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Yohimbine Potentiates Active Defensive Responses to Threatening Stimuli in Swiss-Webster Mice

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BLANCHARD, R. J., H. K. TAUKULIS, R. J. RODGERS, L. K. MAGEE AND D. C. BLANCHARD. *Yohimbine potentiates active defensive responses to threatening stimuli in Swiss-Webster mice.* PHARMACOL BIOCHEM BEHAV 44(3) 673–681, 1993. – Yohimbine HCl, an antagonist at α_2-noradrenaline receptors with putative panicogenic effects in human subjects, was administered to Swiss-Webster mice at doses of 0.5, 1.0, and 2.0 mg/kg. Animals were then tested in two defense test batteries. Yohimbine produced increases in flight from an approaching/contacting human and potentiated animals' reactions to dorsal contact. During a 5-min exposure to a cat (separated from the mouse by a wire-mesh screen) and the 15-min period thereafter, yohimbine produced a dose-dependent pattern of changes in defensive behaviors that included increases in locomotion, transits from one segment of the test chamber to another, fore- and hindpaw wall climbing, screen climbing and hanging, and roof pushing. Crouching (relative immobility while in a hunched-back posture) was notably decreased at all doses. During the postcat period, two different response patterns, "high-escape" and "low-escape," characterized in part by high and low frequencies of wall climbing, were observed in cat-exposed groups. In yohimbine-injected mice, the low-escape behavior pattern also included a tendency to avoid the segment of the test chamber closest to the cat compartment. Both patterns differed from the crouching and immobility generally exhibited by vehicle-injected, cat-exposed controls. It was suggested that yohimbine effected these behavioral changes by either potentiating neural mechanisms mediating flight or inhibiting mechanisms mediating freezing. This model may have some utility for the investigation of panicogenic and antipanic compounds and may contribute insights into the etiology of panic disorder.

Defense Swiss-Webster mice Yohimbine Panic Escape Potentiated flight

DYSREGULATION of central noradrenergic function has been suggested as a mechanism underlying panic disorder. Evidence for this proposal depends heavily upon the observed behavioral effects of yohimbine, an α_2-adrenoreceptor antagonist, in humans and animals [for reviews, see (12,31,38,44)]. Yohimbine has been reported to induce anxiety-like states in humans, with panic patients showing stronger reactions than individuals who are not panic prone (11,25,32,37). Further, in several studies drug-induced anxiety has been positively correlated with serum increases in 3-methoxy 4-hydroxy phenylglycol (MHPG), the principal metabolite of noradrenaline (10, 26).

In animal studies, yohimbine has been found to increase behavioral analogs of anxiety in stump-tailed macaques (39), squirrel monkeys (30), and bonnet macaques (13,40). In rats, putatively anxiogenic effects of this compound (typically in the range of 1.0–4.0 mg/kg) have been reported in the potentiated startle paradigm (16), social interaction model (23,27), and the elevated plus-maze test (14,21,22,28,43,45).

It has recently been suggested that the spontaneous activation of neuronal systems mediating the flight component of defense reactions may underlie human panic disorder (17, 18,24). If rodent defensive behavior is directed by parallel neurophysiological mechanisms, then it may be that flight in these animals can be potentiated by agents known to generate panic-like states in humans. In this laboratory, two test batteries have been designed to elicit and measure the defensive reactions of rats to a range of nonpainful threat stimuli: a) the Fear/Defense Test Battery (F/DTB), in which animals are confronted with immediate, discrete threat stimuli; and b) the Anxiety/Defense Test Battery, in which potential threat stimuli are presented. Both have proven useful in the analyses of

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the effects of various drugs and brain lesions on defensive behavior patterns in wild and laboratory rats (4,9). The present study extended the application of these test batteries to the laboratory mouse, and the effects of yohimbine on defensive behavior in this species were examined.

Defensive patterns of behavior in mice differ somewhat from those exhibited by rats. To accommodate these differences, certain aspects of the Anxiety/Defense Test Battery were changed. The modified version is herein referred to as the Proximics/Activity Test (P/AT).

METHOD

Subjects

Subjects were 79 Swiss-Webster mice, 40 male and 39 female, obtained from Simonsen Laboratories (CA) and were 60–75 days old at the beginning of the experiment. They were housed singly in polycarbonate cages in a room maintained on a 12 L : 12 D cycle.

Drugs and Treatment Groups

Yohimbine HCl (Sigma Chemical Co., St. Louis, MO) was dissolved in a vehicle of isotonic saline plus Tween-80 (1 drop in 4.0 ml) to various concentrations so that IP injections were always at a constant volume of 10.0 ml/kg. Mice were randomly assigned to groups (eight males and eight females per group) that were treated with yohimbine at doses of 0.5, 1.0, or 2.0 mg/kg or the drug vehicle alone. For the second portion of the experiment (P/AT), a further group was added (eight males and seven females) that also received the vehicle.

Apparatus

F/DTB. The apparatus for the F/DTB with rat subjects, described in detail in previous reports (4,5), was adapted here for use with mice. Two sets of tests were conducted in sequence, the first in an oval runway and the second in a plastic barrel 44.5 cm wide at its base and 67.0 cm deep.

