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Effects of Scopolamine on Antipredator Defense Reactions in Wild and Laboratory Rats

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RODGERS, R. J., D. C. BLANCHARD, L. K. WONG AND R. J. BLANCHARD. *Effects of scopolamine on antipredator defense reactions in wild and laboratory rats.* PHARMACOL BIOCHEM BEHAV **36**(3) 575–583, 1990.—Two experiments were designed to investigate the effects of scopolamine hydrobromide (0.25–1.0 mg/kg), and its methyl derivative, on the defensive reactions of rats to nonpainful threat stimuli. In the first experiment, over the dose range studied neither compound significantly altered avoidance, freezing, defensive threat or attack in wild *Rattus rattus* confronted by the experimenter and other predator-related stimuli. Scopolamine hydrobromide did, however, produce a dose-dependent increase in flight distance; this effect was not seen with the methyl compound, confirming central cholinergic mediation. In the second experiment, no dose of either compound significantly altered the behaviour of Long-Evans rats prior to cat exposure. During cat exposure, however, scopolamine hydrobromide (but not methyl scopolamine) increased the amount of time spent in the vicinity of the cat, increased scanning and rearing, and reduced grooming behaviour. Although reliable, the latter effects were not pronounced. Together, these data do not support a major involvement of central muscarinic receptor mechanisms in the regulation of defensive patterns in wild or laboratory rats.

Cholinergic Muscarinic Scopolamine Methyl scopolamine Defensive behaviour Fear Anxiety Wild rats Long-Evans rats

THE involvement of muscarinic cholinergic mechanisms in the facilitatory modulation of aggressive behaviour in animals is well established in the literature [for reviews: (2, 5, 20, 21, 27–29)]. However, while there is little doubt that manipulation of cholinergic function can rather specifically alter offensive behaviour in a variety of species (3, 16, 18, 19, 26, 35), evidence also supports a role for central muscarinic receptors in defensive responding.

In both rats and cats, central injections of muscarinic agonists (e.g., carbachol, acetylcholine, D-tubocurarine) elicit affective defense ('sham rage') and fear-like escape reactions; these effects can be antagonized by atropine and/or scopolamine [for review: (1,32)]. Antimuscarinics have also been reported to inhibit shock-induced defensive fighting in rats and mice, whereas their quarternary analogues are ineffective at comparable doses (23, 24, 30). Similar effects of scopolamine have been observed following direct drug application to amygdaloid and hypothalamic sites (4,31). Under more naturalistic test conditions, Mollenauer, Plotnik and Snyder (22) and Plotnik, Mollenauer and Snyder (25) reported that scopolamine reduced fear reactions in laboratory rats confronted with a cat, an effect that again could not be attributed to peripheral muscarinic blockade. In these studies, reduced fear was indicated by consummatory behaviour in the presence of the cat, more approaches to the cat enclosure and less freezing.

To further assess the influence of muscarinic receptor blockade on antipredator defensive reactions, the present study examined the effects of scopolamine (and its methyl derivative) on responses of wild rats *(Rattus rattus)* in a Fear/Defense Test Battery (F/DTB). This set of procedures has been designed to measure responses to nonpainful threat stimuli (14), and has been successfully used to study the effects of brain lesions (6, 10, 17), benzodiazepines (8), 5-HT_{1A} agonists (9) and ethanol (13) on the natural defensive repertoire of this species. In addition, a second experiment assessed the effects of both compounds on the responses of Long-Evans rats to the presence of a cat. This study, similar in nature to those of Plotnik and colleagues (22,25), derived from an Anxiety/Defense Test Battery (A/DTB) recently developed in our laboratory (7).

METHOD

EXPERIMENT 1

Subjects

Twenty male and 19 female wild *Rattus rattus*, trapped on the island of Oahu, served as subjects. Animals were singly housed in suspended metal cages (19 × 27 × 15 cm) for 30–60 days prior to testing, and maintained under a normal 12:12 light-dark cycle.

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Food and water were freely available. Weight range at the time of testing was 104–258 grams.

