3.17$. Further post-hoc comparisons indicated that the SALINE–SALINE group froze significantly more than the SCH–SCH group and the SCH–SALINE group (p 's < 0.05) and marginally more than the SALINE–SCH group ($p = 0.06$). However, the groups that received SCH 23390 at any time were not significantly different from one another (all p 's > 0.05).

3.1.3.3. Specificity of freezing. An additional analysis was calculated to determine if the freezing behavior observed during CS presentations, at least in part, represented freezing specific to the CS rather than simply freezing to background contextual cues in this paradigm. Freezing during the PRE-CS periods (i.e., background contextual freezing) was compared to freezing during the CS presentations in the SALINE control group. A *t*-test for related measures revealed a significant increase in freezing during the CS presentations compared to freezing during the PRE-CS periods $t(8) = 6.45$. This suggests that freezing during CS presentations was specifically elicited by the CS.

3.1.4. Activity levels

The activity levels of animals that received pre-training infusions of SCH 23390 ($n = 8$) were not significantly

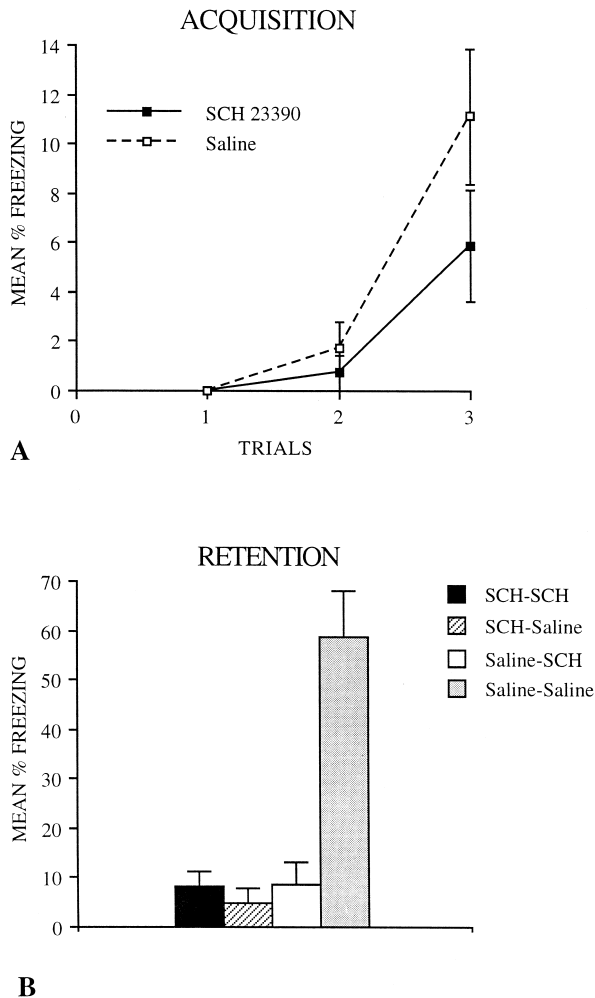


Fig. 2. (A) Freezing during acquisition training for Experiment 1A. Mean percent freezing across trials during the acquisition session to each 24 s tone presentation. The SCH 23390 group ($n = 15$) received SCH 23390 ($2.0 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$) prior to the training session and the SALINE group ($n = 16$) received SALINE prior to the training session. There were no statistically significant differences between the groups. (B) Freezing during retention testing for Experiment 1A. Mean percent freezing to the tone presentations, collapsed across trials during the retention test. The SCH–SCH group ($n = 6$) received pre-training and pre-testing SCH 23390. The SCH–SALINE group ($n = 9$) received only pre-training SCH 23390. The SALINE–SCH group ($n = 7$) received only pre-testing SCH 23390. The SALINE–SALINE group ($n = 9$) received pre-training and pre-testing SALINE. The SALINE–SALINE group froze significantly more than all three drug groups. Standard error bars represent standard error of the mean.

3.1.5. Dorsal cannulae placements

A number of animals were not included in the previous analyses due to inaccurate cannula placements. A subgroup ($n = 6$) of these animals had cannulae placements that were located dorsally, primarily within the caudate–putamen. The data from these animals, together with data from seven additional animals that were prepared with cannulae intentionally positioned in the caudate–putamen were analyzed to determine if SCH 23390 had any effect when injected dorsal to the amygdala. These analyses were

identical to those described in the previous section. All of the animals in the dorsal placement group received SCH 23390 at some time (SCH–SCH, $n = 5$; SCH–SALINE, $n = 4$; SALINE–SCH, $n = 4$). There were no differences in freezing to either CS presentations or background contextual cues between the SALINE–SALINE group and any of the dorsal control groups during acquisition training (all p 's > 0.05) or retention testing (all p 's > 0.05).

