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Effects of D-amphetamine on defensive behaviors related to fear and anxiety

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Abstract

In rodents, the administration of amphetamine has been associated with increased locomotor activity and stereotypy, and an emerging body of evidence suggests that it also enhances anxiety-like behavior in a number of animal models. Ethoexperimental analyses have outlined an array of defensive behaviors to threat that are responsive to anxiolytic, panicolytic-like and panicogenic agents, suggesting that the characterization of amphetamine effects on defense may provide further insights into the emotionality consequences of this drug.

In Experiment 1, intraperitoneal administration of amphetamine (1 and 5 mg/kg, i.p.) on defensive behavior elicited by a predatory threat stimulus was assessed via time sampling analysis. Amphetamine dose-dependently suppressed freezing while potentiating locomotor activity. In Experiment 2, amphetamine was administered intravenously and animals were tested in a Rat Runway Test (RRT), designed to individually elicit a variety of defensive behaviors to a conspecific threat. All three doses of amphetamine (1, 2 and 5 mg/kg) produced robust changes in defensive responding by increasing directional flight behavior, jump escapes and upright/orientations. The results are in agreement with those of another psychostimulant, cocaine, and support a previously hypothesized link between flight and panic.

Keywords: Amphetamine; Defense; Fear; Anxiety

1. Introduction

Enhanced locomotor activity and stereotypical behaviors are commonly reported effects of amphetamine administration in rodents. Low doses of amphetamine induce an increase in locomotor activity (Schiorring, 1979; Antoniou and Kafetzopoulos, 1991; Antoniou et al., 1998), while high doses of amphetamine elicit behavioral stereotypy, including sniffing, circling, biting, gnawing, rearing and backward locomotion (Taylor et al., 1974; Pechnick et al., 1979; Fray et al., 1980; Antoniou et al., 1998). Stereotypy has been used as a model for amphetamine psychosis, a condition virtually indistinguishable from schizophrenia and commonly induced by high and/or chronic doses of amphetamine (Flaum and Schultz, 1997).

Among amphetamine users, psychiatric disorders such as anxiety and panic are commonly reported (Hall et al., 1996; Williamson et al., 1997). A recent study (Williamson et al., 1997) found that among various psychostimulant compounds (including amphetamines, cocaine and ecstasy), amphetamine produced the highest occurrence of adverse effects among abusers (30%), with feelings of paranoia, psychosis, mood swings, anxiety and panic. Hall et al. (1996) have also reported that anxiety, mania and panic were among the most commonly reported psychiatric disturbances among first time amphetamine users. Stereotypical behavioral patterns such as the repeated assembling and disassembling of complex mechanical devices are also frequently observed following high dose amphetamine intake and have been used as a model of amphetamine psychosis (Segal and Janowsky, 1978; Segal and Kuczenski, 1987; Angrist, 1994; Kuczenski and Segal, 1999).

Continuous sniffing behavior is a key component of psychostimulant-induced stereotypy in rodents. It is also observed in undrugged animals in response to novel or biologically meaningful (e.g., predator) odors. Sniffing in the latter situation is often considered as part of risk assessment, the information gathering process associated with defensive behaviors (Blanchard and Blanchard, 1989). While cocaine-elicited increases in sniffing were observed in rats not exposed to a threat stimulus, exposure to a cat dramatically reduced sniffing, while increasing other defensive behaviors (see Blanchard et al., 1999, 2000 for full description of procedure).

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This was interpreted as suggesting that the sniffing seen after cocaine, or, potentially other psychostimulant administration may reflect risk assessment behavior, which is suppressed by the presence of a high level threat source, such as a live predator. This view is consonant with findings that in non-drugged animals risk assessment behaviors are suppressed by a potent threat stimulus (such as a cat), but reappear when the threat stimulus is removed or if the animal is confronted with an ambiguous or low-level threat source, such as predator odors (Blanchard et al., 1990).

Like cocaine, the administration of amphetamine in rodents has been shown to produce effects on emotionality (Antoniou and Kafetzopoulos, 1991; Sams-Dodd, 1998) and to induce intense levels of sniffing (Pechnick et al., 1979; Fray et al., 1980; Antoniou and Kafetzopoulos, 1991; Antoniou et al., 1998). However, unlike cocaine, its effect has not been investigated in the context of defensive behaviors. The aim of Experiment 1 was to assess amphetamine-induced stereotypy in animals exposed to a live cat and to assess amphetamine's effect on defensive behaviors.

2. Method and procedure—Experiment 1

2.1. Subjects and treatment groups

Subjects were 72 experimentally naive Long Evans hooded rats (36 males and 36 females) from the University of Hawaii Laboratory Animal Services. All subjects were single housed in clear polycarbonate cages with wood chip bedding and free access to food and water. The rooms were kept under constant temperature and humidity. All animals were maintained under a 12-h light/dark cycle, with lights on at 06:00 h. Testing was conducted under the light phase between 09:00 and 14:00 h.

2.2. Drug administration

D-Amphetamine sulfate (Research Biochemicals International, Boston, MA) was dissolved in a vehicle of physiological saline and administered i.p. at 0, 1, or 5 mg/kg at a constant volume of 1 ml/kg.

2.3. Apparatus

The cat exposure apparatus for the toy, control and real cat conditions consisted of a cat compartment (55 cm long × 40 cm wide × 35 cm high) constructed of Plexiglas. The wire-mesh floor of the compartment was elevated 20 cm so that the subjects' homecage could be slid underneath. Subjects were videotaped from a camera positioned 0.5 m from the apparatus.

