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Role of dopamine D_3 receptors in the expression of conditioned fear in rats

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Abstract There has been considerable interest in the role of dopamine D3 receptors in appetitive conditioning but few studies have examined their role in aversive conditioning. The present study examined the effect of the dopamine D3 receptor-preferring partial agonist BP 897 (1-(4-(2-naphthoylamino) butyl)-4-(2-methoxyhenyl)-1A-piperazine hydrochloride) and the selective dopamine D3 receptor antagonist SB-277011A (trans-N-[4-[2- (6-cyano-1,2,3,4-tetrahydroisoquinolin-2-yl)ethyl]syclohexyl]4-quinolininecarboxamide]) on the expression and acquisition of fear conditioning. Rats (N=143) received 3 conditioned stimulus–shock pairings and then received 15 conditioned stimulus-alone presentations (3 per day) while lever pressing for food. Response suppression was taken as the behavioral measure of fear. Rats showed strong suppression to the conditioned stimulus after it had been paired with shock and suppression progressively weakened over conditioned stimulus-alone presentations. In experiment 1, rats that received BP 897 (1.0, 2.0 mg/kg i.p.) or SB-277011A (10.0 mg/kg i.p.) prior to conditioned stimulus-alone presentation sessions showed reduced suppression to the conditioned stimulus as compared to rats that received vehicle or lower doses of drug (0, 0.1 mg/kg BP 897; 0, 0.5, 5.0 mg/kg SB-277011A). Injections of BP 897 (1.0, 2.0 mg/kg) or SB-277011A (10.0 mg/kg) prior to conditioned stimulus–shock pairings did not significantly affect subsequent response suppression. Thus, BP 897 and SB-277011A dose-dependently attenuated the expression but not the acquisition of conditioned fear. These findings suggest that BP 897 and SB-277011A reduce the control of responding by aversively conditioned stimuli.

Keywords: BP 897; SB-277011A; Fear; Conditioning; Dopamine; D3 receptors

1. Introduction

Dopamine is a central nervous system neurotransmitter that has been implicated in a wide range of behaviors, including appetitive (e.g., Papp et al., 2002) and aversive (e.g., Inoue et al., 2000) conditioning. Molecular biological studies have identified two distinct families of dopamine receptors, all of which are Gprotein coupled (Jaber et al., 1996). The dopamine D1-like family (D1, D5) stimulate adenylyl cyclase activity and do not contain introns whereas the dopamine D2-like family (D2, D3, D4) inhibit adenylyl cyclase activity and have a discontinuous gene sequence. Recently, due to the advent of highly selective dopamine D3 receptor ligands, there has been considerable interest in the role of these receptors in appetitive conditioning.

Studies have differentially implicated dopamine D3 receptors in the acquisition vs. expression of appetitive conditioning. Thus, pre-test injections of the dopamine D3 receptor partial agonist BP 897 (1-(4-(2-naphthoyl-amino)butyl)-4-(2-methoxyhenyl)- 1A-piperazine hydrochloride) blocked the expression of cocaine-, morphine-, amphetamine-, or nicotine-induced conditioned place preference (Duarte et al., 2003; Frances et al., 2004; Aujla and Beninger, 2005; Le Foll et al., 2005) and the dopamine D3 receptor-preferring agonist 7-OH-DPAT (7- hydroxy-N,N-di-n-propyl-2-aminotetralin) blocked morphine-induced conditioned place preference (De Fonseca et al., 1995). Pre-test injections of the selective dopamine D3 receptor antagonist SB-277011A (trans-N-[4-[2-(6-cyano-1,2,3,4-tetrahydroisoquinolin- 2-yl)ethyl]syclohexyl]4-quinolininecarboxamide]) blocked heroin-, cocaine-, or nicotine-induced conditioned

place preference (Vorel et al., 2002; Ashby et al., 2003; Le Foll et al., 2003; Pak et al., 2006). In contrast, pre-conditioning injections of similar doses of BP 897 failed to affect the acquisition of a conditioned place preference based on cocaine, morphine or amphetamine (Gyertyan and Gal, 2003; Duarte et al., 2003; Aujla and Beninger, 2005). Similarly, the dopamine D3 receptor-preferring agonists 7-OH-DPAT or PD128907 (R- (+)-trans-3,4,4a,10b-tetrahydro-4-propyl-2H,5H[1]benzopyrano[4,3-b]-1,4-oxazin-9-ol) failed to affect acquisition of a cocaine-induced conditioned place preference (Gyertyan and Gal, 2003). Some reports however showed that dopamine D3 receptor-preferring agents given during acquisition blocked conditioning; this was found for the effects of BP 897 on conditioning based on cocaine (Duarte et al., 2003), 7-OHDPAT on conditioning based on amphetamine (Khroyan et al., 1998), and SB-277011A on conditioning based on cocaine (Vorel et al., 2002) and heroin (Ashby et al., 2003). Thus, it remains to be clearly assessed whether dopamine D3 receptors are differentially implicated in the acquisition vs. expression of conditioning processes.

Previous studies have shown that pre-test injections of BP 897 or SB-277011A blocked expression of conditioned activity based on amphetamine (Aujla et al., 2002), cocaine (Le Foll et al., 2002) or nicotine (Le Foll et al., 2003; Pak et al., 2006). In local injection studies, pre-test injections of BP 897 into the nucleus accumbens or basolateral amygdala blocked the expression of conditioned activity based on intra-nucleus accumbens amphetamine (Aujla and Beninger, 2004). In contrast, preconditioning injections of BP 897 either systemically (Aujla et al., 2002) or into the nucleus accumbens or basolateral amygdala failed to affect the expression of conditioned activity (Aujla and Beninger, 2004). Altogether these results suggest that dopamine D3 receptors, including those in the nucleus accumbens and basolateral amygdala, are implicated in the expression but not in the acquisition of appetitive conditioning in the conditioned activity paradigm.

Results from conditioned place preference and conditioned activity are consistent with those from drug self-administration studies. BP 897 or SB-277011A attenuated responding for drug seeking-associated conditioned cues but did not affect drug-taking responses (Pilla et al., 1999; Cervo et al., 2003; Di Ciano et al., 2003). SB-277011A also attenuated drug- (Vorel et al., 2002; Andreoli et al., 2003), cue- (Gilbert et al., 2005; Cervo et al., 2006; Vengeliene et al., 2006), and stress- (Xi et al., 2004) induced relapse of drug-seeking responses following extinction of drug taking (for reviews, see Heidbreder et al., 2005; Micheli and Heidbreder, 2006). Taken together, results from conditioned place preference, conditioned activity and drug self-administration studies suggest that dopamine D3 receptors may play a more important role in controlling the expression of responding to cues associated with appetitive conditioning than in the acquisition of information about those cues.