The oval runway, 0.46 m wide, 1.23 m high, and 12.0 m in total length, consisted of two 4.0-m straight segments joined by two 2.0-m curved segments. Walls were made of plywood and the floor was concrete, marked off at 1.0-m intervals to facilitate distance measurements. Maze activity was recorded with videocameras mounted above the apparatus.

P/AT. The test apparatus for this procedure was a cat exposure box that consisted of two adjacent subject chambers (each 29.0 × 21.2 × 23.0 cm), both of which abutted a single cat compartment (57.0 × 36.3 × 42.5 cm). All walls and roofs of the cat and subject chambers were made of Plexiglas, with the exception of a wire-mesh wall that separated the cat chamber from subject chambers. The roof and outer walls of the subject chambers were clear while the remaining walls were opaque. A separate, laterally mounted videocamera was used to film the activities of each of the mice.

Procedure: F/DTB

Oval runway procedures. Thirty minutes after injection of yohimbine or vehicle, a subject was placed into the runway and the number of lines it crossed during a 5-min period was counted. Immediately thereafter, the experimenter entered the runway and approached the subject at a speed of approximately 0.5 m/s. Approach was terminated when contact with the subject was made or the subject ran away from the experimenter. If the subject fled, avoidance distance (the distance from the experimenter to the subject at the point of flight) was recorded, as was the distance fled. This was repeated five times.

The experimenter then approached the subject at a speed of approximately 1.2–2.0 m/s and recorded the time it took to chase the subject a distance of 24.0 m. Flight speed (m/s) was subsequently calculated from these measures.

Proximal Test Procedures. The subject was placed on the floor of the test barrel and its reactions to each of three types of threatening stimuli were measured.

Dorsal contact. With a wooden dowel, the experimenter lightly tapped the subject on its dorsal flank, and the animal's reaction was scored on a 5-point rating scale in which 1 represented a flinch reaction localized at the body area of contact and 5 represented a rapid jump in which all four limbs left the floor. Vocalizations in response to this stimulation were also recorded. This procedure was repeated four more times, and the scores for each behavior were summed over these trials.

Vibrissal stimulation. The subject's vibrissae were stimulated with two small brushes mounted adjacently on a long dowel. For each of five trials of this type, the following behaviors were recorded: jump attack, boxing, biting, and vocalizing.

Anesthetized rat. A Long-Evans male rat was terminally anesthetized and presented to the subject. Held snout-forward in the experimenter's gloved hand, the rat was moved toward the mouse at the rate of approximately 5.0 cm/s until contact occurred. The subject's boxing, biting, and jump attacks toward the rat's head were recorded, as well as vocalizations. This procedure was repeated four more times.

Procedure: P/AT

Seven to 9 days after the F/DTB procedure, each mouse was injected with the same dose of yohimbine (or vehicle) it had received before. Thirty minutes thereafter, it was placed into a subject compartment in the cat exposure apparatus for a total of 30 min. Following a 10-min precat period, a neutered male or spayed female domestic cat was placed into the cat compartment for 5 min. After the cat's removal, the mouse remained in the apparatus for an additional 15 min. Vehicle-injected, no-cat control subjects were included in this test; these subjects experienced only the opening and closing of the cat compartment door at appropriate times.

Subsequent analysis of the videotape records involved a time sampling method in which the activities of mice were noted at 30-s intervals. This provided frequency measures of rearing, grooming, locomotion, crouching (defined as sitting or standing still in a curved-back posture), orientation (toward or away from the cat compartment), and location in the chamber (in the near, middle, or far segment relative to the cat compartment) or on the wire-mesh screen between the cat and subject compartments. A real-time analysis of the videotapes was also performed to measure the number of transits (movements between segments of the subject compartment), episodes of screen poking (snout pushed through the holes in the wire mesh) and screen gnawing, instances of roof pushing (climbing to the top of the screen and pushing with snout and/or paws against the lid of the chamber), and instances of jumping and wall climbing. Wall climbing was defined as vertical movements of the forepaws alone or fore- and hindpaws together against any of the Plexiglas sides of the chamber. A jump was defined as an abrupt vertical leap, usually against a wall, in which all four paws left the floor.

RESULTS

F/DTB

The results of the F/DTB are presented in Table 1. Scores for male and female subjects combined are shown. Significant differences between sexes were not found except for a single exception as noted below.

Oval runway

Line crossings. A 2 × 4 × 5 (sex × dose × time) analysis of variance (ANOVA) performed on the number of line crossings during the initial 5-min free-roaming period in the runway yielded an effect of drug dose, $F(3, 56) = 4.47$, $p < 0.01$, which a subsequent Newman-Keuls analysis showed to be due to a significant decrease in line crossings by the group receiving the highest dose (2.0 mg/kg) of yohimbine. Line crossings increased in all groups from minute one through minute five, $F(4, 224) = 105.52$, $p < 0.001$, with a dose × time interaction, $F(12, 224) = 2.34$, $p < 0.01$, reflecting the fact that this increase was greater in the 2.0-mg/kg yohimbine group than it was in the others.