2.4. Procedure

In order to minimize contamination of the control group by cat odors, the experiment was conducted in two separate, but identical, rooms. On the day of testing, subjects were transported from the holding room to either of two testing rooms, one for the Real Cat (RC) testing, and the other for testing with a toy plush cat (TC). Although exposure to a TC will typically elicit low level defensiveness in rodents (Blanchard et al., 1998), it serves as an excellent control to the RC since it neither produces the movement nor emits the odor or sounds associated with a live cat. This procedure has been used in a similar study comparing the effects of cocaine administration on defensive behavior (Blanchard et al., 1999).

In the appropriate test room, each subject was removed from its homecage and injected (i.p.) with saline, 1 or 5 mg/kg amphetamine then returned immediately to its cage. Thirty minutes later, the subject, in its own cage, was placed under the stimulus compartment. Following a 5-min 'pre-cat' period the cat or toy cat (as appropriate) was introduced into the apparatus for a 15-min 'cat exposure' period. The cat/toy was then removed and subjects were videotaped for an additional 15-min 'post-cat' period. At the end of testing, subjects were removed from the apparatus and returned to the holding room. The apparatus was then cleaned thoroughly with a 70% ethanol solution and allowed to air dry prior to the next subject. Experimenters were blind to the drug dose being administered.

2.5. Scoring of behaviors

Videotapes were analyzed using time sampling, a procedure whereby behaviors are rated every 30 s over a 1 s time interval. Behaviors were scored for the entire duration of the 35-min test. The following behaviors were scored:

Locomote	Movement greater than 1 cm over the 1-s sampling period.
Crouch Immobile (*Freezing*)	Animal is immobile, in a sitting posture with forelimbs elevated off the floor.
Stand	Animal is immobile with both fore and hind limbs extended.
Rear	Forelimbs are off the floor.
Lie	The animal's weight is on the floor of the apparatus with no elevation due to either its forepaws or hind legs.
Groom	The animal licks, rubs or strokes its own body or fur.

2.6. Sniffing analysis

Sniffing was defined as one of four types of movements: polypnea (rapid respiration), tip of snout movement, vibrissa movement, and head movement. Because individual sniffing movements were virtually impossible to score, sniffs were recorded in bouts, defined as a continuous occurrence of sniffing and separated by periods of at least 5 s. In addition to the frequency of sniffing behaviors, the duration of each bout was scored.

Scorers had been trained to 95% or greater agreement on these behaviors using training tapes, and were blind to the drug condition of the subject.

2.7. Statistical analysis

All time sampling and sniffing measures during the stimulus period and post-stimulus period were evaluated using analysis of variance (ANOVA), with exposure condition (RC vs. TC), dose (0, 1 and 5 mg/kg) and sex as factors. All subsequent tests used Newman-Keuls post hoc analysis.

3. Results—Experiment 1

Figs. 1 and 2 present time sampling data for cat exposure and post-cat periods, respectively. There was no significant effect of sex on any behavior across these time periods and data for males and females were combined in all subsequent analyses. In addition, pre-cat data are not presented, since no significant differences were found in any measures.

3.1. Freezing

3.1.1. Cat period

The effect of exposure condition (cat vs. toy cat) was not significant. However, the main effect of amphetamine was significant during this period, $F(2, 59)=8.25$; $p<0.001$, as was the dose×exposure interaction, $F(2, 59)=5.63$, $p<0.01$. Subsequent analysis indicated that subjects administered each dose of amphetamine and exposed to the real cat engaged in significantly less freezing compared to saline, cat-exposed subjects ($p<0.05$ for each). In addition, subjects in the RC saline group exhibited more freezing compared to the TC saline group, $p<0.01$.

3.1.2. Post-cat period

ANOVA revealed a significant main effect of amphetamine on freezing during the post-cat period, $F(2, 59)=8.25$;