Drugs

Scopolamine hydrobromide and scopolamine methylbromide (Sigma Chemical Co., St. Louis) were dissolved in physiological (0.9%) saline which, alone, served for control injections. Drugs (0.25, 0.5 and 1.0 mg/kg) were administered intraperitoneally (IP) in a volume of 1 ml/kg, 30 minutes prior to testing. Doses cited refer to the salts.

Procedure

A separate experiment was conducted with each compound; scopolamine hydrobromide (10 males, 9 females), scopolamine methylbromide (10 males, 10 females). Order of testing was counterbalanced within each study and a minimum intertest interval of 4 days was employed. The experimenter remained blind to treatment groups until all testing was complete. Subjects were tested on a set of procedures specifically designed to elicit a relatively complete array of species-typical defensive reactions to nonpainful threat stimuli (6, 10, 14). Subjects were tested during the light portion of the light-dark cycle.

Because wild rats are extremely sensitive to the presence of humans, these tests are run with only a single experimenter in the test alley or close enough to make behaviour ratings. It is therefore difficult to run reliability checks as part of the ongoing test procedure. However, these procedures have been run for over a decade in this laboratory and reliability testing is an integral component of the training of each new experimenter. New experimenters are required to reach a criterion of 95% or better agreement on all behavioural measures with the Hawaii laboratory director (R.J.B.) before being allowed to run subjects independently.

Oval runway.

Apparatus. The oval runway was formed by enclosing a 6×2 meter area with plywood. The runway consisted of a 4×2 meter straight section centrally divided by a partition, making each side 4 m long $\times$ 1 m wide. Both ends of the runway were rounded by a curved radius of 1 meter to keep the width constant throughout. The floor of the runway was marked at 1 meter intervals.

Five-minute pretest. The experimenter gently slid each subject out of its home cage into the runway, then left the runway area to observe and record the subject's line crossings during a 5-minute pretest period.

Avoidance. After the pretest, the experimenter entered the runway at the end opposite to the subject and made 5 approaches (approach speed was approximately 0.5 m/sec) toward the subject, until contact (a light touch with the experimenter's shoe) was recorded, or the subject ran away. If the subject avoided by running away, the distance between the experimenter and subject (avoidance distance) and the distance the subject fled (flight distance) were recorded. An intertrial interval of 30 seconds was employed.

Flight speed. Flight measurement was conducted immediately following the avoidance test. The experimenter rapidly approached the subject from the opposite end of the runway at a speed of roughly 1.5 to 2 meters per second and, using a stopwatch, recorded the time taken to chase the subject a standard distance of 36 meters. If the subject did not flee, the experimenter remained in contact with the subject for 60 seconds. If no flight was elicited, a chase time of 300 seconds was recorded and the trial terminated. Chase time was converted to flight speed for statistical analysis.

Inescapable runway.

Apparatus. The oval runway was converted into an inescapable runway by closing a partition at both ends of the straight section. This produced a 4×1 meter straight runway with no escape possible from either end.

Responses to an approaching experimenter. The experimenter made 5 approaches toward the subject from the far end of the runway, making a mild noise by clapping his hands before each approach to ensure the subject was aware of his presence. The experimenter approached the subject at a speed of 0.5 meters per second, pausing for 30 seconds at distances of 4, 3, 2, 1 and 0.5 meters from the subject. Subject freezing and flight, as well as defensive threat and attack behaviours—box, vocalize, jump attacks and bites—were recorded at each distance. If flight or other types of active defense were seen, the experimenter moved in to lightly contact the subject, and recorded defensive threat or flight if these occurred to the contact.

Proximal testing.

Apparatus. At the conclusion of runway testing, subjects were placed into an aluminum barrel, 50 cm in diameter and 120 cm in height. The following defensive tests were conducted while the subject was in the barrel.

Reaction to handclap and dorsal contact. After the subject had been in the barrel for 1–2 minutes, the experimenter clapped his hands once (4 trials, ITI = 30 seconds), and the subject's response was noted. One minute later, a series of 4 trials was begun that assessed flinch/jump reactions to light dorsal (flank) contact with a 1 meter wooden dowel. Scores were recorded as 1) Startle 1—a local flinch reaction; 2) Startle 2—a flinch reaction of the animal's entire body; 3) Jump 1—a rapid movement in which two of the animal's paws left the floor; 4) Jump 2—rapid movement in which all four of the animal's paws left the floor; 5) Jump 3—rapid movement in which the animal jumped 10 cm or higher. Each of these scores was assigned a value, with 1 for 'Startle 1' through 5 for 'Jump 3,' and a total startle score calculated by adding together these values for all 4 trials. Reaction to handclap was similarly scored.