3.2. Experiment 1B

This experiment was conducted to assess the dose-dependent effects of pre-training intra-amygdaloid infusions of SCH 23390 on conditioned freezing. The methods and procedures were similar to those of Experiment 1A. Because a potent effect on retention testing was observed following pre-training infusions in Experiment 1A, infusions in this experiment were made only prior to acquisition training. The animals were randomly assigned to one of four drug treatment groups: (1) high dose— $2.0 \mu\text{g}/0.5 \mu\text{l}$; (2) medium dose— $1.0 \mu\text{g}/0.5 \mu\text{l}$; (3) low dose— $0.5 \mu\text{g}/0.5 \mu\text{l}$; or (4) saline. A total of 34 animals were used in this experiment, which were run in two replications.

3.2.1. Histology

Similar to the results of Experiment 1A depicted in Fig. 1, all cannula placements judged to be accurate were located no more than 0.7 mm dorsal to the surface of the amygdala in the region of the amygdaloid central nucleus. A total of 6 of the 34 animals were not included in the statistical analyses because of inaccurate cannula placements or illness.

3.2.2. Acquisition training

3.2.2.1. CS-freezing. As depicted in Fig. 3A, the animals that received any dose of SCH 23390 or SALINE prior to training demonstrated a significant increase in freezing to

Table 1
Mean percent freezing to the context during acquisition for Experiments 1A–2B

Group	Trial 1	Trial 2	Trial 3
SCH 23390	0 (0.0) ^a	0.72 (0.5)	5.16 (2.1)
SALINE	0 (0.0)	0 (0.0)	9.36 (3.1)
SCH-high	0 (0.0)	2.12 (2.1)	33.25 (12.6)
SCH-med	0 (0.0)	0 (0.0)	16.67 (16.7)
SCH-low	0 (0.0)	14.28 (14.3)	34.00 (13.0)
SALINE	0 (0.0)	0 (0.0)	12.00 (4.8)
SKF 82958	0 (0.0)	3.33 (2.4)	17.80 (7.7)
SALINE	0 (0.0)	0.80 (0.8)	20.71 (7.0)
SKF-high	0 (0.0)	0 (0.0)	2.42 (2.4)
SKF-med	0 (0.0)	3.40 (2.1)	16.60 (5.8)
SKF-low	0 (0.0)	2.12 (2.1)	29.00 (11.7)
SALINE	0 (0.0)	0 (0.0)	20.44 (12.4)

^aNumbers in parenthesis are S.E.M.

the CS presentations during acquisition training. An ANOVA revealed a significant main effect of Trials $F(2,48) = 17.13$, but no significant main effect of Group $F(3,24) < 1.0$ or Group \times Trials interaction $F(6,48) < 1.0$. These results indicate that all four groups demonstrated a similar increase in freezing to the CS across trials during acquisition training.

3.2.2.2. Context-freezing. The animals that received any dose of SCH 23390 or SALINE prior to training demonstrated a significant increase in freezing to the context during acquisition training (Table 1). An ANOVA revealed a significant main effect of Trials $F(2,48) = 12.19$, but no significant main effect of Group $F(3,24) < 1.0$ or Group \times Trials interaction $F(6,48) < 1.0$. These results indicate that all four groups demonstrated a similar increase in freezing to the context across trials during acquisition training.

3.2.2.3. Specificity of freezing. A *t*-test for related measures did not reveal a significant increase in freezing during the CS presentation compared to the PRE-CS period for Trial 3 $t(6) = 1.32$. This suggests that freezing during acquisition training was not elicited by the CS but reflected a more general fear associated with the contextual cues of the conditioning chamber.

3.2.3. Retention testing

3.2.3.1. CS-freezing. As depicted in Fig. 3B, the animals in the SALINE group demonstrated significantly more freezing to the CS presentations during the retention test than the animals that received the high dose of SCH 23390. An ANOVA revealed a significant main effect of Group $F(3,25) = 4.33$. Further post-hoc comparisons indicated

Table 2

Mean percent freezing to the context collapsed across trials during retention testing for Experiments 1A–2B

Group	Means
SCH–SCH	4.83 (2.7) ^a
SCH–SALINE	0 (0.0)
SALINE–SCH	7.14 (4.9)
SALINE–SALINE	24.11 (9.9)
SCH-high	0.63 (0.6)
SCH-med	4.33 (1.2)
SCH-low	2.86 (1.7)
SALINE	5.29 (2.1)
SKF–SKF	10.00 (9.5)
SKF–SALINE	20.75 (9.4)
SALINE–SKF	3.00 (1.9)
SALINE–SALINE	3.30 (2.0)
SKF-high	6.67 (4.3)
SKF-med	27.80 (17.3)
SKF-low	25.28 (6.7)
SALINE	4.28 (2.1)

^aNumbers in parenthesis are S.E.M.