Surprisingly, no information presently exists about the possible differential role of dopamine D3 receptors in the acquisition vs. expression of aversive conditioning, despite the fact that numerous studies have implicated dopaminergic involvement in fear conditioning. For instance, Suzuki et al. (2002) found increased dopamine release in the amygdala following fear conditioned-induced freezing in rats. Systemic injections of the dopamine D1-like receptor antagonist SCH23390 (R-(+)-7- chloro-8-hydroxy-3-methyl-1-phenyl-2,3,4,5-tetrahydro-1H-3- benzazepine hydrochloride) attenuated the acquisition but not expression of conditioned fear as measured by freezing (Inoue et al., 2000) and amygdalar infusion of the dopamine D2 receptor-preferring antagonist raclopride prior to fear conditioning attenuated fear potentiated startle in rats (Greba et al., 2001). Thus, evidence suggests that D1-like and D2 dopamine receptors in the amygdala mediate the acquisition of conditioned fear yet there is a lack of studies that have examined the role of dopamine D3 receptors in aversive conditioning.

In this study, rats were trained to lever press for food and then received several presentations of a tone conditioned stimulus followed by mild electrical foot-shock. During subsequent lever-pressing sessions, the tone conditioned stimulus was presented periodically but shock was no longer given. The tone produced conditioned suppression of responding that gradually extinguished with repeated presentations. The effects on conditioned suppression of the dopamine D3 receptor partial agonist BP 897 (Pilla et al., 1999) and the selective dopamine D3 receptor antagonist SB-277011A (Reavill et al., 2000; Stemp et al., 2000), given prior to the conditioning session (acquisition) or prior to the test session (expression) were evaluated. BP 897 was used as it was the dopamine D3 receptor-preferring agent that was available to us when we began this study and we then repeated the study with the selective dopamine D3 receptor antagonist SB-277011A when it became available to us. Based on the findings of appetitive conditioning paradigms, we examined the hypothesis that dopamine D3 receptors play a more important role in the expression vs. acquisition of aversive conditioning.

2. Materials and methods

2.1. Subjects

Experimentally naïve male albino Wistar rats (N=143), bred by Charles River Laboratories (St. Constant, Quebec), were housed separately or in pairs with food (LabDiet 5001, PMI Nutrition Intl, Brentwood, MO) freely available or restricted (see Procedure). They were housed on bedding material (Beta Chip; Northeastern Products Corp., Warrensburg, NY) in clear plastic cages (45.0 cm°—25.0 cm°—22.0 cm) in an environmentally controlled colony room and had free access to water. Behavioral testing was conducted during the dark portion of a reversed 12-hour light–dark cycle, where dark began at 7:00 a. m. Rats were maintained according to the guidelines of the Canadian Council on Animal Care and the Animals for Research Act.

2.2. Apparatus

Four identical Skinner boxes (29.0°—23.0°—19.0 cm) were each housed in a sound-attenuating and light-resistant shell outfitted with a 2.5-Watt light bulb and a speaker located at the rear wall. The walls of each box were made of Plexiglas and the floor was made of a series of 0.3 cm diameter parallel stainless-steel rods that were 1.0 cm apart. The grid floor was able to deliver scrambled foot-shocks (0.5 s) that served as the unconditioned stimulus. There was a recessed food cup in the center of one sidewall of each box and a lever (1.5°—5.0°—1.0 cm) was 2.0 cm to the right of the magazine at a height of 6.0 cm above the floor. After testing the 0 and 2.0 mg/kg BP 897 groups in experiment 1A (see below), these levers were replaced by new ones with the following dimensions: 3.0°—3.5°—0.2 cm. A 75 dB, 3200 Hz tone emitted from the speaker served as the conditioned stimulus. Dustless precision food pellets (45 mg) from Bio-serv (Frenchtown, NJ; product number: F0021) were used as rewards. Experimental events were controlled and recorded by computers located in the same room as the chambers, and data were downloaded for analysis to a computer located in a different room than the chambers. The shock source was an A-615A Master Shocker (Lafayette Instruments; Lafayette, IN). The shock level was set at 0.5 mA.

2.3. Drug injections

BP 897 (Sigma; Oakville, ON, Canada) and SB-277011A (GlaxoSmithKline; Verona, Italy) were dissolved in dimethyl sulfoxide (DMSO) vehicle immediately prior to experimental testing. Rats received i.p. injections of BP 897 (0.1, 1.0, 2.0 mg/ kg) or SB-277011A (0.5, 5.0, 10.0 mg/kg) 30 min prior to behavioral testing.

2.4. Procedure — Experiments 1 and 2

Rats in both experiments received the same procedure outlined below. Upon arrival at the colony room, rats (N=143) were housed in pairs and had food continuously available for one week. This allowed them to gain weight and habituate to the colony room. For the last 3 days of this period rats were handled approximately 3 min per day.

Rats were then housed separately for the remainder of the experiment and their weight was monitored daily. They were reduced to 85% of their free-feeding weight by giving them no food on the first day, and then giving them 5 g of food per day on subsequent days until they reached their target weight. This took 2–5 days. Rats were then given approximately 12–15 g of food per day to maintain them at target weight. After leverpressing training, rats were restricted to approximately 17 g of food per day to allow for growth.

Each rat was trained to lever press for food reward using a shaping technique, whereby the researcher dispensed a food pellet when the rat sniffed or approached the lever. Eventually, the rat pressed the lever and learned that it dispensed reward. Food was available on a fixed ratio 1 schedule. That is, every time the rat pressed the lever, one food pellet was dispensed. The rat was considered trained when it pressed the lever at least 30 times in 30 min. All subsequent sessions were 30 min in duration and there was one session per day, seven days a week.

Trained rats were placed in the chamber for one session on a variable interval 15-s schedule and five sessions on a variable interval 30-s schedule. During the last of these sessions a tone was turned on three times, at random (see below), for a 15-s period each time to assess the level of response suppression to the stimulus before conditioning. The first presentation of the stimulus occurred at a random time between 5 and 12 min into a session, the second random presentation occurred between 13 and 19 min into a session, and the third random presentation occurred between 20 and 27 min into a session for all sessions in which stimuli were presented. Rats were required to press the lever a minimum of two times during a 15-s period before each stimulus presentation in order to receive presentation of the stimulus. If a rat failed to press the lever two or more times within the 15-s period, an identical pre-stimulus 15-s period would

commence and so on until the rat received the stimulus. This contingency remained in effect for all subsequent stimuli presentations (see below).

2.5. Procedure — Effect of BP 897 (experiment 1A) and SB- 277011A (experiment 1B) on expression of fear conditioning

Acquisition. The next day, experimental testing began. With the variable interval 30-s food reward schedule in effect, rats were exposed to the conditioned stimulus (tone) three times, each immediately followed by the unconditioned stimulus, a 0.5-s, 0.5-mA foot-shock. Suppression of lever pressing (see below) during the conditioned stimulus was calculated to assess the amount of conditioning.