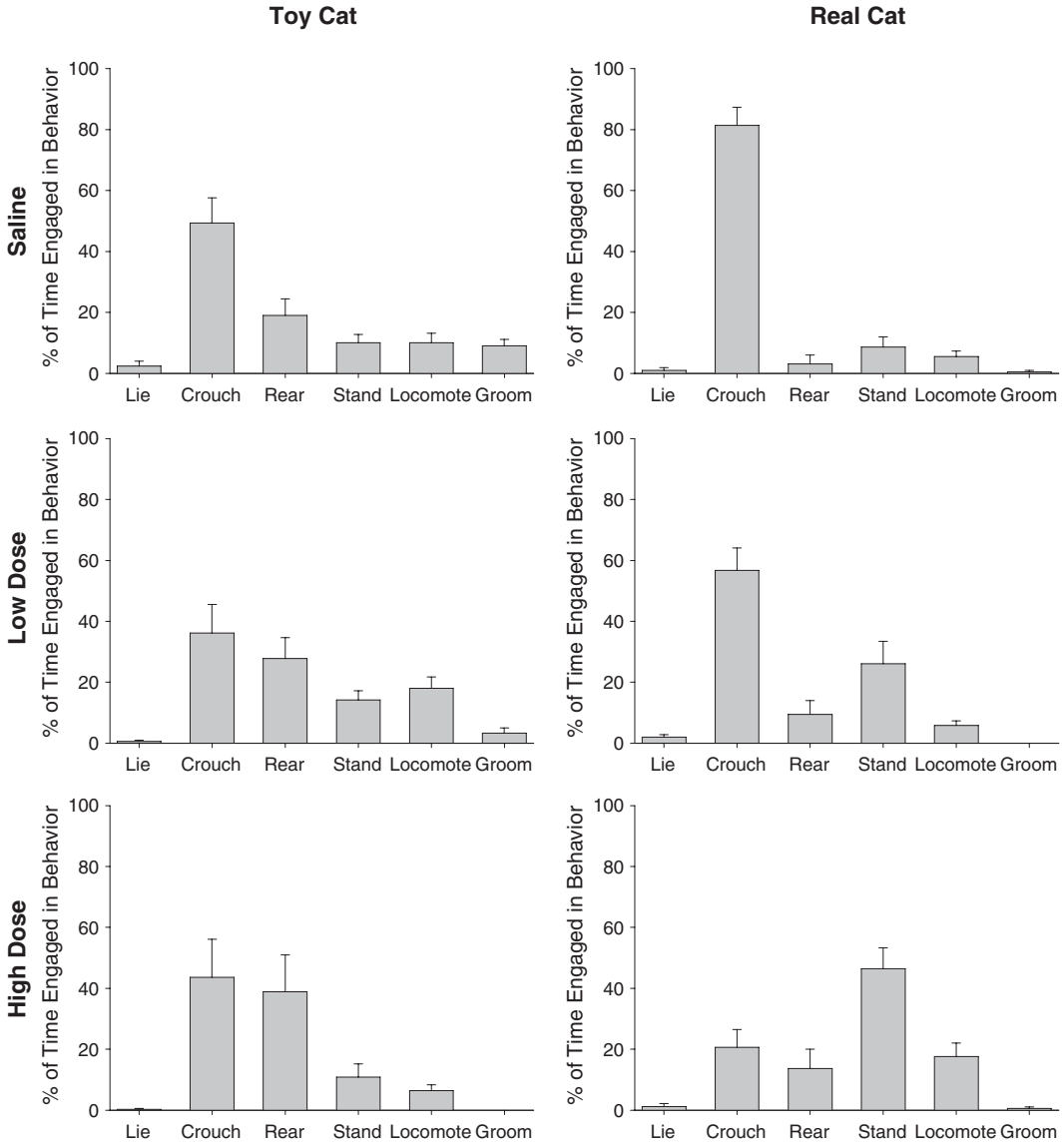

Fig. 1. Percentage of ratings of each behavior during the 15-min cat exposure period, for subjects under saline or amphetamine (1 or 5 mg/kg, ip). Ratings were made every 60 s of the behaviors occurring during a 1-s period.

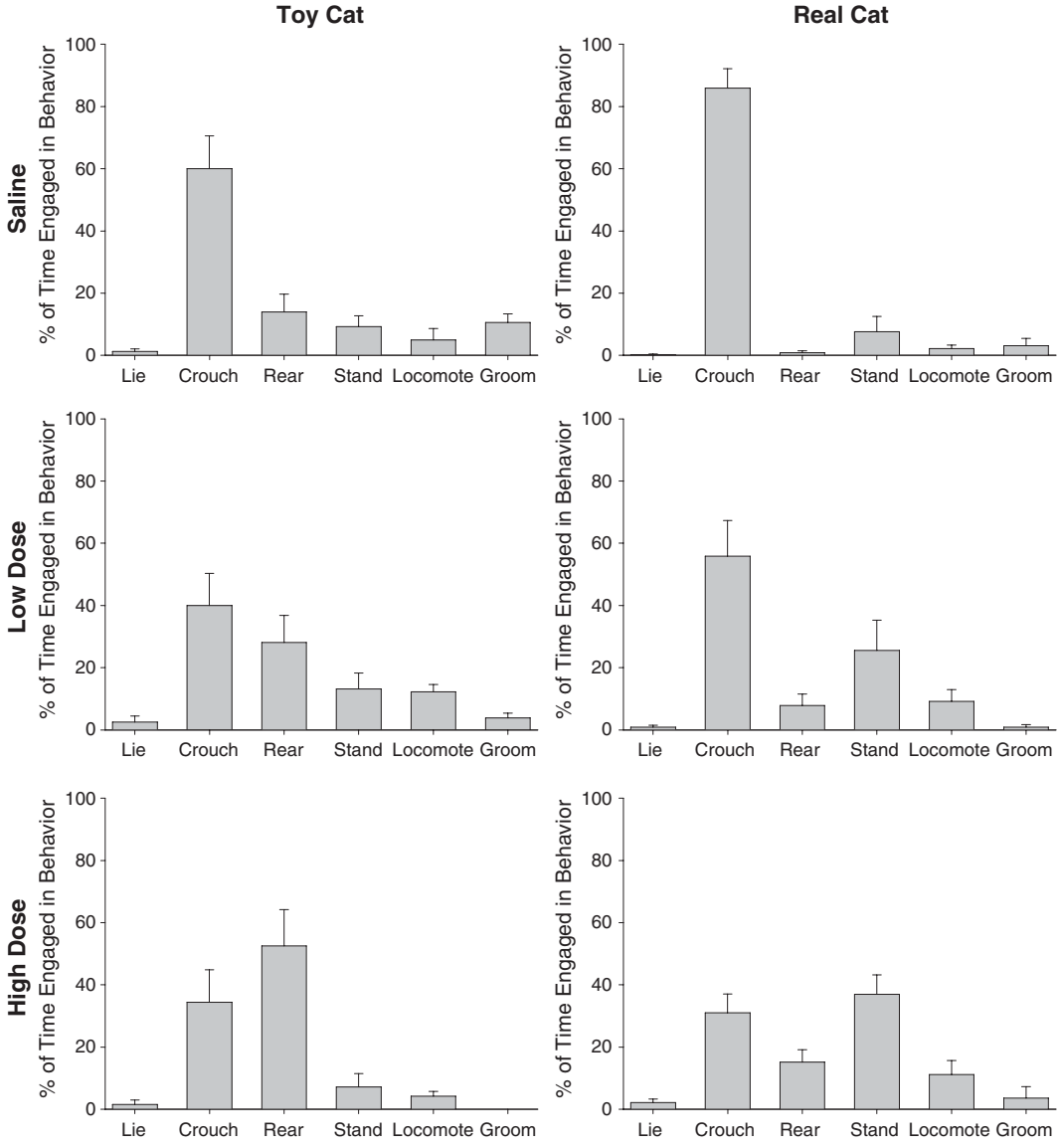

Fig. 2. Percentage of ratings of each behavior during the 15-min post-cat period, for subjects under saline or amphetamine (1 or 5 mg/kg, ip). Ratings were made every 60 s of the behaviors occurring during a 1-s period.