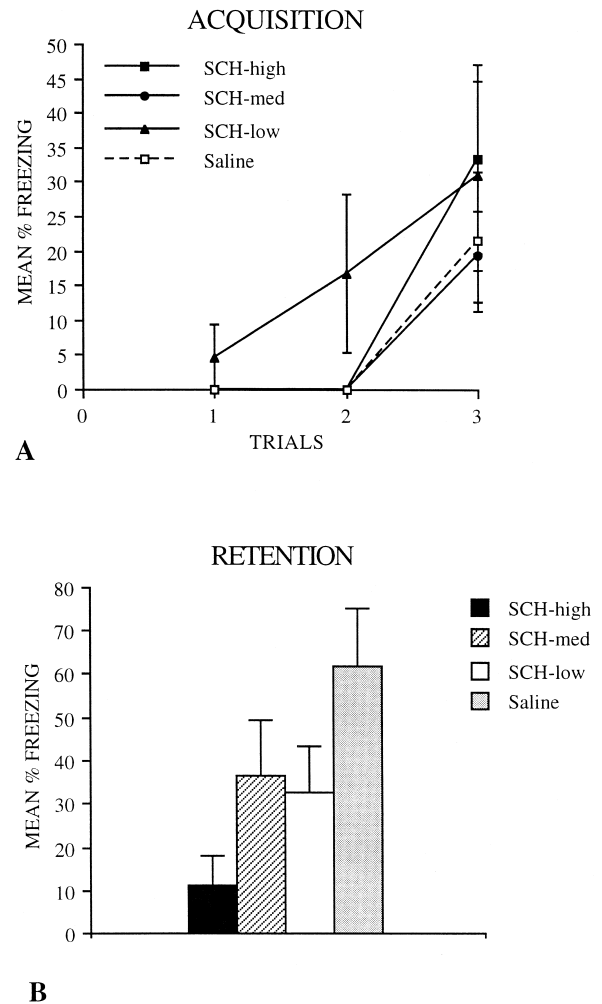


Fig. 3. (A) Freezing during acquisition training for Experiment 1B. Mean percent freezing across trials during the acquisition session to each 24 s tone presentation. The SCH-high group ($n = 8$) received pre-training SCH 23390 ($2.0 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$). The SCH-med group ($n = 6$) received pre-training SCH 23390 ($1.0 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$). The SCH-low group ($n = 7$) received pre-training SCH 23390 ($0.5 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$). The SALINE group ($n = 7$) received pre-training SALINE. There were no statistically significant differences between the groups. (B) Freezing during retention testing for Experiment 1B. Mean percent freezing to the tone presentations collapsed across trials during the retention test to the tone presentations. The SCH-high group ($n = 8$) received pre-training SCH 23390 ($2.0 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$). The SCH-med group ($n = 6$) received pre-training SCH 23390 ($1.0 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$). The SCH-low group ($n = 7$) received pre-training SCH 23390 ($0.5 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$). The SALINE group ($n = 7$) received pre-training SALINE. The SALINE group froze significantly more than the SCH-high group.

that the SALINE group froze significantly more than the group that received the high dose of SCH 23390 ($p < 0.05$). No other group comparisons were significant (all p 's > 0.05).

3.2.3.2. Context-freezing. The animals in the SALINE group demonstrated significantly more freezing to the context during the retention test than the animals that received

the high dose of SCH 23390 (Table 2). The main effect of Group $F(3,25) = 2.15$ approached significance ($p = 0.10$). Further post-hoc comparisons indicated that the SALINE group froze significantly more than the group that received the high dose of SCH 23390 ($p < 0.05$). No other group comparisons were significant (all p 's > 0.05).

3.2.3.3. Specificity of freezing. A t -test for related measures revealed a significant increase in freezing during the CS presentations compared to the PRE-CS periods $t(6) = 3.81$. This suggests that freezing during CS presentations was specifically elicited by the CS.