Flight from and avoidance of the experimenter. Yohimbine at all doses caused an increase in the percentage of flight responses away from the experimenter, $F(3, 56) = 4.51$, $p < 0.01$. A Newman-Keuls analysis indicated that each drug group differed from the saline control group with $p < 0.01$ (0.5 mg/kg), $p < 0.05$ (1.0 mg/kg), and $p < 0.02$ (2.0 mg/kg). The distance at which flight occurred varied across groups, $F(3, 56) = 4.22$, $p < 0.01$, with the greatest flight distance exhibited by the group administered the lowest dose of yohimbine (Newman-Keuls, $p < 0.01$); the medium- and high-dose groups did not differ from the saline controls. No group differences were found in the distances to which mice fled, the duration of flight, or speed of flight.

Proximal Tests. Effects of yohimbine were noted in only one of the proximal tests: reaction to dorsal contact, $F(3, 56) = 3.66$, $p < 0.02$. An exaggerated reaction was observed in all drug groups relative to saline controls and reached significance in the low-dose ($p < 0.02$) and medium-dose ($p < 0.04$) groups (Newman-Keuls test).

Although no effect of yohimbine was found on vocaliza-

TABLE 1

EFFECTS OF YOHIMBINE ON THE FEAR/DEFENSE TEST BATTERY

	Yohimbine (mg/kg)			
	0.0	0.5	1.0	2.0
Line crossing	68.75 (5.07)	64.38 (3.61)	61.94 (4.92)	46.69* (4.22)
Percent avoidance	31.88 (5.49)	48.75† (0.85)	41.88* (3.68)	46.25* (2.02)
Avoidance distance	1.57 (0.28)	2.84† (0.27)	1.76 (0.27)	2.15 (0.25)
Flight distance	2.27 (0.25)	1.97 (0.27)	2.63 (0.39)	2.24 (0.21)
Flight speed (m/s)	0.34 (0.02)	0.37 (0.02)	0.33 (0.02)	0.34 (0.02)
Dorsal Contact reaction	1.25 (0.63)	5.69* (1.27)	5.00* (1.18)	3.56 (0.93)
Dorsal Contact vocalization	0.13 (0.09)	0.81 (0.41)	0.44 (0.27)	0.25 (0.14)
Vibrissae boxing	4.25 (0.34)	4.63 (0.20)	4.63 (0.20)	4.5 (0.27)
Vibrissae vocalization	1.13 (0.43)	2.00 (0.56)	2.81 (0.50)	2.19 (0.48)
Vibrissae biting	0.13 (0.09)	0.48 (0.27)	0.81 (0.28)	0.56 (0.22)
Vibrissae jump attack	0.75 (0.41)	0.63 (0.30)	0.50 (0.27)	0.25 (0.11)
Boxing to rat	4.50 (0.24)	4.75 (0.11)	4.88 (0.85)	4.56 (0.24)
Vocalization to rat	4.19 (0.33)	4.38 (0.43)	4.81 (0.19)	4.44 (0.30)
Biting to rat	1.69 (0.53)	2.88 (0.49)	1.88 (0.54)	1.75 (0.44)
Jump attack toward rat	1.81 (0.51)	2.63 (0.49)	2.00 (0.50)	1.88 (0.41)

Data are presented as means (±SEM).

*$p < 0.05$, †$p < 0.01$, significant differences from vehicle-injected, cat-exposed controls.

tion to dorsal contact, female subjects overall showed a reliable tendency to vocalize more frequently, $F(1, 56) = 8.03$, $p < 0.01$. Female vocalizations to vibrissal contact and presentations of the anesthetized rat were also more frequent, but these differences did not reach statistical significance.

P/AT

Cat exposure period. The behavior patterns of mice during the 5-min cat exposure period are shown in Table 2. Data from two animals (1 male and 1 female) in the high-dose (2.0 mg/kg) yohimbine group was inadvertently lost, and so the means for this group were derived from the remaining 14 animals.

The presence of the cat altered the behavior of mice in a variety of ways, as was apparent when the saline and no-cat control groups were compared with 2 × 2 (groups × sex) ANOVAs. Significant decreases were observed in general locomotion, $F(1, 27) = 16.25$, $p < 0.001$, transits from one area of the compartment to another, $F(1, 27) = 4.45$, $p < 0.05$, on-screen hanging and locomotion, $F(1, 27) = 59.04$, $p < 0.001$, and screen poking, $F(1, 27) = 10.97$, $p < 0.001$. Forepaw wall climbing also decreased somewhat, with the difference between groups approaching significance, $F(1, 27) = 3.71$, $p = 0.06$. Roof pushing and fore- and hindpaw wall climbing occurred infrequently in vehicle-treated, cat-exposed animals and were not observed at all in many animals. For this reason, groups were compared using the nonparametric Mann–Whitney *U*-test, which revealed that vehicle-treated, no-cat controls exhibited significantly more roof pushing, $U = 70$, $p < 0.01$, but not fore- and hindpaw wall climbing, $U = 90$, $p < 0.11$, than cat-exposed subjects.