Expression. During the next five sessions, the variable interval 30-s food reward schedule remained in effect. In experiment 1A, 30 min prior to each session, rats received an injection of either BP 897 (0.1, 1.0, 2.0 mg/kg; ns=9, 8, 12, respectively) or DMSO (n=12). In experiment 1B, 30 min prior to each session, rats received an injection of either SB-277011A (0.5, 5.0, 10.0 mg/kg; ns=14, 13, 12, respectively) or DMSO (n=18). There were three conditioned stimulus-alone presentations per session. Thus, rats received 15 conditioned stimulus-alone presentations over five sessions. Suppression of lever pressing during the conditioned stimulus was calculated to assess expression of conditioning.

2.6. Procedure — Effect of BP 897 (experiment 1A) and SB- 277011A (experiment 1B) on acquisition of fear conditioning

Acquisition. The procedure was identical to experiment 1, except rats in experiment 2A received an injection of BP 897 (1.0, 2.0 mg/kg; ns=8, 7, respectively) or DMSO (n=8) 30 min prior to the session, and rats in experiment 2B received SB- 277011A (10.0 mg/kg, n=11) or DMSO (n=11) 30 min prior to the session.

Expression. The procedure was identical to experiment 1, except 30 min prior to each session all rats in experiments 2A and 2B received an i.p. injection of DMSO.

2.7. Data analysis

To assess the level of fear during a stimulus presentation, suppression ratios were calculated. Ratios took the form A/(A+B), where A was the number of responses during the conditioned stimulus and B was the number of responses during the same period of time (15 s) just before conditioned stimulus onset. Thus, a ratio of 0.5would indicate no conditioned fear to the conditioned stimulus whereas ratios less than 0.5 would indicate the degree of response suppression during the conditioned stimulus. The lower this ratio, the greater the fear of the conditioned stimulus. Alpha was set at 0.05 for all statistical analyses and all analyses were done using SPSS software.

For all experiments, 2-way analyses of variance (ANOVA) were conducted to assess lever-pressing rates and suppression ratios before, during and after conditioning. Suppression ratios were averaged across sessions for all analyses. Where appropriate, significant effects were further analyzed with Newman– Keuls post-hoc tests and linear contrasts.

3. Results

3.1. Experiment 1A — Effect of BP 897 on expression of fear conditioning

Mean (±S.E.M.) lever-pressing rates (responses/5 min) for each 30-min session were calculated for each group (Table 1A). Response rates increased over sessions and were higher for the 0 and 2.0 mg/kg groups. A 2-way ANOVA with session (11 levels) as a within- and group (0, 0.1, 1.0, 2.0 mg/kg) as a between subjects factor revealed a significant interaction ($F(30, 370)= 2.53$, $Pb.001$). There was also a significant main effect of session ($F(10, 370)=17.87$, $Pb.001$), and group ($F(3, 37)= 3.71$, $Pb.05$). Newman–Keuls post-hoc comparisons confirmed that the 0 and 2.0 mg/kg BP 897 groups had significantly higher response rates than the 0.1 and 1.0 mg/kg groups ($Pb0.05$). These differences were attributable to the different levers used in the two pairs of groups (see Materials and methods).

To assess acquisition of fear conditioning, suppression ratios were averaged (±S.E.M.) across the second and third conditioned stimulus-alone presentations (before conditioning) and compared to suppression ratios calculated by averaging (±S.E.M.) across the second and third conditioned stimulus–unconditioned stimulus presentations (Table 2A). The first conditioned stimulus– unconditioned stimulus presentation was not included because suppression would not be expected before shock had ever been presented. Suppression to the conditioned stimulus increased (i.e., suppression ratios decreased) during conditioned stimulus– unconditioned stimulus pairings. A 2-way ANOVA with session (2 levels) as a within- and group (0, 0.1, 1.0, 2.0 mg/kg) as a between-subjects factor revealed a significant main effect of session ($F(1, 37)=52.88$, $Pb.001$). There was no significant main effect of group ($F(3, 37)=1.81$, n.s.), or interaction ($F(3, 37)= 0.09$, n.s.). Thus, all groups acquired fear conditioning.

Table 1.
Mean (± S.E.M.) lever pressing responses per 5 min for each VI 30-sec session for groups that received 0, 0.1, 1.0, or 2.0 mg/kg of BP 897 in Experiment 1A (Table 1A) and 0, 0.5, 5.0, or 10.0 mg/kg of SB-277011A in Experiment 1B (Table 1B). BP 897 and SB-277011A were given prior to testing expression of conditioned fear.

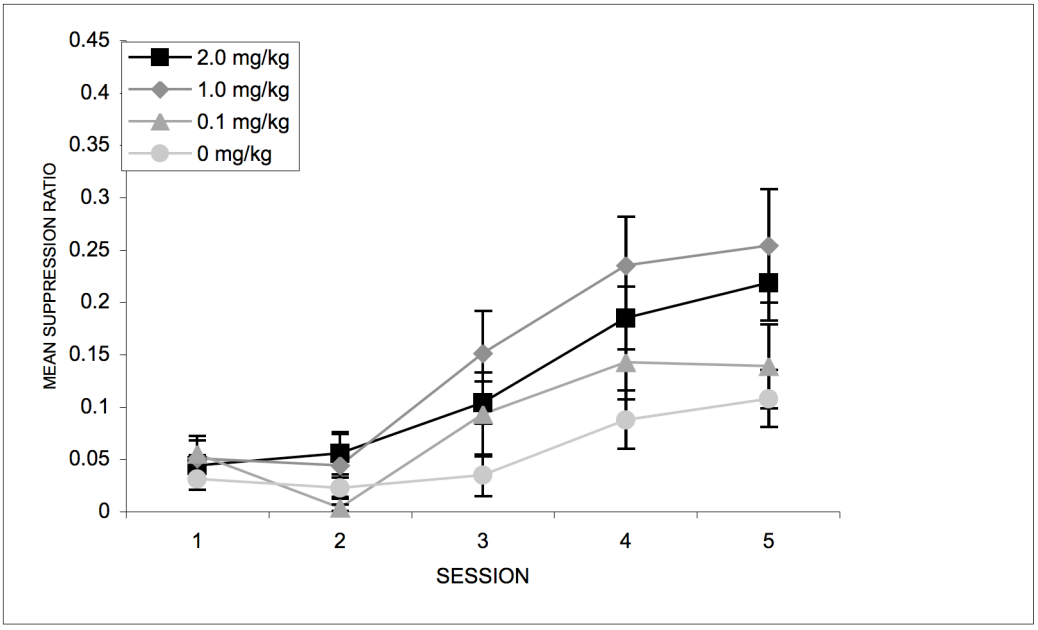
(A) Dose BP 897	**0 mg/kg**	**0.1 mg/kg**	**1.0 mg/kg**	**2.0 mg/kg**
Day*				
1	70.7 (±8.10)	50.2 (±13.39)	65.8 (±10.30)	83.5 (± 7.80)
2	95.0 (± 10.47)	72.0 (± 13.24)	79.2 (± 12.81)	102.0 (± 9.04)
3	118.1 (± 13.25)	102.5 (± 17.85)	97.8 (± 18.52)	135.1 (± 10.69)
4	157.0 (± 18.31)	119.5 (± 21.39)	127.9 (± 28.51)	168.9 (± 15.33)
5	178.3 (± 25.66)	97.7 (± 13.86)	93.4 (± 14.78)	177.8 (± 21.27)
6	177.6 (± 32.31)	59.2 (± 6.74)	60.5 (± 12.96)	175.2 (± 22.89)
7	163.2 (± 35.02)	82.5 (± 14.77)	95.2 (± 19.85)	125.6 (± 18.37)
8	188.3 (± 35.38)	91.3 (± 14.64)	115.4 (± 19.50)	169.3 (± 28.74)
9	196.9 (± 39.95)	103.3 (± 13.96)	116.8 (± 17.59)	191.6 (± 29.84)
10	213.5 (± 38.15)	117.4 (± 21.86)	127.8 (± 20.28)	192.6 (± 27.97)
11	229.0 (+ 35.87)	101.7 (+17.66)	94.3 (+ 24.12)	205.4 (+ 30.11)