3.3. Experiment 2A

If the effects of SCH 23390 observed in Experiments 1A and 1B were due to a selective effect on amygdaloid dopamine transmission, then one prediction would be that a dopaminergic agonist would facilitate conditioned fear. Experiment 2A was designed to test the validity of this prediction by examining the effects of intra-amygdaloid administration of the D_1 receptor agonist, SKF 82958 (2.0 $\mu\text{g}/0.5 \mu\text{l}$; RBI) on the acquisition and expression of conditioned fear. The methods and procedures of Experiment 2A were similar to those of Experiment 1A. However, two procedural changes were made. The intensity of the footshock was reduced from 0.5 to 0.4 mA during acquisition training and two additional CS presentations were administered during retention testing. These changes were made in order to mitigate against the possibility of observing a ceiling effect in freezing for the SALINE–SALINE control animals, which could mask any drug-induced facilitation of conditioned fear. A total of 50 animals were used in this experiment, which was run in three replications.

3.3.1. Histology

Similar to the results of Experiment 1A depicted in Fig. 1, all cannulae placements judged to be accurate were located no more than 0.7 mm dorsal to the surface of the amygdala in the region of the amygdaloid central nucleus. A total of 14 of the 50 animals were not included in the statistical analyses because of inaccurate cannula placements or illness.

3.3.2. Acquisition training

3.3.2.1. CS-freezing. As depicted in Fig. 4A, the animals that received SKF 82958 or SALINE prior to training demonstrated a significant increase in freezing to the CS presentations during acquisition training. An ANOVA revealed a significant main effect of Trials $F(2,68) = 23.70$, but no significant main effect of Group $F(1,34) < 1.0$ or Group \times Trials interaction $F(2,68) < 1.0$. These results indicate that both groups demonstrated a similar increase in freezing to the CS across trials during acquisition training.

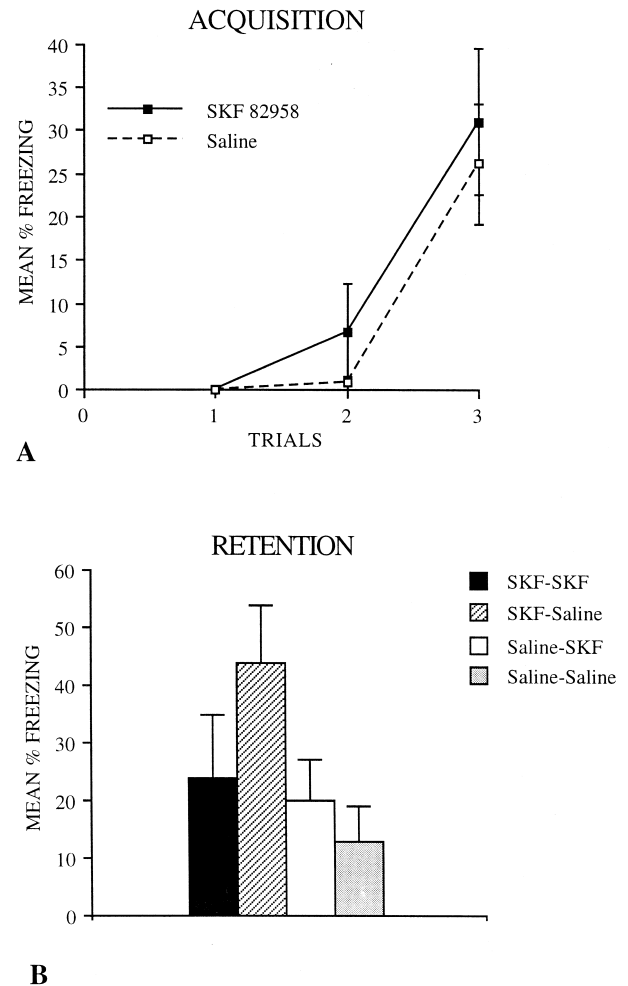


Fig. 4. (A) Freezing during acquisition training for Experiment 2A. Mean percent freezing across trials during the acquisition session to each 24 s tone presentation. The SKF 82958 group ($n = 15$) received SKF 82958 (2.0 $\mu\text{g}/0.5 \mu\text{l}$ /hemisphere) prior to the training session and the SALINE group ($n = 21$) received SALINE prior to the training session. There were no statistically significant differences between the groups. (B) Freezing during retention testing for Experiment 2A. Mean percent freezing to the tone presentations collapsed across trials during the retention test. The SKF–SKF group ($n = 8$) received pre-training and pre-testing SKF 82958. The SKF–SALINE group ($n = 8$) received only pre-training SKF 82958. The SALINE–SKF group ($n = 10$) received only pre-testing SKF 82958. The SALINE–SALINE group ($n = 10$) received pre-training and pre-testing SALINE. The SKF–SALINE group froze significantly more than the SALINE–SALINE group.