Vibrissal stimulation. Two circular brushes, 2.5 cm in diameter, fixed perpendicularly to a 1 meter long wooden dowel, were used to stimulate the subject's vibrissae, in a series of four trials. The experimenter made short upward strokes with the hairs to the brush, making extensive contact with the vibrissae, and being very careful not to touch the subject's snout. Four defensive threat and attack reactions were recorded: boxing, biting, vocalizing and jump attacks.

Anaesthetized conspecific. A terminally anaesthetized conspecific, held and presented at ground level with its snout facing the subject, was moved toward the subject at a rate of 5 cm per sec, and until contact occurred. Four trials were given. Boxing, biting, vocalizing and jump attacks toward the head and snout of the anaesthetized conspecific were recorded.

Reaction to handling. The final procedure measured the subject's defensiveness in response to an attempt by the experimenter to pick it up. Only one pickup attempt was made. Boxing, biting, vocalizing and jump attacks toward the experimenter's gloved hand were recorded. The experimenter also rated subject defensiveness during pickup on a scale of 0 to 5, with a score of 0 given to a totally docile animal that was easily picked up and showed no defensive reaction and 5 to subjects that could not be picked up and showed a full range of defensive threat and attack behaviours.

Statistical Analysis

Data were initially analysed by 2- or 3-factor analysis of variance (ANOVA), except as noted below. Follow-up tests were performed using Dunnett's procedure for comparing treatment means with control. In view of the lack of significant effects for

TABLE 1

	Scopolamine HBr				Scopolamine MBr			
Behaviour	0.0	0.25	0.50	1.0	0.0	0.25	0.50	1.0 mg/kg
Avoidance Distance (m)	3.52 (0.25)	3.76 (0.20)	3.61 (0.24)	3.24 (0.33)	3.09 (0.30)	2.96 (0.33)	3.01 (0.30)	1.00 (0.24)
Flight Distance (m)	1.33 (0.28)	2.23* (0.45)	2.57† (0.48)	3.02† (0.51)	1.23 (0.24)	1.73 (0.38)	1.30 (0.21)	1.15 (0.23)
Flight Speed (m/sec)	15.17 (1.51)	15.02 (1.88)	14.76 (0.56)	16.57 (1.97)	15.14 (3.03)	15.53 (4.69)	14.47 (0.37)	15.88 (2.11)
% Avoidance	95.00	90.00	95.00	80.00	85.00	90.00	85.00	85.00

Effects of scopolamine hydrobromide and scopolamine methylbromide, 0.25–1.0 mg/kg (IP) on defensive behaviour in wild rats tested in the oval runway. Data (except % avoidance) are expressed as means (s.e.m.), *$p<0.05$, †$p<0.01$ versus saline control.

sex on any measure, subsequent analyses were collapsed across this factor.

Behavioural elements which were recorded as present/absent, or by subjective intensity (i.e., boxing, defensive threat vocalization, biting, jump attacks and overall defensiveness rating) were analyzed using Cochran's Q-test. Although each experiment was analyzed separately, results were presented in terms of procedure.

EXPERIMENT 2

Subjects

Twenty-seven adult male Long-Evans rats (273–405 grams) served as subjects. These animals were individually housed and maintained under a normal 12:12 light-dark cycle with food and water freely available. A 4.5 kg house cat, which served as the predator stimulus in this study, was housed separately and, during testing, sat quietly and showed little interest in the rats.

Apparatus

The test apparatus consisted of a rectangular enclosure, measuring 120 cm long, 180 cm high and 50 cm wide. A stimulus cat chamber (50 × 30 × 70 cm) was located at one end of the enclosure but separated from it by a wire mesh screen. Swing doors, at either end of the apparatus, facilitated introduction of subject and cat to their respective regions of the apparatus. The floor of the subject chamber was marked at 20 cm intervals, providing a total of 6 equal zones, zone 1 being closest to the cat chamber through zone 6 (farthest).