The one variable found to be significantly increased by the presence of the cat was crouching, $F(1, 27) = 9.07$, $p < 0.001$. The positions of animals in the compartment (near, middle, far) were unaffected, as were rearing, grooming, screen gnawing, and digging at corners (an infrequent behavior). Finally, no sex differences were detected for any variable.

The effects of yohimbine were assessed with 4 × 2 (dose × sex) ANOVAs, which included the four groups exposed to

TABLE 2

EFFECTS OF YOHIMBINE ON PROXIMICS/ACTIVITY DURING CAT PRESENTATION

	Yohimbine (mg/kg)				
	0.0	0.5	1.0	2.0	0.0 (no cat)
Near	63.13 (8.20)	51.88 (8.57)	55.63 (8.37)	40.71 (6.50)	69.33 (4.63)
Middle	10.63 (2.66)	10.63 (3.22)	15.63 (3.29)	20.71 (4.25)	16.67 (3.19)
Far	26.25 (7.85)	37.50 (8.49)	28.75 (8.70)	38.57 (8.44)	14.0 (2.54)
On screen	0.38 (0.15)	1.31 (0.69)	1.50 (0.52)	0.64 (0.31)	2.93* (0.30)
Crouch	4.63 (0.97)	3.5 (1.01)	2.56 (0.79)	1.86 (0.59)	1.40* (0.27)
Rear	3.44 (0.65)	2.5 (0.58)	3.69 (0.66)	3.36 (0.52)	3.33 (0.35)
Locomote	1.63 (0.42)	2.31 (0.45)	2.94 (0.50)	4.21† (0.50)	3.73* (0.28)
Transits	2.19 (0.53)	2.75 (0.55)	2.75 (0.39)	4.29‡ (0.52)	3.93‡ (0.61)
Groom	0.06 (0.06)	0.75‡ (0.30)	0.19 (0.10)	0.42 (0.23)	0.40 (0.19)
Wall climb: forepaws	2.19 (0.90)	3.00 (1.29)	2.25 (0.87)	2.71 (0.79)	4.60 (0.83)
Wall climb: Fore- and hindpaws	0.13 (0.09)	2.25‡ (0.85)	1.63 (0.62)	0.29 (0.22)	2.00 (0.77)
Digging at corner	0.25 (0.19)	0.31 (0.20)	0.50 (0.33)	0.00 (0.00)	0.40 (0.16)
Screen poke	4.31 (1.40)	5.00 (1.59)	7.06 (1.91)	5.93 (1.57)	13.80† (2.44)
Screen gnaw	1.50 (0.87)	1.50 (1.13)	0.88 (0.40)	0.07 (0.07)	3.47 (0.88)
Roof push	0.06 (0.06)	0.38 (0.18)	0.13 (0.09)	0.29 (0.22)	0.87† (0.32)

Data are presented as means (± SEM).

*$p < 0.001$, †$p < 0.01$, ‡$p < 0.05$, significant differences from vehicle-injected, cat exposed controls.