3.3.2.2. Context-freezing. The animals that received SKF 82958 or SALINE prior to training demonstrated a significant increase in freezing to the context during acquisition training (Table 1). An ANOVA revealed a significant main effect of Trials $F(2,68) = 12.00$, but no significant main effect of Group $F(1,34) < 1.0$ or Group \times Trials interaction $F(2,68) < 1.0$. These results indicate that both groups demonstrated a similar increase in freezing to the context across trials during acquisition training.

3.3.2.3. Specificity of freezing. A t -test for related measures did not reveal a significant increase in freezing

during the CS presentation compared to the PRE-CS period for Trial 3 $t(20) = 1.47$. This suggests that freezing during acquisition training was not elicited by the CS but reflected a more general fear associated with the contextual cues of the conditioning chamber.

3.3.3. Retention testing

3.3.3.1. CS-freezing. As depicted in Fig. 4B, the animals in the SKF–SALINE group demonstrated significantly more freezing during the retention test to the CS presentations than the animals in the SALINE–SALINE group. The main effect of Group $F(3,32) = 2.15$ approached significance ($p = 0.09$). Further post-hoc comparisons indicated that the SKF–SALINE group froze significantly more than the SALINE–SALINE group ($p < 0.05$). No other group comparisons were significant (all p 's > 0.05).

3.3.3.2. Context-freezing. The animals in the SKF–SALINE group demonstrated significantly more freezing to the context during the retention test than the animals in the SALINE–SALINE group (Table 2). An ANOVA revealed a significant main effect of Group $F(3,32) = 3.08$. Further post-hoc comparisons indicated that the SKF–SALINE group froze significantly more than the SALINE–SALINE group ($p < 0.05$). No other group comparisons were significant (all p 's > 0.05).

3.3.3.3. Specificity of freezing. A t -test for related measures revealed a significant increase in freezing during the CS presentations compared to the PRE-CS periods $t(9) = 2.32$. This suggests that freezing during CS presentations was specifically elicited by the CS.

3.3.4. Activity levels

The activity levels of animals that received pre-training infusions of SKF 82958 ($n = 7$) were not significantly different from the activity levels of animals that received pre-training infusions of saline ($n = 8$). A between groups t -test revealed no significant differences between the groups $t(13) = 0.59$.

3.3.5. Dorsal cannulae placements

A number of animals were not included in the previous analyses due to inaccurate cannula placements. A subgroup ($n = 7$) of these animals had cannulae placements that were located dorsally, primarily within the caudate–putamen. The data from these animals, together with data from six additional animals that were prepared with cannulae intentionally positioned in the caudate–putamen, were analyzed to determine if SKF 82958 had any effect when injected dorsal to the amygdala. These analyses were identical to those described in the previous section. All of the animals in the dorsal placement group received SKF 82958 at some time (SKF–SKF, $n = 5$; SKF–SALINE, $n = 4$; SALINE–SKF, $n = 4$). There were no differences

in freezing to either CS presentations or background contextual cues between the SALINE–SALINE group and any of the dorsal control groups during acquisition training (all p 's > 0.05) or retention testing (all p 's > 0.05).

3.4. Experiment 2B

This experiment was conducted to assess the dose-dependent effects of pre-training intra-amygdaloid infusions of SKF 82958 on conditioned freezing. The methods and procedures were similar to those of Experiment 2A. Because effects were observed following pre-training infusions in Experiment 2A, infusions in this experiment were made only prior to acquisition training. The animals were randomly assigned to one of four drug treatment groups: (1) high dose—4.0 $\mu\text{g}/0.5 \mu\text{l}$; (2) medium dose—2.0 $\mu\text{g}/0.5 \mu\text{l}$; (3) low dose—1.0 $\mu\text{g}/0.5 \mu\text{l}$ or (4) saline. A total of 32 animals were used in this experiment, which was run in two replications.

3.4.1. Histology

Similar to the results of Experiment 1A depicted in Fig. 1, all cannulae placements judged to be accurate were located no more than 0.7 mm dorsal to the surface of the amygdala in the region of the amygdaloid central nucleus. A total of 7 of the 32 animals were not included in the statistical analyses because of inaccurate cannula placements or illness.

3.4.2. Acquisition training

3.4.2.1. CS-freezing. As depicted in Fig. 5A, the animals that received any dose of SKF 82958 or SALINE prior to training demonstrated a significant increase in freezing to the CS presentations during acquisition training. An ANOVA revealed a significant main effect of Trials $F(2,50) = 18.49$, but no significant main effect of Group $F(3,25) = 1.06$ or Group \times Trials interaction $F(6,50) < 1.0$. These results indicate that all four groups demonstrated a similar increase in freezing to the CS across trials during acquisition training.