(B)Dose SB-277011A	**0 mg/kg**	**0.5 mg/kg**	**5.0 mg/kg**	**10.0 mg/kg**
Day*				
1	62.2 (± 7.28)	84.4 (± 8.57)	87.6 (± 6.38)	56.4 (± 8.01)
2	86.4 (± 6.84)	99.2 (± 8.94)	98.8 (± 6.79)	78.2 (± 9.06)
3	106.3 (± 9.64)	115.6 (± 10.99)	104.9 (± 7.01)	98.3 (± 9.42)
4	123.4 (± 10.10)	102.3 (± 9.23)	100.1 (± 5.39)	106.3 (± 11.59)
5	106.6 (± 11.53)	108.6 (± 6.31)	106.4 (± 4.82)	102.1 (± 12.88)
6	96.3 (± 9.52)	95.3 (± 10.30)	82.4 (± 6.35)	79.1 (± 9.40)
7	97.4 (± 10.89)	93.3 (± 9.52)	75.5 (± 6.03)	45.2 (± 4.22)
8	118.4 (± 12.44)	108.1 (± 16.32)	83.5 (± 9.04)	58.5 (± 6.60)
9	148.2 (± 16.31)	118.6 (± 17.33)	91.6 (± 7.93)	71.2 (± 8.25)
10	148.2 (± 16.12)	127.7 (± 9.63)	91.3 (± 12.07)	87.5 (± 11.29)
11	134.6 (± 17.36)	116.3 (± 8.88)	87.2 (± 8.26)	71.6 (± 9.56)

* Day 1-4 = Training; Day 5 = CS-Alone; Day 6 = CS-US pairing; Day 7-11 = CS-Alone

To assess expression of fear conditioning, mean (±S.E.M.) suppression ratios to the conditioned stimulus averaged across each session were calculated for each dose group (Fig. 1A). All groups, including the control group, showed reduced suppression to the conditioned stimulus over sessions and this effect was greatest for the 1.0 and 2.0 mg/kg groups. A 2-way ANOVAwith session (5 levels) as a within- and group (0, 0.1, 1.0, 2.0 mg/kg) as a between-subjects factor revealed a significant main effect of group ($F(3, 37)=4.50$, $Pb.01$), and session ($F(4, 148)=29.44$, $Pb.001$). There was no significant interaction ($F(12, 148)= 1.49$, n.s.). In Newman–Keuls post-hocs comparisons, the 1.0 mg/kg group showed significantly less suppression to the conditioned stimulus than the 0 mg/kg group ($Pb.05$). The difference between the 2.0 mg/kg group and the 0 mg/kg group approached significance ($P=.051$). To further analyze the main effect of session, collapsing across groups, a linear contrast revealed that suppression was reduced over the sessions ($F(1, 37)= 57.52$, $Pb.001$).

A



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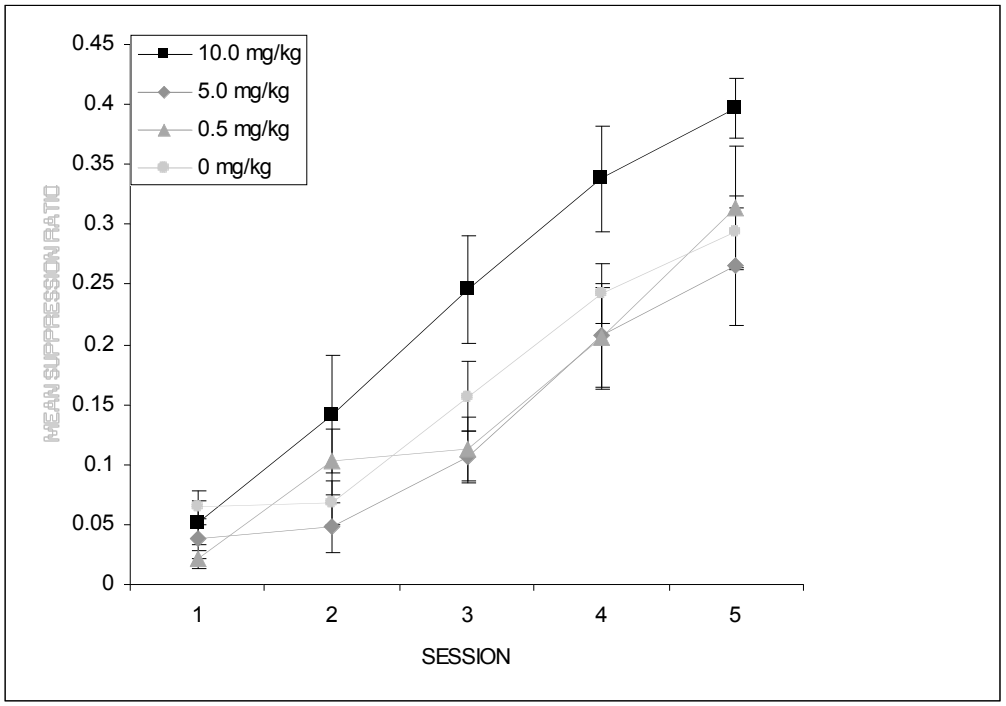


Figure 1. Mean (± *S.E.M.*) suppression ratio during expression (CS-alone presentations) of fear conditioning for groups that received BP 897 (0, 0.1, 1.0, and 2.0 mg/kg i.p.) in Experiment 1A (Figure 1A) and SB-277011A (0, 0.5, 5.0, and 10.0 mg/kg i.p.) in Experiment 1B (Figure 1B). BP 897 and SB-277011A were given prior to testing for expression of fear conditioning.

Table 2.
Mean (± S.E.M.) suppression ratio to the second and third CS before conditioning and during the second and third CS-US pairings for groups that received 0, 0.1, 1.0 or 2.0 mg/kg BP 897 in Experiment 1A (Table 2A) and 0, 0.5, 5.0 or 10.0 mg/kg SB-277011A in Experiment 1B (Table 2B). BP 897 and SB-277011A were given prior to testing for expression of conditioned fear.