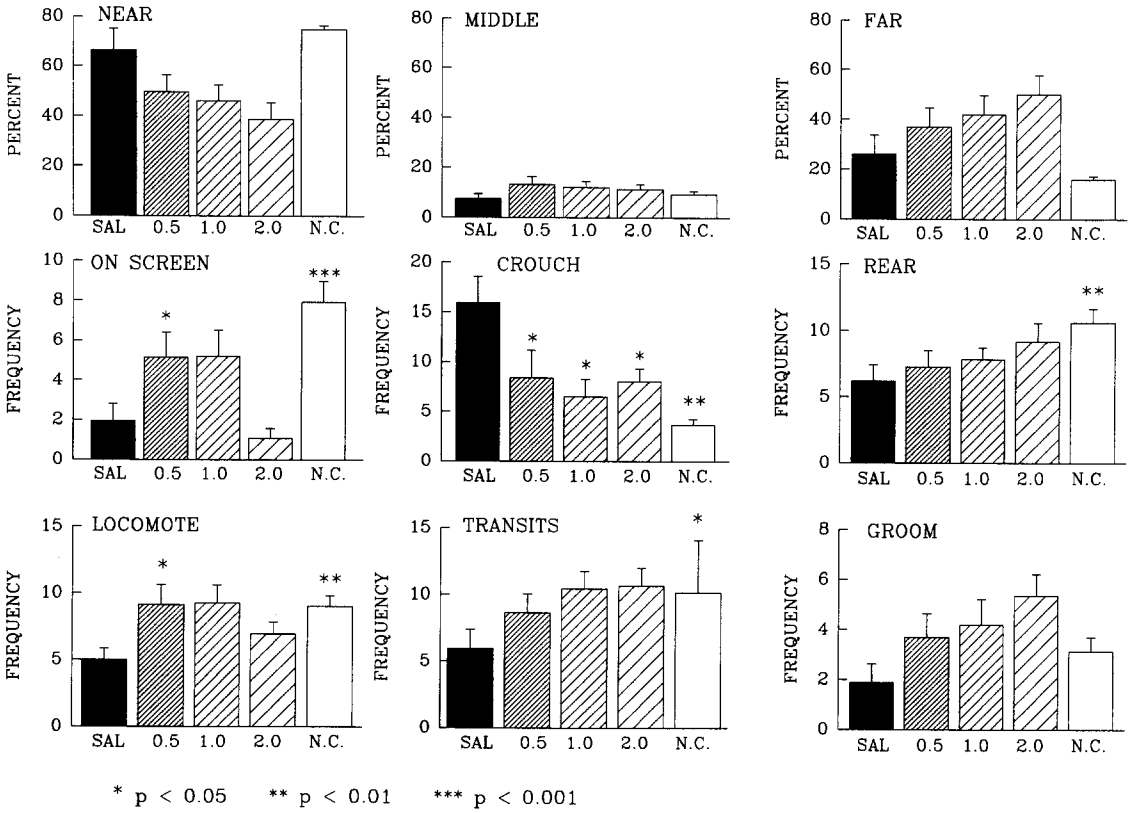

FIG. 1. Effects of yohimbine on proximics/activity following cat exposure. Data are presented as means ± SEM. SAL, cat-exposed, vehicle-injected control group; N.C., no-cat control. Asterisks indicate a significant difference in comparison with the SAL control: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

the cat. When significant main effects were obtained, subsequent Newman-Keuls analyses were performed comparing each yohimbine-injected group with the vehicle-injected, cat-exposed control group. Dose-dependent increases were seen in locomotion, $F(3, 54) = 5.28$, $p < 0.01$, with a significant increase detected for the high-dose (2.0 mg/kg) group ($p < 0.01$, Newman-Keuls). Transits also increased with dose, $F(3, 54) = 2.94$, $p < 0.05$, and reached significance in the high-dose group ($p < 0.05$, Newman-Keuls). Yohimbine raised the frequency of fore- and hindpaw wall climbing, but only reliably in the 0.5-mg/kg group, Mann-Whitney $U = 75$, $p < 0.02$. The only other significant effect was noted in grooming: A greater frequency of this behavior was found in the 0.5-mg/kg group relative to the control group, which displayed little, Mann-Whitney $U = 79$, $p < 0.02$. For none of the variables were reliable sex differences detected.

Frequency of jumping was not included in Table 1 because it occurred rarely. Only three animals exhibited this behavior: One was in the 1.0-mg/kg yohimbine group (two jumps) and two were in the 0.5-mg/kg group (two and four jumps).

Postcat period. The patterns of behavior observed in all groups during the entire 15-min postcat period are shown in Fig. 1 and Table 3. Behaviors assessed via the time-sampling method were analyzed in three 5-min blocks using three-way (dose × sex × time) ANOVAs. Less frequently seen behaviors were measured continuously over the 15-min period, and these were analyzed using two-way (dose × sex) ANOVAs.

A comparison of the vehicle-treated, cat-exposed group with the vehicle-treated, no-cat control group found that the residual effects of cat exposure were decreases in general locomotion, $F(1, 27) = 11.84$, $p < 0.01$, transits, $F(1, 27) = 5.82$, $p < 0.05$, rearing, $F(1, 27) = 8.75$, $p < 0.01$, on-screen activity, $F(1, 27) = 18.64$, $p < 0.001$, and locomotion, $F(1, 27) = 18.64$, $p < 0.001$, forepaw wall climbing, $F(1, 27) = 13.37$, $p < 0.01$, screen poking, $F(1, 27) = 26.71$, $p < 0.001$, and screen gnawing, $F(1, 27) = 4.62$, $p < 0.05$. Both fore- and hindpaw wall climbing and roof pushing occurred with such low frequencies in the cat-exposed group that use of a nonparametric statistic was warranted. Analysis by the Mann-Whitney U-test found that the no-cat group displayed significantly more instances of fore- and hindpaw wall climbing, $U = 48$, $p < 0.01$, and roof pushing, $U = 45$, $p < 0.01$. Frequency of crouching was higher in the cat-exposed group, $F(1, 27) = 20.94$, $p < 0.001$, while differences were not detected for three other variables: position in the compartment, grooming, and digging at corners. No sex or time effects were found for any variables except rearing: The overall frequency of rearing tended to be higher for males than for females, $F(1, 27) = 7.23$, $p < 0.02$.

When compared with vehicle-treated, cat-exposed animals, the various yohimbine-treated groups exhibited generally higher rates of most activities while showing a decreased tendency to remain in a relatively immobile, crouched position, $F(3, 54) = 3.66$, $p < 0.02$, an effect that was found to be significant at all doses by Newman-Keuls analyses, $p < 0.02$–0.05. Locomotion increased, $F(3, 54) = 2.79$, $p < 0.05$, as