Drugs

Scopolamine hydrobromide and its methyl derivative were each used at two dose levels, 0.6 and 1.2 mg/kg. Vehicle, route of administration, injection volume and injection-test interval were identical to those used in Experiment 1.

Procedure

A separate experiment examined the effects of each compound; scopolamine hydrobromide (n = 18), scopolamine methylbromide (n = 9). Treatments were administered in counterbalanced order with a minimum intertrial interval of 7 days. The experimenter remained blind to treatment conditions until all testing was complete.

Behavioural observations (see below) were made both before and during cat exposure. In the pre-cat condition, the subject was allowed to exit its home cage into zone 6 of the main enclosure, following which the swing door was closed. Two minutes later, the stimulus cat was introduced into its chamber for a 10-minute cat exposure period. Following the introduction of subject and stimulus cat, the experimenter retired to an adjacent room from which the session was monitored.

Between trials, the subject enclosure was swept and mopped to remove any residual odour due to urine and/or faeces. The cat chamber was also thoroughly cleaned using hot soapy water. Experimental sessions (pre-cat and cat periods) were videotaped. Behaviours recorded from videotape were: 1) *location*: the duration of time the subject spent in zones 1 and 6 of the enclosure; 2) *scanning*: the frequency of sideways head movements directed towards the cat chamber; 3) *rearing* (frequency); and 4) *grooming* (frequency).

Reliability checks for these measures indicated interscorer agreement of 87% or above with reference to all behaviours.

Statistical Analysis

Data were analyzed by 2-factor analyses of variance and, where indicated by the nature of the data, by Wilcoxon matched pairs tests.

RESULTS

EXPERIMENT 1

Oval Runway Test

Data are summarized in Table 1 and Fig. 1.

Five-Minute Pretest

Line crossings were not reliably altered by either compound (data not shown). For scopolamine HBr, ANOVA failed to reveal either a significant main effect for drug, $F(3,54) = 2.54$, ns, or a drug × time interaction, $F(12,216) = 0.34$, ns. However, a significant main effect for time was found, $F(4,72) = 3.5$, $p<0.05$, reflecting a group-independent reduction in line crossings over the test session. No significant main effects or interactions were apparent for scopolamine MBr; drug, $F(3,57) = 0.03$, ns, time, $F(4,76) = 1.25$, ns, and drug × time, $F(12,228) = 1.03$, ns.

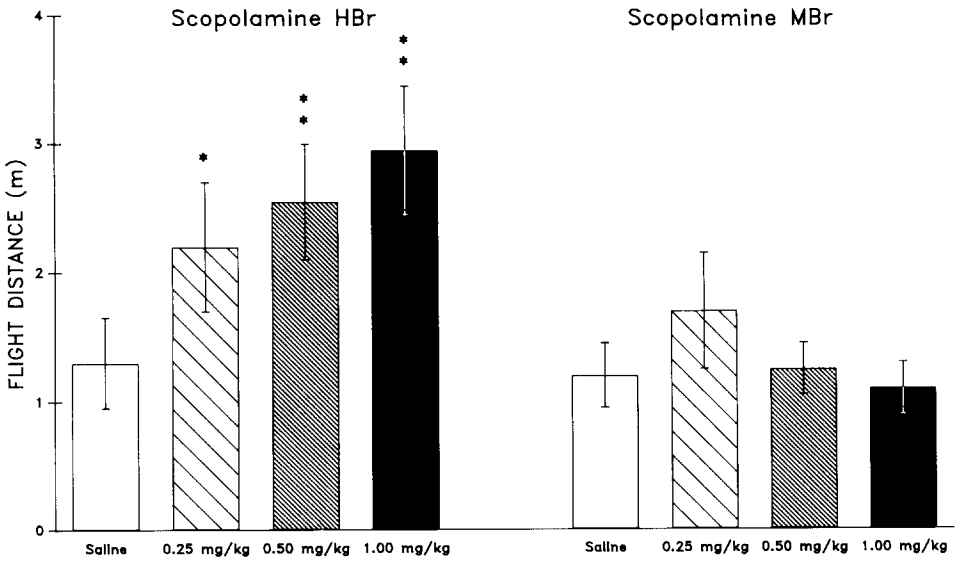

FIG. 1. Effects of scopolamine hydrobromide and scopolamine methylbromide, 0.25–1.0 mg/kg (IP), on flight distance in the oval runway test. Data are presented as mean distance in metres (±s.e.m.), averaged over 5 trials. $*p<0.05$, $**p<0.01$ versus saline control.