$p<0.001$. Post hoc analysis indicated that both the low and high dose groups showed less freezing compared to the saline control group ($p<0.05$). No other effects were significant.

3.2. Locomote

3.2.1. Cat period

Although ANOVA failed to detect a significant main effect of either exposure condition or dose alone, there was a significant dose × exposure interaction effect, $F(2, 59)=9.56$; $p<0.0005$. In the TC group, low dose subjects exhibited more locomotor activity than the high dose group ($p<0.05$). This pattern was reversed in the RC condition with the high dose group exhibiting more locomotor activity than the low dose group ($p<0.05$). High dose RC animals showed more locomotor activity than the high dose TC group, but low dose RC subjects exhibited lower levels of locomotion than the low dose TC group ($p<0.05$). For control animals, although the TC group showed higher levels of locomotion compared to the RC group, this difference was not significant.

3.2.2. Post-cat period

There was no significant main effect of dose or exposure condition or dose by exposure interaction on locomotor activity during this period.

3.3. Stand

3.3.1. Cat period

ANOVA revealed significant main effects of both exposure condition and dose on the number of stands during the cat exposure period, $F(2, 59)=8.43$; $p<0.001$, and $F(2, 59)=14.01$; $p<0.0005$, respectively. Subjects exposed to the RC exhibited a greater number of stands compared to subjects in the TC

condition ($p<0.05$). In the RC group both the low and high dose groups exhibited higher levels of standing compared to the saline group ($p<0.05$ for each). Amphetamine showed no significant effect in the TC group. The dose × exposure interaction was also significant, $F(2, 59)=5.94$; $p<0.005$. Among high dose subjects, animals exposed to the RC had higher levels of stands compared to the TC group ($p<0.001$).

3.3.2. Post-cat period

ANOVA revealed a significant main effect of exposure condition on stands during the post-cat period, $F(2, 59)=6.86$; $p<0.05$, with more stands for subjects in the RC condition, $p<0.05$. No other differences were significant.

3.4. Rear

3.4.1. Cat period

Subjects exposed to the RC exhibited significantly fewer rears during the cat exposure period compared to the TC group, $F(2, 59)=12.04$; $p<0.001$, indicating a significant condition effect. The main effect of dose and the dose × condition interaction was not significant.

3.4.2. Post-cat period

Subjects in the RC group exhibited fewer rears compared to animals in the TC group, $F(2, 59)=19.22$; $p<0.00001$. The effect of dose was also significant, $F(2, 59)=8.03$; $p<0.001$. Post-hoc analysis showed that the high dose group exhibited more rearing than either the saline ($p<0.0005$) or the low dose groups ($p<0.05$). The dose × exposure interaction was not significant.

3.5. Groom

3.5.1. Cat period

ANOVA revealed significant main effects of both exposure condition and dose on grooming during the cat period, $F(2, 59)=18.47$; $p<0.0001$, $F(2, 59)=8.75$; $p<0.001$, respectively. Subjects exposed to the RC showed significantly less grooming compared to the TC control groups ($p<0.05$). There was also a significant dose × exposure interaction, $F(2, 59)=9.32$; $p<0.001$, with the RC saline group showing fewer grooms than the TC saline group ($p<0.05$).

3.5.2. Post-cat period

There was no significant main effect of dose, exposure condition or a dose × exposure condition interaction on grooming during the post-cat period.

3.6. Lie

ANOVA did not indicate a main effect of dose, exposure condition or a dose × exposure condition interaction on lying during either the cat or post-cat period.

3.7. Sniffing analysis

Fig. 3 presents sniffing data for both the cat and post-cat periods.

3.7.1. Cat period

ANOVA revealed a reliable effect of exposure condition, $F(2, 59)=11.83$; $p<0.0001$, with more sniffs in the TC condition compared to the RC condition. Dose also had a significant effect, $F(2, 59)=14.94$; $p<0.00001$, with high dose amphetamine subjects showing significantly more bouts of sniffing compared to both the saline and low dose groups ($p<0.001$ for each). The dose × exposure interaction was not significant.

3.7.2. Post-cat period

The post-cat period produced a similar pattern: ANOVA revealed a significant main effect of both exposure condition and dose on the mean number of sniffing bouts during this period, $F(2, 59)=4.76$; $p<0.05$, and $F(2, 59)=14.98$; $p<0.0000$, respectively. Real Cat exposed animals showed less sniffing than animals in the Toy Cat condition ($p<0.01$ for each). Post-hoc tests indicated that both amphetamine doses produced more sniffing than did saline ($p<0.01$). The