(A) Dose BP 897	Before Conditioning	CS-US Pairings
0 mg/kg	0.39 (± 0.17)	0.11 (± 0.07)
0.1 mg/kg	0.31 (± 0.07)	0.07 (± 0.03)
1.0 mg/kg	0.33 (± 0.04)	0.08 (± 0.08)
2.0 mg/kg	0.41 (± 0.03)	0.17 (± 0.04)

(B) Dose SB-277011A	Before Conditioning	CS-US Pairings
0 mg/kg	0.31 (± 0.05)	0.10 (± 0.02)
0.5 mg/kg	0.33 (± 0.06)	0.10 (± 0.03)
5.0 mg/kg	0.36 (± 0.03)	0.07 (± 0.02)
10.0 mg/kg	0.27 (± 0.05)	0.03 (± 0.02)

3.2. Experiment 1B — Effect of SB-277011A on expression of fear conditioning

Mean (±S.E.M.) lever-pressing rates (responses/5 min) for each 30-min session were calculated for each group (Table 1B). All the groups showed similar rates of responding over days one through six, but on days seven through eleven the groups that received 5.0 or 10.0mg/kg SB-277011A showed reduced rates. A 2-way session°—group ANOVA revealed a significant main effect of session (F (10, 520)=12.09, Pb.001), group (F (3, 52)=3.48, Pb.05), and interaction (F (30, 520)=3.53, Pb.05). The interaction reflected the general increase in rates over sessions for the 0 and 0.5mg/kg groups vs. the generally lower rates during sessions 7–11 for the 5.0 and 10.0 mg/kg groups.

To assess acquisition of fear conditioning, suppression ratios were averaged (±S.E.M.) across the second and third conditioned stimulus-alone presentations (before conditioning) and compared to suppression ratios calculated by averaging (±S.E.M.) across the second and third conditioned stimulus–unconditioned stimulus presentations (Table 2B). Suppression to the conditioned stimulus increased (i.e., suppression ratios decreased) during conditioned stimulus–unconditioned stimulus pairings, as compared to before conditioning. A session°—group ANOVA revealed a significant main effect of session (F (1, 53)=78.84, Pb.001). There was no significant main effect of group (F (3, 53)=1.17, P=n.s.) or interaction (F (3, 53)=0.48, P=n.s.). Thus, all groups acquired fear conditioning.

During expression of fear conditioning all groups, including the control group, showed reduced suppression over sessions but the 10.0 mg/kg group showed the greatest effect (Fig. 1B). ANOVA revealed a significant main effect of group (F (3, 53)=3.50, Pb.05) and session (F (4, 212)=77.32, Pb.001), but no interaction (F (12, 212)=1.21, n.s.). In Newman–Keuls tests, the 10.0 mg/kg group showed significantly less suppression to the conditioned stimulus than the other groups that did not differ from one another. To further analyze the main effect of session, collapsing across groups, a linear contrast revealed that suppression was reduced over the sessions (F (1, 53)=208.09, Pb.001).

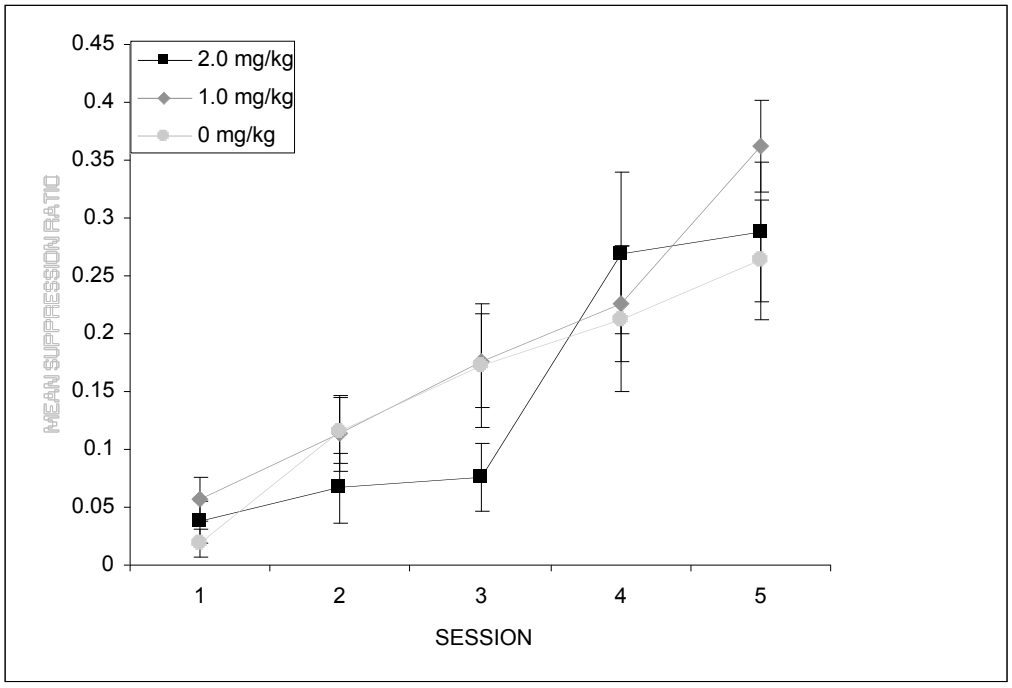
3.3. Experiment 2A — Effect of BP 897 on acquisition of fear conditioning

Mean (±S.E.M.) lever-pressing rates (responses/5 min) for each 30-min session were calculated for each group (Table 3A). Response rates increased over sessions. ANOVA revealed a significant main effect of session (F (10, 200)=7.19, Pb.001). There was no significant main effect of group (F (2, 20)=0.75, n.s.), or interaction (F (20, 200)=1.53, n.s.).

Groups acquired fear conditioning and there did not appear to be an effect of drug treatment (Table 4A). ANOVA revealed a significant main effect of session (F (1, 20)=88.72, Pb.001), but no significant effect of group (F (2, 20)=0.43, n.s.), or interaction (F (2, 20)=0.55, n.s.).

Groups did not differ during expression (Fig. 2A). ANOVA revealed a significant main effect of session (F (4, 80)=25.11, Pb.001), but no significant effect of group (F (2, 20)=0.51, n.s.), or interaction (F (8, 80)=1.03, n.s.). To further analyze the main effect of session, collapsing across groups, a linear contrast revealed that suppression was reduced over the sessions (F (1, 20)=73.47, Pb.001).