3.4.2.2. Context-freezing. The animals that received any dose of SKF 82958 or SALINE prior to training demonstrated a significant increase in freezing to the context during acquisition training (Table 1). An ANOVA revealed a significant main effect of Trials $F(2,50) = 9.89$, but no significant main effect of Group $F(3,25) = 1.11$ or Group \times Trials interaction $F(6,50) = 1.15$. These results indicate that all four groups demonstrated a similar increase in freezing to the context across trials during acquisition training.

3.4.2.3. Specificity of freezing. A t -test for related measures did not reveal a significant increase in freezing during the CS presentation compared to the PRE-CS pe-

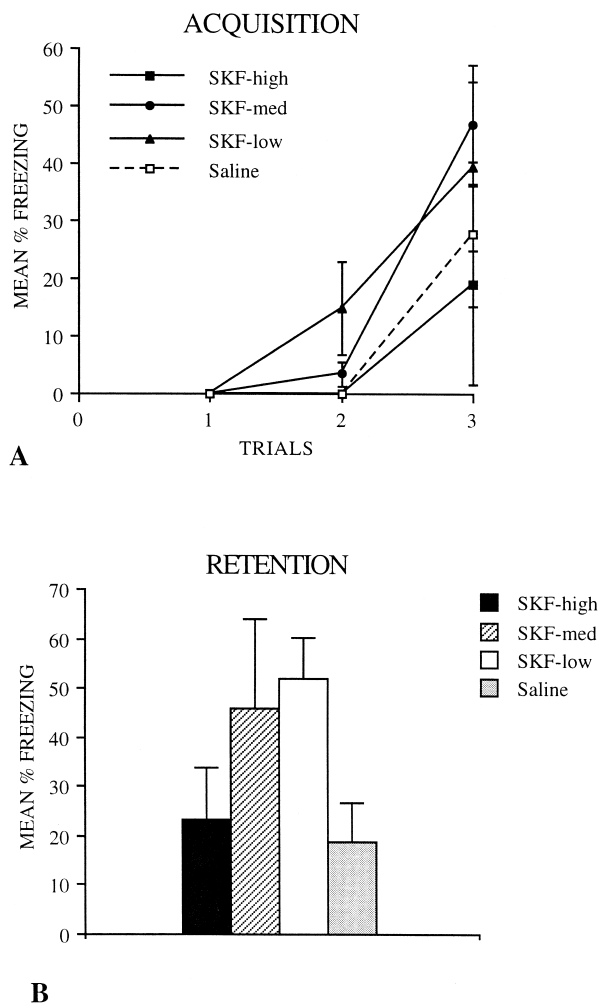


Fig. 5. (A) Freezing during acquisition training for Experiment 2B. Mean percent freezing across trials during the acquisition session to each 24 s tone presentation. The SKF-high group ($n = 6$) received pre-training SKF 82958 ($4.0 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$). The SKF-med group ($n = 5$) received pre-training SKF 82958 ($2.0 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$). The SKF-low group ($n = 7$) received pre-training SKF 82958 ($1.0 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$). The SALINE group ($n = 7$) received pre-training SALINE. There were no statistically significant differences between the groups. (B) Freezing during retention testing for Experiment 2B. Mean percent freezing to the tone presentations collapsed across trials during the retention test. The SKF-high group ($n = 6$) received pre-training SKF 82958 ($4.0 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$). The SKF-med group ($n = 5$) received pre-training SKF 82958 ($2.0 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$). The SKF-low group ($n = 7$) received pre-training SKF 82958 ($1.0 \mu\text{g}/0.5 \mu\text{l}/\text{hemisphere}$). The SALINE group ($n = 7$) received pre-training SALINE. The SKF-low group froze significantly more than the SALINE group.

riod for Trial 3 $t(8) = 1.83$. This suggests that freezing during acquisition training was not elicited by the CS but reflected a more general fear associated with the contextual cues of the conditioning chamber.

3.4.3. Retention testing

3.4.3.1. CS-freezing. As depicted in Fig. 5B, the animals in the low dose SKF 82958 group demonstrated significantly

more freezing to the CS presentations during the retention test than the animals that received SALINE. The main effect of Group $F(3,24) = 2.49$ approached significance ($p = 0.09$). Further post-hoc comparisons indicated that the low dose SKF 82958 group froze significantly more than the group that received SALINE ($p < 0.05$). No other group comparisons were significant (all p 's > 0.05). A quadratic trend analysis was also calculated comparing the four groups because the group means demonstrated an inverted U-pattern. A significant quadratic trend was found between groups $F(1,23) = 5.87$.