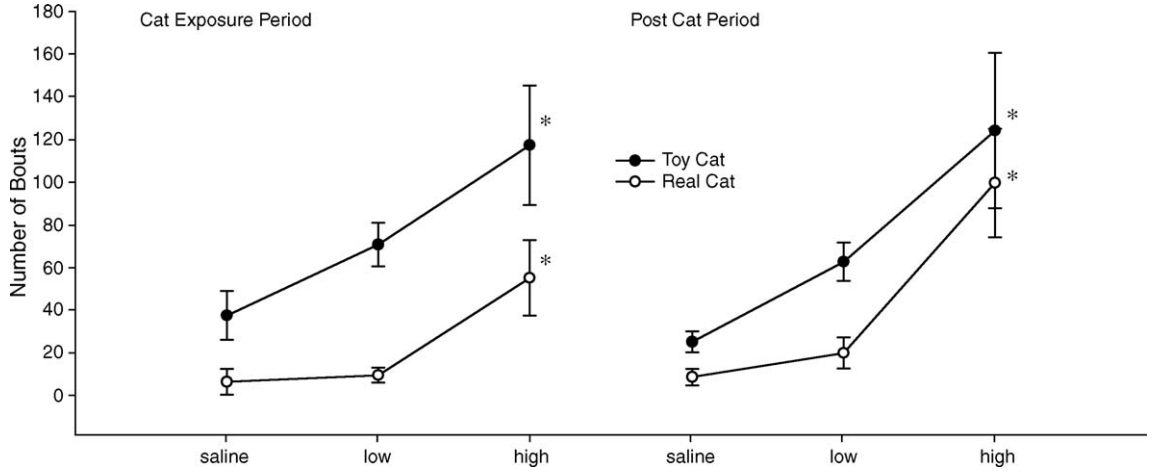

Fig. 3. Effect of amphetamine on number of sniff episodes during cat exposure and post-cat period. * indicates difference from control and low dose animals at $p<0.01$.

dose × exposure interaction during this period was not significant.

4. Discussion—Experiment 1

The administration of amphetamine produced clear behavioral effects that differed with exposure conditions. While the low dose TC groups exhibited high levels of locomotor activity, high dose animals exposed to the toy cat showed a dramatic reduction in locomotion. These behavioral results are consistent with reports of low-dose amphetamine induced increases in locomotor activity in a neutral or minimally threatening environment (Antoniou and Kafetzopoulos, 1991; Schiorring, 1979), whereas the reduced locomotion of TC high dose animals may be due to the concurrent increase in stereotypy (sniffing) observed under these non-threatening conditions.

In contrast to the amphetamine TC group, animals in the RC group that were administered amphetamine showed the opposite effect: while low dose animals showed a suppression of locomotor activity, high dose animals showed a significant potentiation of locomotion. These findings suggest that the low dose animals were responding appropriately to the presence of the cat by freezing. Although the small test arena used did not permit a differentiation between locomotion and flight, the potentiation of locomotion observed in the high dose animals might be related to flight potentiation previously observed in cocaine treated rats and mice (Blanchard and Blanchard, 1999; Blanchard et al., 1999, 2000; Hebert et al., 1999).

The suppression of freezing in RC exposed animals treated with amphetamine compared to animals administered saline is consistent with findings that cat exposure reduced freezing in animals given a high dose of cocaine, compared to a toy cat group (Blanchard et al., 1999). In this case, a similar decrease in freezing was observed in high dose animals exposed to the RC, compared to the cocaine-TC group.

In the context of the present study, the results indicate that amphetamine had strong effects on responsivity to a predator threat, but these effects did not fit a pattern of systematically reduced, or systematically increased, defensiveness. Reduced freezing and enhanced locomotion suggest decreased responsivity to the cat, but reduced grooming and enhanced rearing suggest otherwise. These results may reflect some relatively specific effects of amphetamine on particular defensive behaviors.

The striking dose-related increase of standing in amphetamine animals exposed to the cat is consonant with similar increases seen in cocaine dosed animals on cat exposure (Blanchard et al., 1999). Standing is not typically regarded as a defensive behavior, but its clear increase in RC, but not in TC animals, suggests that it may be related to the defense changes of RC-exposed animals with increasing doses of amphetamine. Previous studies have shown that conditions which reduce freezing (for example through the removal of the threat source), commonly increase standing, supporting the view that the standing posture often occurs when the freezing response is reduced (Blanchard et al., 2005). This transitional state may function to re-establish normal patterns of activity. Indeed, one possibility suggested by the topography of the behavior as well as by its relation to freezing (decreasing sharply with increasing amphetamine doses) and with locomotion (which increased with increasing amphetamine doses), is that it may, in this context, reflect a transition-state between freeze and locomote. Freezing involves support by hind limbs only, a posture incompatible with locomotion in the rat. Standing is observed when an animal is not locomoting, but has all four feet on the ground. It may thus represent a state in which the animal is transitioning from freeze to locomote, or vice versa. In terms of this interpretation, what is particularly interesting is that this intermediate state was of such high magnitude. This suggests an amphetamine dose-related reduction in the celerity of the transition between the two clear defensive behaviors under the presence of a high-magnitude threat stimulus.

Finally, the sniffing analysis portion of the study provides further evidence of a suppression of stereotypy under threatening conditions. Indeed, the purpose of the sniffing analysis of Experiment 1 was to determine the effect of predatory exposure on amphetamine elicited sniffing, a major form of psychostimulant induced 'stereotypy'. Findings that rats exposed to the RC exhibited significantly less sniffing compared to animals in the TC condition indicate that environmental conditions strongly influence the incidence of this 'stereotypical' response, raising questions about the function of this behavior.