A



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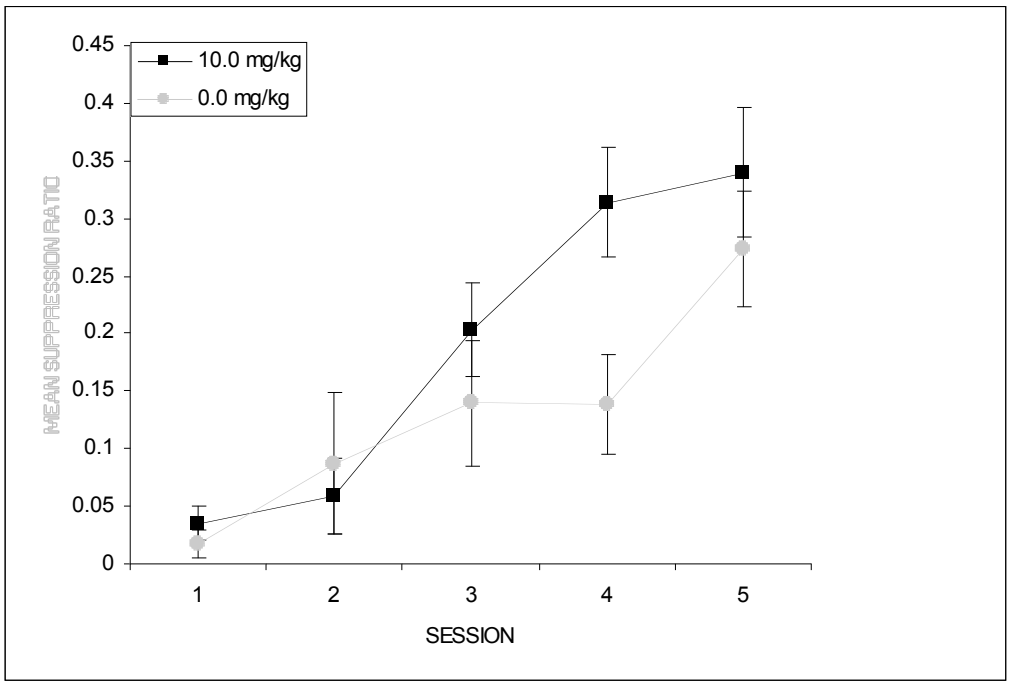

Figure 2. Mean (± *S.E.M.*) suppression ratio during expression (CS-alone presentations) of fear conditioning for groups that received BP 897 (0, 1.0, and 2.0 mg/kg i.p.) in Experiment 2A (Figure 2A) and SB-277011A (0 and 10.0 mg/kg i.p.) in Experiment 2B (Figure 2B). BP 897 and SB-277011A were given prior to testing for acquisition (CS-US pairings) of fear conditioning.

Table 3.
Mean (± S.E.M.) lever pressing responses per 5 min for each VI 30-sec session for groups that received 0, 1.0 or 2.0 mg/kg of BP 897 in Experiment 2A (Table 3A), and 0 or 10.0 mg/kg of SB-277011A in Experiment 2B (Table 3B).

(A) Dose BP 897	**0 mg/kg**	**1.0 mg/kg**	**2.0 mg/kg**
Day*			
1	79.0 (± 10.21)	50.6 (± 9.14)	76.5 (+ 17.62)
2	82.1 (± 9.92)	67.3 (± 12.69)	94.1 (+ 17.34)
3	87.6 (± 9.19)	79.0 (± 10.00)	100.5 (+ 24.35)
4	105.4 (± 9.82)	87.2 (± 12.33)	110.00 (+ 27.39)
5	98.8 (± 12.73)	85.4 (± 9.94)	116.7 (+ 30.32)
6	76.8 (± 8.36)	70.2 (± 9.20)	71.6 (+ 22.86)
7	54.9 (± 9.40)	58.2 (± 11.95)	107.6 (+ 27.26)
8	81.6 (± 10.29)	74.4 (± 14.25)	119.5 (+ 28.51)
9	85.8 (± 10.62)	83.0 (± 13.72)	110.4 (+ 35.53)
10	91.1 (± 8.19)	99.4 (± 13.15)	106.9 (+ 14.20)
11	97.5 (± 17.57)	106.9 (± 11.26)	113.4 (+ 18.42)

(B) Dose SB-277011A	**0 mg/kg**	**10.0 mg/kg**
Day*		
1	44.4 (± 6.26)	54.2 (± 6.08)
2	63.8 (± 6.98)	64.4 (± 5.50)
3	81.3 (± 8.94)	71.9 (± 8.23)
4	82.4 (± 7.67)	89.5 (± 10.55)
5	77.8 (± 10.23)	71.9 (± 8.67)
6	48.0 (± 8.05)	51.8 (± 10.63)
7	42.4 (± 10.28)	86.9 (± 14.58)
8	67.4 (± 9.55)	90.7 (± 11.64)
9	85.4 (± 8.70)	91.6 (± 10.76)
10	104.0 (± 9.90)	107.2 (± 10.78)
11	94.8 (± 8.55)	84.8 (± 9.14)

* Day 1-4 = Training; Day 5 = CS-Alone; Day 6 = CS-US; Day 7-11 = CS-Alone

3.4. Experiment 2B — Effect of SB-277011A on acquisition of fear conditioning

Mean (±S.E.M.) lever-pressing rates (Table 3B) generally increased over sessions (F (10, 200)=17.85, Pb.001), but tended to be lower in the 0 mg/kg group on the first two days of expression testing. This was supported by a significant interaction (F (10, 200)=3.68, Pb.001) in the ANOVA.

Both groups similarly acquired fear conditioning (Table 4B). ANOVA revealed a significant main effect of session (F (1, 20)= 42.99, Pb.001). There was no significant main effect of group (F (1, 20)=0.01, n.s.), or interaction (F (1, 20)=0.05, n.s.).

In expression (Fig. 2B), suppression decreased (i.e., suppression ratios increased) across sessions for both groups and ANOVA revealed a significant main effect of session (F (4, 80)=20.90, Pb.001). There was no significant main effect of group (F (1, 20)=1.84, n.s.), or interaction, although the interaction approached significance (F (4, 80)=2.31, P=0.065). To further analyze the significant main effect of session, collapsing across groups, a linear contrast revealed that suppression was reduced over the sessions (F (1, 20)=53.97, Pb.001).

Table 4.
Mean (± S.E.M.) suppression ratio to the second and third CS before conditioning and during CS-US pairings for groups that received 0, 1.0 or 2.0 mg/kg BP 897 in Experiment 2A (Table 4A), and 0 or 10.0 mg/kg SB-277011-A in Experiment 2B (Table 4B). BP 897 and SB-277011A were given prior to acquisition (CS-US pairings) of conditioned fear.