TABLE 3
EFFECTS OF YOHIMBINE ON PROXIMICS/ACTIVITY DURING THE POSTCAT PERIOD

	Yohimbine (mg/kg)				
	0.0	0.5	1.0	2.0	0.0 (no cat)
Wall climb: Forepaws	5.63 (1.32)	9.81 (2.52)	7.19 (1.57)	4.79 (1.53)	12.4* (1.25)
Wall climb: Fore- and hindpaws	2.06 (1.31)	7.44 (2.45)	10.69* (3.47)	2.36 (1.27)	8.07* (2.16)
Digging at corner	0.63 (0.51)	0.88 (0.54)	0.75 (0.32)	0.21 (0.15)	1.20 (0.45)
Screen poke	8.31 (2.10)	15.38 (3.70)	14.25 (3.07)	13.14 (4.75)	26.07* (2.91)
Screen gnaw	5.19 (2.28)	6.06 (3.20)	4.0 (1.35)	2.21 (1.42)	13.93* (3.31)
Roof push	0.44 (0.27)	1.50 (0.76)	2.19* (0.73)	0.21 (0.21)	2.13 (0.50)

Data are presented as means (±SEM).
*$p < 0.05$, significant differences from vehicle-injected, cat-exposed controls.

did on-screen behavior (hanging and locomotor activity), $F(3, 54) = 3.74$, $p < 0.02$, reaching significance in the 0.5-mg/kg group in each case ($p < 0.05$, Newman-Keuls). Fore- and hindpaw wall climbing and roof pushing, behaviors seen infrequently in vehicle-injected, cat-exposed controls, occurred significantly more often in the 1.0-mg/kg yohimbine group ($p < 0.05$, Mann-Whitney U). Transits tended to increase, approaching significance, $F(3, 54) = 2.41$, $p < 0.08$, and a sex × time interaction was detected for this factor, $F(2, 108) = 4.17$, $p < 0.02$. This interaction reflected the fact that male mice tended to make fewer transits during the last 5 min of the 15-min cat period, whereas females tended to make slightly more transits during this time. No group differences were detected in frequencies of screen poking, screen gnawing, corner-digging, and jumping. Jumps were seen in only two mice: One was in the 0.5-mg/kg yohimbine group (two jumps) and one was in the 1.0-mg/kg group (three jumps).

Group differences in the position of mice relative to the cat chamber did not reach significance, as shown by the analyses of the percentage of time the various groups spent in the segments of the test chamber near the cat, $F(3, 54) = 2.45$, $p < 0.08$, and farthest from the cat, $F(3, 54) = 1.55$, $p > 0.20$. However, as Fig. 1 shows, mice spent increasingly less time in the near segment and correspondingly more time in the far segment as the dose of yohimbine increased. This tendency was most noticeable in an identifiable subpopulation of subjects revealed through an examination of the behavior of individual animals. Two distinctly different patterns were apparent. Some animals in each group were very active, showing high levels of wall climbing, roof pushing, on-screen activity, and general locomotion, while others exhibited low levels of the same activities. Of these behaviors, wall climbing (both fore- and hindpaw) was selected as an "escape index" because it was thought to be the most clearly escape-directed behavior and its incidence was less likely to be influenced by an animal's position in the test chamber than other escape-related behaviors (on-screen activity or roof pushing). Each instance of wall climbing was assigned a value of "1" or "2" depending upon whether the animal used forepaws only or both forepaws and hindpaws in its climbing attempts. The latter was judged to be a more vigorous attempt at escape and was therefore given a greater weight. A composite score was obtained for each animal, and the mean derived from all 77 animals was 20.4. Mice were then classified as *low escape* (scores of 20 or less) or *high escape* (scores of 21 or more). The number of cat-exposed animals falling into each of these categories is shown in Table 4. Also shown is the percentage of time that each of the low-escape and high-escape subgroups remained within the segments of the test chamber nearest to and farthest from the cat. (No-cat control animals were not included here because many of their scores clustered around the mean and hence did not fall into two clear categories.) Yohimbine increased the ratio of high-escape to low-escape animals significantly at both 0.5 and 1.0 mg/kg ($p < 0.03$, Fisher's Exact Test) when compared with vehicle-injected, cat-exposed controls, but had no effect at 2.0 mg/kg. In all four cat-exposed groups, low-escape mice were more likely to remain in the segment of the test chamber farthest from the cat. Yohimbine seemed to increase this tendency as indicated by ANOVA performed on the percentages of near- and far-segment activity seen in *only* low-escape animals. Group differences approached significance with $F(3, 34) = 2.77$, $p < 0.06$, for the near segment and $F(3, 34) = 2.43$, $p < 0.09$, for the far segment.

DISCUSSION

F/DTB and P/AT: Behavioral analysis of drug-free controls

Consistent patterns of flight, avoidance, and defensive attack by laboratory and wild rats in the F/DTB have repeatedly been reported, with wild rats in general showing more intense reactions (6,9). In comparison with the Long-Evans laboratory strain, wild rats avoid an approaching experimenter on a greater percentage of trials and maintain a greater distance between themselves and this perceived threat. Wild rats also show a far greater response to dorsal contact and respond to an anesthetized conspecific with bites and jump attacks, behaviors not normally seen in male Long-Evans rats. The Swiss-Webster mice in the present study tended to exhibit behavior patterns that more closely resembled those of wild rats.