5. Experiment 2

Experiment 1 indicated that amphetamine reduced freezing, against a background that otherwise suggests enhanced defensiveness (e.g., reduced grooming), enhanced rearing and, in the presence of the cat, enhanced locomotion. In order to evaluate amphetamine effects on defensiveness in situations differentially eliciting these behaviors, Experiment 2 utilized the Rat Runway Test (RRT), a modification of the well-characterized and pharmacologically validated (for a review, see Blanchard et al., 2003) Mouse Defense Test Battery (MDTB). The RRT/MDTB allows the experimenter to elicit, via a hand-held threat source (a terminally anesthetized rat) various defensive behaviors, including flight in an endless oblong runway. Tests utilizing the MDTB/RRT have demonstrated that panicolytic-like compounds have been shown to specifically decrease flight, while panicogenic-like (i.e., panic-inducing) compounds have been shown to specifically increase it (Griebel et al., 1995a,b). In addition, intravenous administration of cocaine elicits high levels of flight in rats tested in the RRT (Hebert et al., 1999).

In Experiment 2, rats were given amphetamine intravenously and confronted with a conspecific threat source. In view of studies suggesting that amphetamine use may be associated with manifestations of anxiety and panic in human users (Hall et al., 1996; Taylor et al., 1974), as well as findings that intravenous cocaine enhances flight in rodents (Hebert et al., 1999), it was hypothesized that the intravenous administration of amphetamine would also facilitate flight behavior to an oncoming threat source.

6. Method and procedures—Experiment 2

6.1. Subjects

Subjects were 12 experimentally naive Long Evans hooded rats approximately 110 days old at the time of testing. Animals were individually housed in clear plastic cages with free access to food and water in a temperature and humidity controlled room. They were kept on a 12-h light/dark cycle, with lights on at 06:00 h.

6.2. Experimental design

Each subject received saline, or 1.0, 2.0 or 4.0 mg/kg of amphetamine administered intravenously, via an indwelling venous catheter. Drug doses were administered according to a pre-determined, quasi-random schedule. Animals were tested each day during the light cycle for four consecutive days between 13:00 and 15:00 h with injections at least 24 h apart.

6.3. Drugs

D-Amphetamine sulfate (Research Biochemicals International) was dissolved in physiological saline and administered intravenously (i.v.) at doses of 0, 1, 2 and 4 mg/kg at a constant volume of 1 ml/kg.

6.4. Apparatus

Behavioral testing was conducted in an oval runway made of black Plexiglas (0.40 m wide × 0.30 m high × 4.8 m in length). It consisted of two 2-m straight segments connected to two 0.4-m curved segments and separated by a median wall. In order to minimize the subjects' visual contact with the experimenter, the apparatus was elevated 0.80 m from the ground. The floor of the runway was marked with a line drawn every 20 cm in order to facilitate locomotor and distance measures. Activity was recorded via 2 video cameras mounted above the apparatus.

6.5. Surgery

An indwelling venous catheter was implanted in each subject. Animals were anesthetized with a combination of sodium pentobarbital (65 mg/kg i.p.) and ketamine hydrochloride (40 mg/kg i.p.). A 25 cm length of Micro-Renethane MRE-040 (Braintree Scientific) tubing (0.040 in. o.d. × 0.025 i.d.) containing streptokinase (1.5 mg/kg) dissolved in a sterile 10% heparin/saline solution was inserted into the right jugular vein and moved to within a few millimeters of the right atrium. The catheter was anchored in place with two loops of 3–0 surgical silk. An 8 × 5 cm patch of Velcro was fixed to the back of each subject in the midscapular region using nonabsorbable 3–0 nylon sutures and Vetbond (3 M). The catheter was then passed subcutaneously through the shoulder area and externalized in the middle of the back. The tubing was then coiled within the Velcro patch. Each day following surgery, the catheters were flushed daily with the streptokinase/heparin solution to help maintain patency. Subjects were allowed 48 hours to recover before testing began.

6.6. Drug administration

The infusion of amphetamine was conducted in a closed off portion at the end of the runway. The catheter was uncoiled from the Velcro patch and first flushed with 0.5 ml of a 10% heparin/saline solution. Amphetamine (or saline) was then slowly infused through the catheter followed by an additional 0.5 ml heparinized saline to ensure complete delivery of the drug. The entire drug administration procedure took less than 2 min. After drug infusion, subjects were restricted in the closed off section for two minutes before behavioral testing began.

6.7. Behavioral testing

The following sub-tests were conducted by highly trained experimenters. During the 4 days of testing, subjects received the following battery of tests in the RRT:

1. Pre-test: Following the drug infusion, the subject was confined to the enclosed area for 2 min, after which the barriers were removed and the subject was allowed to explore the apparatus for an additional 3 min before the threat stimulus was introduced. The total number of line crossings and wall climb/rears were recorded.
2. Avoidance Test: The subject was approached by a hand held, terminally anesthetized rat at an approximate speed of 1.0 m/s. If flight was elicited, approach was terminated and the distance at which the subject began to flee from the stimulus rat (avoidance distance) and the total distance the subject fled (escape distance) was recorded. In cases where the subject failed to flee, a score of zero was recorded for both measures. The test was repeated, and the stimulus rat was removed from the runway for 5 s between the two trials.
3. Flight Test: The subject was approached by the stimulus rat at an approximate speed of 2.0 m/s. Approach speeds were kept consistent by the use of a stopwatch, such that ten 20-cm segments were covered in the span of 1 s. If the subject did not flee, the experimenter continued to make repeated contact attempts with the subject until flight was elicited or 2 min had elapsed. If flight was elicited, the subject was chased with the stimulus rat until three laps around the runway was completed, with a constant distance of 20 cm between stimulus rat and test subject. In addition to the time required to complete the three laps, the total number of stops, orientations (subject stops and turns to face the stimulus rat), reversals (changes in flight direction) and jump escapes were recorded. Following this initial test, a second flight test was conducted to assess whether the flight was directed in nature. The second trial was identical to the first, except the subject was approached from the opposite direction from the initial flight test.
4. Closed Door Test: A door at one end of the alley was closed and the subject was placed in the 'first square' (nearest the door). Starting at the opposite end of the alley,

Table 1
Number of line crossings during Pre-test period

Dose	Number of crossings	SEM
0	13.8	4.30
1	13.4	2.61
2	17.5	3.26
4	45.1*	14.30

* Indicates significant difference from control, $p<0.05$.

the stimulus rat was held stationary for 15-s periods at 1.2, 0.8, and 0.4 m. During each pause, time out of the first square, immobility time, average number of jump escapes and the closest distance between the stimulus rat and subject were scored.