Flight and Avoidance to the Experimenter

Avoidance distance. Neither compound produced significant effects on avoidance distance (Table 1): for scopolamine HBr, drug, $F(3,54)=1.62$, ns, trial, $F(4,72)=1.29$, ns, drug × trial interaction, $F(12,216)=1.17$, ns. A similar pattern was observed for scopolamine MBr, drug, $F(3,57)=0.01$, ns, trial, $F(4,76)=2.15$, ns, drug × trial interaction, $F(12,228)=0.86$, ns. In both studies, the percentage of subjects showing avoidance was unaltered by drug treatment.

Flight distance. For scopolamine HBr, ANOVA failed to show significant effects for trial, $F(4,72)=1.35$, ns, or the drug × trial interaction, $F(12,216)=0.61$, ns. However, a significant main effect for drug, $F(3,54)=8.75$, $p<0.01$, was observed. Follow-up analyses indicated this effect to be dose-dependent (see Table 1 and Fig. 1), with 0.25 mg/kg producing significant increases on the first 2 trials ($p<0.05$ and $p<0.01$, respectively), 0.5 mg/kg on trials 1–3 ($p<0.05$, $p<0.01$ and $p<0.05$, respectively) and 1.0 mg/kg on all 5 trials ($p<0.01$ in each case). For clarity, Table 1 and Fig. 1 present mean flight distance over the five test trials.

For scopolamine MBr, ANOVA also indicated a significant main effect for drug, $F(3,57)=3.26$, $p<0.05$. However, follow-up analyses failed to reveal any significant differences between saline control and drug groups. The effects of trial, $F(4,76)=2.46$, ns, and the drug × trial interaction, $F(12,228)=0.64$, ns, were not reliable.

Flight speed. Neither compound produced significant effects on flight speed (Table 1); scopolamine HBr, $F(3,54)=2.34$, ns, scopolamine MBr, $F(3,57)=0.56$, ns.

Inescapable Runway Test

Results are summarized in Tables 2 and 3.

Freezing. Duration of freezing to experimenter approach was not reliably altered by either compound (Table 2); scopolamine HBr, $F(3,54)=0.46$, ns, scopolamine MBr, $F(3,57)=0.34$, ns. However, in both studies, the effect of experimenter distance was highly significant, with freezing initially increasing and then

TABLE 2

Treatment Group	Experimenter-Subject Distance (m) 4.0	3.0	2.0	1.0	0.5
Saline	16.11 ± 3.19	27.42 ± 1.47	25.84 ± 2.21	15.31 ± 3.53	6.68 ± 2.87
0.25 mg/kg HBr	13.78 ± 3.22	18.94 ± 3.24	15.78 ± 3.62	15.78 ± 3.63	10.26 ± 3.34
0.50 mg/kg HBr	18.42 ± 3.37	24.78 ± 2.52	23.00 ± 2.78	13.78 ± 3.50	9.05 ± 3.24
1.00 mg/kg HBr	12.47 ± 3.13	20.94 ± 3.07	19.57 ± 3.25	17.15 ± 3.25	14.11 ± 3.29
Saline	19.81 ± 2.89	26.00 ± 1.87	26.73 ± 2.22	24.10 ± 2.79	20.57 ± 3.31
0.25 mg/kg MBr	17.31 ± 3.15	26.00 ± 1.83	28.21 ± 1.61	23.05 ± 2.91	14.21 ± 3.62
0.50 mg/kg MBr	20.63 ± 2.91	27.21 ± 1.69	23.47 ± 2.94	22.05 ± 3.19	12.05 ± 3.38
1.00 mg/kg MBr	17.15 ± 3.09	24.68 ± 2.61	26.78 ± 2.22	26.15 ± 2.22	16.78 ± 3.35

Effects of scopolamine hydrobromide (HBr) and scopolamine methylbromide (MBr), 0.25–1.0 mg/kg (IP), on freezing as a function of experimenter distance in the inescapable runway. Data are presented as mean seconds (±s.e.m.).