TABLE 4

INCIDENCE OF HIGH-ESCAPE AND LOW-ESCAPE BEHAVIOR PATTERNS AND PERCENTAGE OF TIME (±SEM) THAT SUBGROUPS OF MICE EXHIBITING THESE PATTERNS SPENT IN THE SEGMENTS OF THE TEST CHAMBER NEAREST TO AND FARTHEST FROM THE CAT DURING THE 15-min POSTCAT PERIOD

	Number of Mice/Escape Level		Percentage of Time in Near Segment		Percentage of Time in Far Segment	
Escape Level	High	Low	High	Low	High	Low
Saline	2	14	81.6 (1.7)	64.3 (9.8)	13.3 (0.0)	27.9 (8.7)
YOH 0.5 mg/kg	8	8	61.3 (3.8)	37.9 (1.2)	22.5 (2.9)	51.3 (13.3)
YOH 1.0 mg/kg	8	8	63.3 (4.3)	28.8 (8.5)	19.6 (2.1)	64.2 (10.4)
YOH 2.0 mg/kg	2	12	58.3 (1.5)	35.3 (7.3)	26.7 (10.0)	53.9 (8.6)

They maintained a greater distance from the experimenter than is usually observed in Long-Evans rats, their reaction to tactile stimulation was greater, and they exhibited a variety of defense/attack reactions when confronted with an anesthetized rat. For these reasons, the Swiss-Webster mouse may be a more suitable subject than the laboratory rat for studies concerned with behavioral reactions to an immediate threat.

As a complement to the F/DTB, the P/AT was designed to assess defensive reactions to a potential rather than an immediate threat. Here, mice in the present study reacted to the presence of a cat in several ways that were reminiscent of those shown by laboratory rats. Crouching, rearing, grooming, moving from one section of the compartment to another, and general locomotion declined in frequency both during the cat exposure and the postcat periods. A variety of behaviors seen in mice but rarely in adult rats were also reduced: screen climbing, wall climbing, screen gnawing and poking, and roof pushing. In a notable divergence from rat behavior, mice did not tend to avoid the portion of their compartment closest to the cat; instead, they spent two thirds of their time in the area nearest the cat compartment. A similar pattern of investigation of a threatening stimulus has also been observed by Blanchard and Parmigiani (in preparation) in a visible burrow system. Shortly after a cat is placed into an arena adjacent to the entrances of several burrows, mice will linger near these openings, orienting themselves toward the predator. Thereafter, a long period of freezing follows. This investigatory behavior has been interpreted as a form of risk assessment, similar to that exhibited by rats encountering a potential threat.

Effects of yohimbine

F/DTB. In the F/DTB, yohimbine potentiated two indices of flight. The percentage of trials in which flight occurred increased at all doses, as did the experimenter-to-subject distance at which flight occurred, with maximal changes appearing at 0.5 mg/kg. It is unlikely that these effects were attributable to a general increase in locomotor activity because line crossings during the 5-min pretest decreased as a function of dose, with a reliable change evident at 2.0 mg/kg.

Yohimbine-treated animals were also more reactive to dorsal contact, an effect that may be related to the drug's known impact on startle reflexes. Like other drugs that facilitate noradrenergic transmission, yohimbine can potentiate acoustic startle (15,16,34) and has been shown to enhance amphetamine-potentiated startle in response to a tactile (airpuff) stimulus (29).

P/AT. In the P/AT, yohimbine affected behavior patterns somewhat differently during the cat and postcat periods. During cat presentation, significant increases in general locomotion and transits from one segment of the compartment to another were observed in mice given the 2.0-mg dose. These effects did not persist into the subsequent postcat period, and the frequencies of other behaviors were not reliably altered. Increases in wall climbing with fore- and hindpaws were observed at the 0.5- and 1.0-mg/kg doses, and this effect did carry over following cat removal. Although the differences did not reach statistical significance, the time animals spent near the cat compartment tended to decrease with increasing doses and corresponding increases in time spent in the segment farthest from the cat were found.

Behavioral changes induced by yohimbine during the postcat period were more extensive. As a whole, animals administered yohimbine at all doses spent less time inactive in a crouched position and those given 0.5 and 1.0 mg/kg spent more time engaging in activities that can be interpreted as escape related: general locomotion, wall climbing, screen-climbing, and roof-pushing. Other activities also tended to increase, although not to a statistically significant degree at any dose: rearing, transits, grooming, digging at corners, screen-poking, and a shift in position toward the segment of the test chamber farthest from the cat.

A notable departure from the behavior of rats in the P/AT was the absence of sexual dimorphism shown by mice. A number of studies reported substantial differences between male and female rats in the Anxiety/Defense Test Battery (7,8,42). For example, female rats tend to spend more time crouching than do males during the period following cat exposure (5). In the Swiss-Webster mice used in this experiment, however, few gender differences appeared: At times, females exhibited less frequent rearing and fewer transits than males, but no other comparisons were significant.