5. Forced Contact: The door from the previous test remained in the closed position and the removable door was placed 40 cm away, creating an enclosed arena. The stimulus rat was then brought into contact with the subject for 5 s by the experimenter. Behaviors scored included jump attack/escapes, bites, upright defense, vocalizations. These behaviors were chosen because they were easily identifiable and clearly distinct from one another. Jump attacks were scored if the subject jumped toward the stimulus rat, while jump escapes were defined if the subject jumped away from the stimulus. Upright defense was recorded when the subject reared up on its hindlegs and assumed a 'boxing' stance while confronting the stimulus. Bites and vocalizations are self-explanatory.

7. Results—Experiment 2

7.1. Pre-test

One-way ANOVA indicated a significant main effect of amphetamine on the total number of line crossings during the three-minute pretest period, $F(3, 33)=3.92$; $p<0.05$. Analysis using the Fisher's Least Significant Difference test indicated that subjects administered the highest dose of amphetamine (4 mg/kg) showed more locomotor activity compared to either of the two lower doses of amphetamine $p<0.05$, or the control group, $p<0.01$. ANOVA revealed no significant main effect of dose on the number of wall rears during the pre-test period (Table 1).

Table 2
Avoidance and escape distances (cm)

	Dose (mg/kg)			
	0	1	2	4
Mean Avoidance Distances (cm)				
Mean	7.08	51.66	42.08	18.33
SEM	4.86	17.14	14.01	10.28
n	2	7	7	4
Mean Escape Distances (cm)				
Mean	1.33	4.19	6.08	10.41
SEM	0.76	1.46	2.27	5.16
n	3	7	7	4

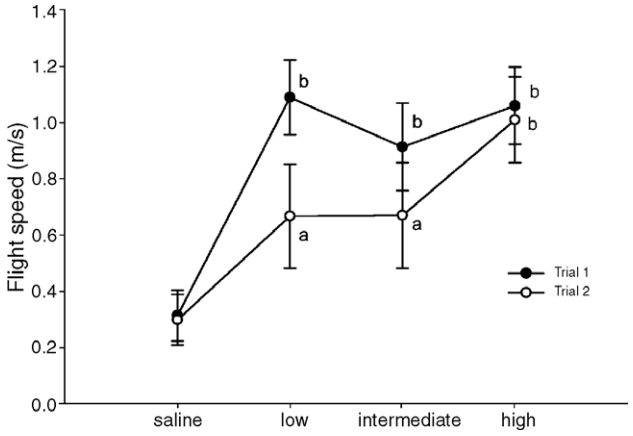

Fig. 4. Mean (and SEM) flight speed during chase test as a function of amphetamine dose for both chase directions. [a]Indicates significant difference from 0 mg/kg dose ($p<0.05$). [b]Indicates significant difference from 0 mg/kg dose ($p<0.001$).

7.2. Avoidance Test

The two avoidance trials were averaged to yield mean avoidance and escape distances. Table 2 presents these data, along with the number (*n*) of animals exhibiting avoidance and escape. ANOVA revealed a significant main effect of dose on the avoidance distance measure, $F(3, 33)=3.63$; $p<0.05$, with higher avoidance distances for the 1 mg/kg and 2 mg/kg groups, but not the 4 mg/kg group compared to the saline subjects, $p<0.01$ and $p<0.05$, respectively. Although subjects administered amphetamine tended to exhibit greater escape distances compared to the control group, ANOVA failed to detect a significant drug effect on this measure (Table 2).

7.3. Chase Test

Overall flight speed during the two trials is shown in Fig. 4. Flight was consistently directed away from the oncoming threat stimulus for all animals. Amphetamine increased flight speed,

Table 3
Behavioral measures during Chase Test, mean frequency of behaviors during Chase Test in experiment 2

		Dose (mg/kg)			
		0	1	2	4
Reversals	Mean	2.83	3.33	2.41	1.08**
	SEM	1.17	1.37	1.04	0.60
	n	6	9	6	4
Orientations	Mean	7.66	4.58	6.08	5.25
	SEM	1.34	1.32	1.29	1.62
	n	12	10	10	11
Stops	Mean	13.3	21.3	9.60	10.3
	SEM	2.69	3.35	2.35	2.39
	n	12	12	11	12
Jump Esc.	Mean	0.08	2.75*	4.50*	2.92*
	SEM	0.08	1.00	1.61	1.00
	n	1	6	7	9

* Indicates significant difference from 0 mg/kg dose, $p<0.05$.
** Indicates significant difference from 1 mg/kg dose, $p<0.05$.