TABLE 3

	Scopolamine HBr				Scopolamine MBr			
Behaviour	0.0	0.25	0.50	1.0	0.0	0.25	0.50	1.0 mg/kg
Box	32.00	21.00	26.00	37.00	57.00	42.00	37.00	42.00
Bite	21.00	16.00	21.00	32.00	47.00	47.00	37.00	37.00
Vocalize	16.00	11.00	11.00	32.00	37.00	32.00	32.00	16.00
Jump Attack	26.00	5.00	16.00	21.00	53.00	47.00	58.00	53.00
Flight	84.00	74.00	84.00	68.00	32.00	58.00	63.00	58.00
Contact	11.00	11.00	21.00	16.00	16.00	0.00	11.00	11.00

Effects of scopolamine hydrobromide and scopolamine methylbromide, 0.25–1.0 mg/kg (IP) on defensive behaviour to close approach by the experimenter in the inescapable runway. Data are presented as the percentage of subjects showing particular defense reactions.

declining as a function of imminent contact; scopolamine HBr, $F(4,72)=18.29$, $p<0.01$, scopolamine MBr, $F(4,76)=19.76$, $p<0.01$.

Defensive threat and attack. On close approach in the inescapable runway (i.e., from 0.5 meters to contact), freezing gives way to more active defensive reactions (Table 3). Cochran's Q-test failed to reveal reliable effects of either compound on the percentage of subjects displaying these responses: Q(HBr/MBr), box (1.42/2.16, ns); bite (1.62/1.26, ns); vocalization (5.6/2.45, ns); jump attack (7.75/0.75, ns); flight (1.97/5.28, ns); contact (1.32/3.35, ns).

Proximal Tests

Data are summarized in Tables 4 and 5.

Auditory startle. Although scopolamine HBr failed to influence the startle reaction to handclap, $F(3,54)=2.02$, ns, a significant effect was found for the methyl compound, $F(3,57)=3.57$, $p<0.05$. Further analysis indicated that, compared to control, startle was reliably reduced at all doses ($p<0.01$ in each instance; Table 4).

Dorsal contact. Neither compound produced significant effects on flinch/jump reactions to dorsal contact (Table 4); scopolamine HBr, $F(3,54)=0.71$, ns, scopolamine MBr, $F(3,57)=0.47$, ns. Similarly, vocalization responses to this stimulation were unaffected (HBr, Cochran's $Q=5.7$, ns; MBr, Cochran's $Q=4.3$, ns).

Vibrissal stimulation. Defensive reactions to stimulation of the mystacial vibrissae were unaffected by either compound (Table 5). Cochran's Q (HBr/MBr), box (0/0.57, ns); bite (2.3/2.23, ns); vocalization (5.66/4.67, ns); jump attack (4.33/2.11, ns).

Anaesthetized conspecific. No significant effects of either compound were found on defensive reactions to an anaesthetized conspecific (Table 5). Cochran's Q(HBr/MBr), box (2.16/1.49, ns); bite (1.63/1.26, ns); vocalization (5.6/2.45, ns); jump attack (7.75/0.75, ns).

Reaction to attempted pickup. No significant effects of either compound were found on reactions to attempted pickup (Table 5). Cochran's Q(HBr/MBr), box (2.0/1.79, ns); bite (3.2/1.45, ns); vocalization (0.27/2.33, ns); jump attack (1.5/0.11, ns). Similarly neither compound significantly influenced the experimenter's ratings of defensiveness to attempted pickup (Table 4); scopolamine HBr, $F(3,54)=0.29$, ns, scopolamine MBr, $F(3,57)=1.14$, ns.

EXPERIMENT 2

Results are summarized in Tables 6 and 7.