In several behavioral measures, nonmonotonic relationships between drug dose and behavioral changes were detected. This was most evident for on-screen activity, locomotion, wall climbing, and roof pushing, where the 0.5- and 1.0-mg/kg doses effected substantially greater changes than the 2.0-mg/kg dose during the postcat period. Yohimbine, although primarily considered a noradrenergic α_2-antagonist, is also thought to interact with dopaminergic (33,41), seroton-

ergic (19,36), and benzodiazepine (35) receptor systems. At the 2.0-mg/kg dose, one of these effects may have predominated, thereby generating a somewhat different pattern of behavioral consequences. This may also have contributed in some as yet unapparent way to the puzzling increase in locomotion noted in these animals during cat presentation. Similar instances of nonlinear dose–response relationships have been reported in other species. In bonnet macaques, Rosenblum et al. (40) noted fewer behavioral changes at 0.75 mg/kg oral yohimbine than were apparent at 0.10 and 0.25 mg/kg; and, Harris and Newman (30) described nonmonotonic effects of dose in their studies of yohimbine modulation of isolation cries in squirrel monkeys. The doses in these studies were far lower than those used in the present experiment, but strain differences in sensitivity to the drug and differences in routes of administration must be taken into account.

A characterization of animals as high escape and low escape was made after it became evident that two distinct behavior patterns had emerged in cat-exposed animals during the postcat period of the P/AT. High-escape mice were in general very active, moving about the test chamber, climbing the screen separating them from the cat and then pushing at the roof of the chamber, and making scrabbling movements with fore- and hindpaws that appeared to be climbing attempts. Low-escape animals exhibited considerably less activity while tending to remain farther from the cat. Of the 16 mice not injected with yohimbine, 2 (12.5%) were classified in the high-escape category. The 0.5- and 1.0-mg/kg doses of yohimbine shifted the percentage of high-escape animals to 50.0%, while at 2.0 mg/kg it was 14.3%.

This bimodal effect of yohimbine within the 0.5- and 1.0-mg/kg groups may be interpreted in several ways. One is that high- and low-escape animals are genetically inclined toward two different modes of antipredator defense: flight vs. immobility. Recent studies of male house mice (*Mus musculus domesticus*) found individual differences in behavioral strategies in subjects confronted with an intrusion or attack by a conspecific [cited in (1,2)] and in subjects confronted with escapable (1) or inescapable (2) electric shock. Some responded passively, exhibiting immobility to the detriment of a variety of other behaviors; others responded actively with increases in (or sometimes no changes in) the frequency of fighting, fleeing, locomotion, exploration, and other activities. In the present experiment, the two lower doses of yohimbine may have potentiated fear of the cat in all animals, but the way in which this fear was expressed may have depended upon the individual defense style. Although the low activity levels in low-escape, yohimbine-injected animals could have simply reflected an insensitivity to the drug, this possibility is considered unlikely because these animals did differ from low-escape, vehicle-injected animals in one telling way: They exhibited a greater tendency to avoid the proximity of the cat (Table 4).

An alternative explanation for the dichotomous behavior in the 0.5- and 1.0-mg/kg yohimbine groups is that low-escape mice were *more* sensitive to the effects of yohimbine than high-escape subjects. The initial response of rats and mice to a distal predator is immobility, especially when the situation does not present a clear avenue for escape (3,20). As the predator approaches, immobility turns to attack and/or flight. At a range of low doses that differs for individual mice, yohimbine may cause a shift from immobility to defensive activity by activating neural, possibly noradrenergic, mechanisms mediating flight or inhibiting those mechanisms that mediate freezing. However, at higher doses perhaps one of the other, nonnoradrenergic effects of yohimbine mentioned earlier predominates, with a resulting suppression of active escape attempts. If low-escape animals were especially sensitive to yohimbine, then, for these animals, the 0.5- and 1.0-mg/kg doses may already have surpassed the threshold at which such suppression occurs. Had lower doses of the drug been administered to these animals, then they too might have exhibited a preponderance of escape behaviors. This analysis assumes that, at 2.0 mg/kg, the suppression threshold for most mice had been reached so that the low-escape/high-escape dichotomy all but disappeared.

The fact that yohimbine can potentiate flight, as demonstrated in this experiment, is of special interest because it suggests that this model may have some utility for investigations into the etiology of panic disorder in humans. According to a recent theory (17,18,24), panic attacks may result from the spontaneous activation of the flight component of primitive (brainstem) defense response in the absence of a threatening stimulus. As noted earlier, yohimbine can evoke a state in human subjects (and especially in those prone to panic attacks) that is autonomically and subjectively akin to panic. Perhaps yohimbine activates flight-mediating neural systems in both humans and rodents, producing inappropriate and maladaptive patterns of behavior. For the mouse, this means being active when it should be immobile. Initial immobility when a predator is detected diminishes the chances of discovery and pursuit by the predator. It also allows the mouse a period during which it may assess risk and subsequently engage in a directed pattern of flight that will most effectively allow it to evade the predator. Yohimbine, at some doses, interferes with this characteristic response.

The hypothesis that panic is linked to neural mechanisms underlying flight will gain credibility if it can be shown that other known panicogenic agents and certain antipanic compounds like alprazolam can potentiate escape in a manner similar to yohimbine. For this purpose, the defensive activities of the Swiss-Webster mouse as elicited by the F/DTB and the P/AT may be well suited. The frequency, intensity, and diversity of animals' reactions to the various threatening stimuli allow for a fine-grained analysis of a drug's effects on various components of the defensive repertoire. Consequently, this model may have greater utility than many that rely upon a single behavioral index of drug action.

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