Table 4
Behavioral measures during Forced Contact Test, mean frequency of behaviors Forced Contact Test in experiment 2

		Dose (mg/kg)			
		0	1	2	4
Upright	Mean	5.58	7.50	5.66	5.75
	SEM	1.71	1.23	1.16	1.37
	n	9	11	9	9
Jump Esc.	Mean	0.08	1.75*	1.42*	2.00*
	SEM	0.08	0.81	0.61	0.90
	n	1	6	6	5

* Indicates significant difference from 0 mg/kg dose, $p<.05$.

with a significant effect for both dose and the first and second chase trials, $F(3, 33)=6.41$; $p<0.01$ and $F(3, 33)=6.66$; $p<0.01$, respectively. Fisher's post hoc analysis indicated a significant difference between the control group and the low ($p<0.001$), intermediate ($p<0.01$) and high doses ($p<0.001$) animals for the first trial. During the second trial, a significant difference was found between the saline/high ($p<0.001$) and the intermediate/high dose group ($p<0.05$).

Results for the other behavioral measures taken during each of the two Chase Trials were combined and are provided in Table 3. Low dose animals exhibited a greater number of reversals compared to the high dose group, $F(3, 33)=3.53$; $p<0.05$. In addition, there was a significant effect of dose on the number of jump escape attempts during the chase test, $F(3, 33)=5.83$; $p<0.05$. All three doses of amphetamine produced an increase in the number of jump escape attempts compared to control animals during the chase; $p<0.05$ for all comparisons.

7.4. Closed Door Test

ANOVA did not reveal a significant effect of amphetamine on any measures (time out of first square, immobility time, number of jump escapes, and closest distance between the subject and stimulus rat) of the closed door test (data not shown).

7.5. Forced Contact Test

A Wilcoxan test indicated a significant increase in the number of jump escape attempts in all three amphetamine dose groups compared to controls, $p<0.05$ (Table 4). Amphetamine effects on upright behaviors were not significant. There were no instances of bites, jump attacks, vocalizations or flight for any of the doses tested.

8. Discussion

The primary finding of Experiment 2 was the potentiation of flight following the intravenous administration of amphetamine. These amphetamine-potentiated flight reactions exhibited directionality since subjects actively fled away from the threat source even when it approached from the opposite direction, requiring them to turn and flee. Thus amphetamine increased flight specifically, and this increase in flight, which occurred at all doses, cannot be attributed to general locomotor activation. Had the effect been attributed to simple activation of motor pathways, the flight response would have been unidirectional. The flight response in the RRT is consonant with the increased activity observed in amphetamine-dosed animals exposed to a cat in Experiment 1. As the threat source in Experiment 2 was a conspecific while in Experiment 1 it was a predator, this consistent potentiation of flight responses suggests that amphetamine may reduce the threshold for flight to a variety of threat stimuli. In addition to its effect on flight, amphetamine also increased jump escape attempts during the Chase Test. Jump escapes normally accompany flight and occur when an animal is cornered or has a limited escape route. These jump escapes often occurred at the ends of the runway, where the animal was required to turn the corner in order to continue fleeing, and may reflect attempts to continue to flee directly away from the oncoming threat source. The dramatic enhancement by amphetamine may indicate the high level intensity of the flight response.

There were both similarities and differences in the effects of amphetamine on stereotypy and defense in the present study, when compared to those of cocaine. Cocaine administration actually potentiated sniffing when the animals were tested either in their homecage (no threat) or when exposed to a low-level threat source (TC; Blanchard et al., 1999). However, exposure to the RC was shown to suppress this response. Similarly, in the present study (as shown in Fig. 3), amphetamine administration caused a suppression of sniffing in animals exposed to the RC compared to animals in the TC group. Indeed, in the TC group, amphetamine resulted in a dramatic potentiation of this response. These results are congruent with other studies showing that psychoactive compounds such as cocaine and amphetamine cause a potentiation of stereotypy (as indexed by sniffing in the present experiment). Furthermore, the present results suggest that this response may be suppressed by the presentation of a high level threat source (RC).

One slight discrepancy between the action of cocaine and amphetamine was in the degree of sniff suppression. The suppression of sniffing in amphetamine-administered animals exposed to the RC was considerably less than that obtained for cocaine animals (Blanchard and Blanchard, 1999). This behavioral difference between cocaine and amphetamine-treated animals that may reflect the fact that amphetamine directly stimulates the release of dopamine (Angrist, 1994; Kuczenski et al., 1997; Kuczenski and Segal, 1999) as well as other neurotransmitters such as norepinephrine (Angrist, 1994), while cocaine serves primarily as a reuptake inhibitor of dopamine (Ross and Renyi, 1967; Hekkila et al., 1975). The consequences of this difference may be a more potent effect on stereotypy (as well as defense) in amphetamine-administered animals compared to cocaine. Thus it is possible that the amphetamine animals may not be as selectively responsive to threat stimuli as are cocaine subjects. When compared to cocaine animals in the identical test situation, amphetamine treated animals exhibited heightened levels of defense. For example, compared to cocaine, amphetamine treated animals exhibited a much higher flight speed in the Chase Test (1.1 m/s

vs. 0.4 m/s, respectively). This, in addition to a reduced degree of suppression of sniffing stereotypy in amphetamine-dosed animals compared to cocaine, may indicate that sniffing was 'overridden' to a greater extent by defensive responding in amphetamine animals compared to cocaine.

In summary, the present series of experiments demonstrate the effects of amphetamine in two models involving different types of natural threat stimuli. In the cat exposure study, amphetamine-dosed rats exhibited heightened defensiveness with a suppression of stereotyped sniffing to the cat. In the RRT model, where a wider choice of behavior was allowed, flight was dramatically increased. These findings, along with previous results on cocaine in similar tests, suggest that psychostimulants may consistently enhance one subset of defensive behaviors, specifically those related to flight/escape, and provide additional support to the view that flight elicited by threat stimuli is selectively sensitive to both pro-panic and anti-panic agents.

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