(A) Dose BP 897	Before Conditioning	CS-US Pairings
0 mg/kg	0.41 (± 0.07)	0.04 (± 0.03)
1.0 mg/kg	0.40 (± 0.03)	0.08 (± 0.05)
2.0 mg/kg	0.48 (± 0.06)	0.06 (± 0.03)

(B) Dose SB-277011A	Before Conditioning	CS-US Pairings
0 mg/kg	0.23 (± 0.04)	0.03 (± 0.02)
10.0 mg/kg	0.24 (± 0.05)	0.02 (± 0.01)

4. Discussion

All groups acquired conditioned fear to the conditioned stimulus; i.e., rats showed strong suppression to the conditioned stimulus during conditioned stimulus–unconditioned stimulus pairings compared to before conditioning. During the expression phase, all groups showed high levels of suppression to the conditioned stimulus during the first conditioned stimulus-alone session and suppression got progressively weaker over the remaining four sessions. In experiment 1, the 1.0 and 2.0 mg/kg doses of BP 897 and the 10.0 mg/kg dose of SB-277011A given prior to testing for expression of fear conditioning led to a significant reduction of suppression to the conditioned stimulus during the expression phase. These findings provide evidence that BP 897 and SB-277011A attenuated the expression of conditioned fear. In experiment 2, when BP 897 or SB-277011A was given prior to acquisition (conditioned stimulus–unconditioned stimulus pairings) of fear conditioning, BP 897 produced no systematic effect and SB-277011A appeared to decrease suppression in session 10. However, suppression ratios during extinction testing of rats that received systemic injections of BP 897 or SB-277011A prior to conditioned stimulus–unconditioned stimulus pairings did not differ significantly from vehicle controls.

Variable interval rates in the 0 and 2.0 mg/kg BP 897 groups in experiment 1A (effect of BP 897 on expression of fear conditioning) were higher than those of the 0.1 and 1.0 mg/kg BP 897 groups as a result of changing levers between experiments. Lower rates observed in the 5.0 and 10.0 mg/kg SB-277011A groups compared to the 0.5 and 0 mg/kg groups revealed a dose-dependent effect of SB-277011A on lever-press responding. Differences in rates are not problematic because overall levels of responding have little effect on suppression ratios; these ratios reflect changes in responding during brief periods. Rats were required to press the lever a minimum of two times during the 15 s preceding conditioned stimulus onset in order for the conditioned stimulus to be presented. Variable interval rates for all rats were high enough to meet this minimum response rate.

For the effects of SB-277011A (experiment 1B) on expression of conditioned suppression we considered the possibility that suppression ratios may have been rate-dependent (Robbins, 1981). For example, if animals with lower response rates showed less suppression than animals with high response rates, a pharmacological manipulation that decreased response rates would lead to less suppression, as was observed in the present study. Such an effect on suppression would reflect rate-dependency and not an effect of the drug on reactivity to the conditioned stimulus. Rate-dependency did not appear to account for the findings in experiment 1B. Correlational analysis revealed no relationship between response rates and suppression ratios. Thus, the attenuation by SB-277011A of expression of conditioned fear during conditioned stimulus presentation was a reliable effect.

The results of the present study revealed that BP 897 (1.0 or 2.0 mg/kg) and SB-277011A (10.0 mg/kg) attenuated expression of conditioned fear, as shown by reduced suppression to the conditioned stimulus during the conditioned stimulus-alone presentations (expression phase), but had no significant effect on the acquisition of fear conditioning. The effect of BP 897 and SB-277011A on the expression of conditioned fear cannot be attributed to the drug producing motor deficits since these groups pressed the lever more when the tone was presented, not less. In the case of SB-277011A, previous studies have also

clearly demonstrated that this compound, in the dose range used in the present experiments, does not induce motoric side effects (Reavill et al., 2000; Xi et al., 2005).

It is unlikely that BP 897 or SB-277011A produced sensory deficits because during the expression phase both drug and vehicle groups showed similarly high levels of suppression to the conditioned stimulus during the first session. It was only in the later expression sessions that differences between the drug and vehicle groups began to emerge. Also, in experiment 2, BP 897 or SB-277011A had no effect on the acquisition of fear conditioning, providing further evidence that the drugs had no effect on the ability of the rats to hear the tone. Thus, the present findings cannot be attributed to sensory deficits.

An alternative explanation of the differences observed in the present study is that state-dependent learning occurred. State-dependent learning occurs when an animal can only express learned behavior when it is in the same physiological state as it was when it acquired that behavior (Overton, 1978). In experiment 1, rats acquired the conditioned stimulus–unconditioned stimulus association in a drug-free state, but expression testing was conducted in a drugged state. Differences only emerged in the later expression sessions whereas a state-dependent learning account would predict differences from the outset of testing. In experiment 2, rats were conditioned in the drug state but tested drug-free and no significant differences in suppression ratios were seen. Thus, state-dependent learning cannot account for our results.

Rats showed a strong fear response to the conditioned stimulus after it had been paired with shock. This finding provides evidence that acquisition of fear conditioning occurred and the data are consistent with previous findings (LeDoux, 2000). During the expression phase, rats showed a strong fear response to the conditioned stimulus that gradually lessened over sessions. Thus, after repeated presentations of the conditioned stimulus-alone, rats showed reduced fear to the conditioned stimulus, i.e., extinction, and this finding is also consistent with previous reports (Davis et al., 2003). Rats that received systemic injections of BP 897 (1.0, 2.0 mg/kg) or SB-277011A (10.0 mg/ kg) showed reduced suppression to the conditioned stimulus alone during expression sessions compared to vehicle controls. This finding provides strong evidence that BP 897 and SB- 277011A attenuated the expression of conditioned fear and lends support to the hypothesis that dopamine D3 receptors play a role in the expression of conditioned fear.

The present findings provide the first evidence that the dopamine D3 receptor partial agonist BP 897 and the selective dopamine D3 receptor antagonist SB-277011A reduce the control of responding by aversively conditioned stimuli. The similarity in effect of the two agents suggests that BP 897 was acting like a dopamine D3 receptor antagonist. These results are consistent with observations from appetitive conditioning paradigms, such as conditioned place preference, conditioned activity, and drug-seeking, where these drugs were found to reduce the control of responding by appetitively conditioned stimuli. For instance, pretest systemic injections of BP 897 (1.0 mg/kg) were shown to block the expression of cocaine- (Duarte et al., 2003), amphetamine- (Aujla and Beninger, 2005), nicotine- (Le Foll et al., 2005), and morphine-induced conditioned place preference in rodents (Frances et al., 2004). Similarly, pre-test systemic administration of SB-277011A has been shown to block the expression of cocaine- (Vorel et al., 2002), heroin- (Ashby et al., 2003), and nicotine-induced conditioned place preference in the rat (Le Foll et al., 2005; Pak et al., 2006).

A previous study from this lab found that pre-test systemic injections of BP 897 (1.0 mg/kg) blocked the expression of amphetamine-induced conditioned activity (Aujla et al., 2002). Pre-test systemic injections of BP 897 (1.0 mg/kg) or SB- 277011A (10.0 mg/kg) were also shown to attenuate the expression of cocaine- (Le Foll et al., 2002) and nicotine-produced conditioned activity (Le Foll et al., 2003; Pak et al., 2006). Finally, BP 897 (1.0 mg/kg) blocked the expression of cue-controlled cocaine-seeking (Cervo et al., 2003). Importantly, selective antagonism at dopamine D3 receptors by SB- 277011A was shown to block the expression of drug-, cue-, and stress-controlled cocaine-seeking behavior (Vorel et al., 2002; Andreoli et al., 2003; Xi et al., 2004; Gilbert et al., 2005; Cervo et al., 2006; Vengeliene et al., 2006). Thus, the present findings are consistent with previous literature and suggest that in addition to reducing control of behavior by appetitively conditioned stimuli, BP 897 and SB-277011A also reduce the control of behavior by aversively conditioned stimuli.