3.4.3.2. Context-freezing. The animals in the low dose SKF 82958 group demonstrated slightly more freezing to the context during the retention test than the animals that received SALINE or the high dose of SKF 82958 (Table 2). The main effect of Group $F(3,24) = 2.29$ approached significance ($p = 0.10$). Further post-hoc comparisons indicated that the animals that received the low or the medium dose of SKF 82958 froze slightly more than the animals that received SALINE but these comparisons only approached significance ($p = 0.06$ and $p = 0.61$, respectively). A quadratic trend analysis was also calculated comparing the four groups because the group means demonstrated an inverted U-pattern. A significant quadratic trend was found between groups $F(1,23) = 5.03$.

3.4.3.3. Specificity of freezing. A t -test for related measures revealed an increase in freezing during the CS presentations compared to the PRE-CS periods $t(6) = 2.43$, however, this result only approached significance with a two-tailed test ($p = 0.06$). This suggests that freezing during CS presentations was specifically elicited by the CS.

4. Discussion

The results of Experiment 1A demonstrate that intra-amygdaloid infusions of the selective D_1 receptor antagonist, SCH 23390, given prior to training, prior to testing or prior to both attenuated the expression of Pavlovian fear conditioning to acoustic and contextual stimuli in rats during retention testing. The statistically significant effects observed on freezing during the CS presentations were more consistent and robust, however, than the effects observed on freezing during the PRE-CS periods (i.e., background contextual freezing). This finding was most likely due to the low level of freezing observed during the PRE-CS periods. The results of Experiment 1B demonstrate that pre-training infusions of SCH 23390 dose-dependently attenuated the expression of conditioned fear during retention testing. However, in both Experiments 1A and 1B pre-training infusions of SCH 23390 had no effect on the emergence of conditioned freezing across acquisition trials.

Given the contribution of the amygdala to conditioned fear [9,22,23,25,26], the rich dopaminergic innervation of the amygdala [2,12,13] and the increased dopaminergic metabolism in the amygdala in response to fear-arousing stimuli [7,21], the most parsimonious interpretation of the results of Experiments 1A and 1B is that the mesoamygdaloid dopaminergic system contributes to associative or mnemonic processes during fear conditioning. However, a number of alternative explanations for the results, including state-dependent learning deficits, disruption of sensory/motor functioning and localization of drug effects to regions other than the amygdala, must be eliminated before any associative or mnemonic interpretation can be adopted. Each of these alternatives is discussed below.

The use of a 2×2 factorial design in Experiment 1A eliminates the possibility that the observed effects were due to state-dependent learning deficits because this design incorporates a control for such effects. Half of the animals were trained and tested in the same drug state and the other half were trained and tested in a different drug state. If the impairments observed in this experiment were due to state-dependent effects, only animals that were trained and tested in different drug states would have been impaired. However, this was not the case since the animals that received SCH 23390 prior to training and prior to testing demonstrated the most pronounced deficit during retention testing, an effect opposite to that predicted by a drug-induced state-dependent learning effect. Therefore, the impairments observed in Experiments 1A and 1B do not appear to be due to state-dependent learning deficits.

The impairments observed in Experiments 1A and 1B are also not likely attributable to disruption of unconditioned sensory processing or motor functioning. First, neither lesions of the amygdala nor intra-amygdaloid infusions of NMDA antagonists have been shown to disrupt unconditioned sensitivity to footshock (e.g., [26,29,34,35]). Moreover, R. Deasy (personal communication) and L. Kokkinidis (personal communication) have found no effect of intra-amygdaloid infusions of SCH 23390 on footshock reactivity. Second, animals with amygdaloid lesions demonstrate normal somatomotor orienting responses to novel acoustic and visual stimuli [16] as well as normal visual pre-pulse inhibition [9]. Third, in the present experiments, there were no effects of pre-training SCH 23390 infusions on the emergence of freezing behavior during acquisition training. Finally, baseline activity data from a subset of animals did not indicate any differences between animals receiving SCH 23390 infusions and animals receiving saline, suggesting that the effects on retention were not due to drug-induced effects on activity. It is unlikely, therefore, that the alterations in freezing during retention testing observed in the present study were due to disruption of unconditioned sensory processing or motor functioning.

With respect to localization of the effects of SCH 23390 infusions to the amygdala, the most obvious extra-amyg-

daloid site where SCH 23390 may have exerted its effect on freezing is the caudate–putamen. The caudate–putamen receives a substantial dopaminergic input [3] and is located immediately dorsal to the amygdala. Infusions made directly into the caudate–putamen were without effect, thereby supporting the interpretation that the results were due to drug effects exerted on the amygdala. To summarize, the effects observed in the present experiments are not likely due to state-dependent effects, altered sensory–motor functioning or diffusion of SCH 23390 into regions dorsal of the amygdala.