BP 897 or SB-277011A given prior to conditioned stimulus– unconditioned stimulus pairings (experiment 2) had little effect on acquisition of fear conditioning. All groups acquired conditioned fear to the conditioned stimulus and during the expression phase showed high levels of suppression to the conditioned stimulus during the first conditioned stimulus-alone session and suppression got progressively lower over the remaining sessions. This pattern of results is consistent with previous findings in fear conditioning paradigms (e.g., LeDoux, 2000). These results suggest that dopamine D3 receptors are not involved in the acquisition of fear conditioning. Some findings from appetitive conditioning paradigms are

consistent with this result. For instance, Gyertyan and Gal (2003) found that preconditioning injections of SB-277011A or the dopamine D3 receptor-preferring agonists BP 897, 7-OH-DPAT, or PD128907 had no effect on the acquisition of cocaine-induced conditioned place preference. In addition, pre-conditioning injections of BP 897 had no effect on the acquisition of amphetamine- (Aujla and Beninger, 2005) or morphine-induced conditioned place preference (Duarte et al., 2003). Systemic injections of BP 897 given prior to training sessions failed to block the acquisition of amphetamine-induced conditioned activity (Aujla et al., 2002). However, other studies have implicated dopamine D3 receptors in the acquisition of appetitive conditioning. Duarte et al. (2003) found that pre-conditioning injections of BP 897 blocked the acquisition of cocaine-induced conditioned place preference, whereas pre-conditioning administration of SB-277011A blocked the acquisition of cocaine- and heroin-induced conditioned place preference (Vorel et al., 2002; Ashby et al., 2003). Since the present study is the first to examine the role of the dopamine D3 receptor in fear conditioning, and there was a near significant (P=0.065) interaction in experiment 2B, caution must be taken in interpreting a null finding. Thus, further studies are necessary to fully understand the role of BP 897 and SB- 277011A in the acquisition of fear conditioning.

During the expression phase, BP 897 and SB-277011A significantly reduced suppression to the conditioned stimulus alone during the later sessions. This allows for the possibility that the drugs facilitated the extinction of fear conditioning, rather than attenuated expression. A large body of evidence has recently led researchers to believe that extinction is not simply 'forgetting' what had previously been learned, but that it is a new form of learning (for a review see Bouton, 2004). There is little evidence to suggest that BP 897 or SB-277011A facilitates new learning in appetitive conditioning paradigms. For instance, BP 897 does not affect the acquisition of conditioned place preference to psychostimulants (e.g., Aujla and Beninger, 2005) and in some cases BP 897 actually blocked the acquisition of conditioned place preference (Duarte et al., 2003). Also, Cervo et al. (2003) found that BP 897 blocked the expression of cue-controlled drug-seeking. Vorel et al. (2002) showed that administration of SB-277011A attenuated cocaine-induced conditioned place preference. Based on these findings, it is difficult to attribute the results of the present study to BP 897 and SB- 277011A facilitating extinction of fear conditioning.

It is unlikely that BP-897 or SB-277011A produced their effects via D2 receptors. They have a 70- and 100-fold higher affinity for dopamine D3 over D2 receptors, respectively (Pilla et al., 1999; Reavill et al., 2000). Blocking D2-like dopamine receptors in the amygdala using raclopride (Greba et al., 2001) or eticlopride (Guarraci et al., 2000) disrupted the acquisition of fear conditioning and intra-central amygdala infusions of the dopamine D2 receptor-preferring antagonist sulpiride blocked the acquisition of morphine-induced conditioned place preference (Rezayof et al., 2002) but BP-897 or SB-277011A had no significant effect on acquisition in the present study. Thus, the present results are unlikely to be the result of blockade of dopamine D2 receptors. BP 897 has been shown to have affinity for neurotransmitter receptors other than dopamine D3 (e.g., D2, α1 adrenergic, α2 adrenergic, 5HT1A, 5HT2A) (Pilla et al., 1999; Cussac et al., 2000; Heidbreder et al., 2005; Xi et al., 2005). Action at one or more of those sites may be responsible for BP 897's effects. Furthermore, BP 897 alone has been reported to produce conditioned place aversion (Duarte et al., 2003; Gyertyan and Gal, 2003) and to inhibit electrical brain stimulation reward, an aversive-like effect (Campos et al., 2004). In contrast, SB-277011A is a highly potent and selective dopamine D3 receptor antagonist with 100-fold selectivity for dopamine D3 over other dopamine receptors, high affinity for the human and rat cloned dopamine D3 receptor, and 100-fold selectivity over 66 other receptors, enzymes, ion channels, and transporters in the central nervous system (Reavill et al., 2000; Stemp et al., 2000).

The effects of SB-277011A in various preclinical animal models (e.g., electrical brain stimulation reward, conditioned place preference, spontaneous locomotor activity, motor coordination, quinelorane-induced decrease in dopamine in the dorsal striatum, catalepsy, hyperprolactinaemia) are significantly different from those produced by dopamine D1- or D2-preferring antagonists (Heidbreder et al., 2005). The effects of SB-277011A might be mediated by interference with general aspects of memory storage and retrieval. This seems unlikely, as SB-277011A has been shown to reverse scopolamine-induced memory deficits in a 3-choice-point water labyrinth test (Laszy et al., 2005), to dose-dependently attenuate the deleterious influence of scopolamine on social memory (Millan et al., 2007), to enhance social memory (Millan et al., 2007), and to significantly increase extracellular levels of acetylcholine in the medial prefrontal cortex (Lacroix et al., 2003, 2006; Millan et al., 2007). Thus, SB-277011A does not induce motoric side effects, and does not impair memory or exhibit appetitive or aversive properties, all of which could have confounded the interpretation of the present results.

The present findings with SB-277011A appear to constitute the first clear demonstration that selective dopamine D3 receptor antagonism attenuates the expression of fear conditioning as assessed by conditioned suppression in the rat. The present study suggests that dopamine D3 receptor-preferring antagonists block the ability of aversive conditioned stimuli to affect behavior without blocking behavior produced by unconditioned aversive stimuli. Many anxiety disorders are believed to develop as a result of fear conditioning (Fyer, 1998). It is possible that in the future dopamine D3 receptor ligands, such as SB-277011A and BP 897 could be clinically useful in attenuating expression of phobias and post-traumatic stress when given alone or in conjunction with other therapies. To conclude, this study provides evidence that the dopamine D3 receptor partial agonist BP 897 and the selective dopamine D3 receptor antagonist SB-277011A attenuate the expression, but not the acquisition of fear conditioning measured by conditioned suppression in rats. Further research may identify brain regions involved and may assess the efficacy of these drugs in the treatment of anxiety disorders, such as phobias and post-traumatic stress disorder.

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