The exact mechanism(s) by which amygdaloid D_1 receptor antagonism affects the expression of conditioned fear are not known. Nevertheless, together with the existing literature, the results of the present study lend some insight into this issue. The impaired expression of conditioned freezing observed during retention testing in Experiment 1A, together with extensive descending projections of the amygdala to brainstem areas believed to be involved in the motoric expression of a number of responses indicative of learned fear [9,22,33], suggests that the intense dopaminergic innervation of the amygdala may contribute to mechanisms necessary for the motoric expression of learned fear behaviors. Since there was no significant effect of SCH 23390 on the emergence of freezing during acquisition trials, the effects of SCH 23390 appear to be selective for the expression of conditioned responses retrieved from long-term memory during retention testing although higher doses may also produce impairments during acquisition. Consistent with this suggestion are the recent results of Lamont and Kokkinidis [24] who found that intra-amygdaloid infusions of SCH 23390 immediately prior to testing blocked the expression of conditioned fear in the fear-potentiated startle paradigm. These combined observations demonstrate that dopamine transmission within the amygdala contributes to the expression of conditioned fear responses retrieved from long-term memory.

In addition to playing a role in the expression of conditioned fear responses retrieved from long-term memory, amygdaloid dopamine transmission may also contribute to the formation and/or consolidation of long-term memory. It is important to note that although SCH 23390 had no significant effect on the emergence of freezing across acquisition trials, pre-training infusions of SCH 23390 did produce a profound effect on freezing during retention testing 24 h later. These results suggest that amygdaloid dopamine transmission may not necessarily be involved in the formation of short-term memory during acquisition, but may play a critical role in the formation and/or consolidation of long-term memory. This suggestion is consistent with the findings that activation of dopaminergic receptors is necessary for long-term synaptic plasticity *in vitro* [14,15,19].

Based on the results of Experiments 1A and 1B, we predicted that pre-training and/or pre-testing infusions of

the selective D₁ receptor agonist, SKF 82958, would facilitate conditioned freezing during retention testing. This prediction was only partially supported by the results of Experiments 2A and 2B since intra-amygdaloid infusions of SKF 82958 facilitated freezing during retention testing in a dose-dependent manner only when administered prior to acquisition training. The finding that infusions of SKF 82958 prior to retention testing or prior to both training and testing did not facilitate the expression of conditioned freezing is puzzling given the results of Experiment 1A demonstrating that SCH 23390 administered at these times attenuated conditioned freezing during retention testing. One possible explanation for this unexpected finding is that SKF 82958 may block the expression of freezing at the time of testing, in addition to facilitating memory formation during acquisition. Future experiments using a wider range of doses may shed some light onto this issue. Further support for this explanation comes from the results of Experiment 2B. There was an inverted U-shaped dose response function for SKF 82958, which was confirmed by a significant quadratic trend analysis. This finding indicates that high doses of SKF 82958 lose the ability to facilitate memory formation. One possible explanation may be that lower doses selectively bind to D₁ receptors, however, higher doses may produce greater non-selective binding. This is similar to opiate modulation of memory [28].

Consistent with the findings of Experiments 1A and 1B, however, are the results demonstrating that pre-training administration of the low or the medium dose of SKF 82958 produced a profound facilitation of retention. Importantly, no effects on freezing were observed during acquisition, a result similar to the results of Experiments 1A and 1B. Taken together, these results suggest that dopamine transmission within the amygdala is not necessarily involved in the formation of short-term memory during acquisition but is involved in the formation and/or consolidation of long-term memory. Given the previous discussion of alternative explanations for the results of Experiments 1A and 1B and the appropriate controls included in Experiment 2A, we are confident that the effects observed following infusions of SKF 82958 are not due to state-dependent effects, altered sensory–motor functioning or drug diffusion into dopamine-rich regions other than the amygdala.

Finally, the design of the present experiments does not permit a definitive conclusion concerning the site of drug action within the amygdala. Although the guide cannulae were implanted just dorsal to the amygdala in the region of the amygdaloid central nucleus, it is quite possible that the drugs diffused into the central as well as the lateral and basolateral amygdaloid nuclei, all of which possess a rich dopaminergic input [2,12,13] and D₁ receptors [36]. Thus, the effects observed in the present experiments may be due to diffusion of either SCH 23390 or SKF 82958 into any or all of these nuclei.

In conclusion, the results of the present study suggest that dopamine transmission within the amygdala plays a role in long-term memory formation and/or consolidation following Pavlovian fear conditioning, as well as for the expression of conditioned fear responses retrieved from long-term memory. The exact mechanisms upon which dopamine acts in exerting this influence is a matter for future research.

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