Pre-Cat Period

Location (i.e., zone 1 vs. zone 6) was not significantly affected by scopolamine HBr, $F(2,51)=0.72$, ns, or by its methyl deriv-

TABLE 4

	Scopolamine HBr				Scopolamine MBr			
Behaviour	0.0	0.25	0.50	1.0	0.0	0.25	0.50	1.0 mg/kg
Auditory Startle	10.6 (2.8)	11.2 (2.8)	11.1 (1.1)	10.8 (1.8)	13.0 (2.9)	11.3* (2.8)	10.6* (2.6)	10.6* (2.6)
Dorsal Contact:								
1) Flinch/jump	14.5 (5.1)	16.0 (2.5)	14.2 (5.1)	14.1 (6.2)	14.9 (4.6)	14.2 (5.2)	13.9 (6.1)	15.4 (3.9)
2) Vocalize	37.00	68.00	42.00	42.00	58.00	63.00	58.00	37.00
Pickup Rating	3.72 (0.20)	3.57 (0.25)	3.59 (0.23)	3.50 (0.24)	3.89 (0.21)	3.77 (0.20)	3.80 (0.20)	3.64 (0.18)

Effects of scopolamine hydrobromide and scopolamine methylbromide, 0.25–1.0 mg/kg (IP), on auditory startle, response to dorsal contact and experimenter rated defensiveness to attempted pickup. Data (except vocalization, expressed as % subjects showing this reaction) are presented as mean scores (±s.e.m.), *$p<0.01$ versus saline control.

TABLE 5

Behaviour	Scopolamine HBr 0.0	0.25	0.50	1.0	Scopolamine MBr 0.0	0.25	0.50	1.0 mg/kg
Vibrissae:								
Box	100	100	100	100	100	95	100	95
Bite	47	53	32	37	35	25	40	45
Vocalize	47	63	58	58	50	55	75	65
Jump Attack	16	16	5	16	15	20	5	15
Conspecific:								
Box	95	100	100	95	95	95	100	95
Bite	95	95	95	90	95	95	100	90
Vocalize	74	63	74	89	80	80	90	90
Jump Attack	42	26	42	32	55	35	35	25
Pick-Up:								
Box	95	95	79	95	95	100	100	95
Bite	84	95	74	84	90	85	95	85
Vocalize	74	84	79	79	85	95	80	85
Jump Attack	21	21	21	32	30	45	15	30

Effects of scopolamine hydrobromide and scopolamine methylbromide, 0.25–1.0 mg/kg (IP), on defensive reactions to vibrissal stimulation, an anaesthetized conspecific and attempted pickup by the experimenter. Data are expressed as the percentage of subjects displaying boxing, biting, vocalization and jump attacks towards the 3 forms of threat stimulation.

ative, $F(1,24) = 1.22$, ns. However, in both studies, a slight but consistent preference for zone 6 was apparent in all treatment groups (Table 6); scopolamine HBr, $F_{crit} = 4.00$, $F(1,51) = 3.45$, ns, scopolamine MBr, $F(1,24) = 16.22$, $p<0.01$. Wilcoxon tests failed to yield significant effects for either drug on rearing or grooming during the pre-cat period.

Cat Period (1–5 Min)

Wilcoxon tests indicated that scopolamine MBr was without significant effect on any behavioural parameter during the first 5 minutes of cat exposure (Table 7). For scopolamine HBr, both doses resulted in a significant increase in time spent in zone 1 ($p<0.05$ for both doses) and in the frequency of scanning ($p<0.05$ for both doses). A reliable increase in rearing was observed with the low dose only ($p<0.05$). Scopolamine HBr also significantly reduced grooming during the first period of cat exposure, an effect observed at both dose levels; 0.6 mg/kg ($p<0.005$) and 1.2 mg/kg ($p<0.05$).

Cat Period (6–10 Min)

In this time frame, the only effect observed with scopolamine MBr was a significant decrease in rearing ($p<0.01$). For scopolamine HBr, many of the effects observed during the first time period remained reliable (Table 7). For location measures, analysis revealed a significant increase in time spent in zone 1 under both doses (0.6 mg/kg, $p<0.005$; 1.2 mg/kg, $p<0.001$). A decrease in time spent in zone 6 was also apparent with the high dose during this period ($p<0.005$). Both doses increased the frequency of scanning (0.6 mg/kg, $p<0.01$; 1.2 mg/kg, $p<0.05$), and rearing (0.6 mg/kg, $p<0.005$; 1.2 mg/kg, $p<0.05$). No effects on grooming were apparent in this second period of cat exposure.

DISCUSSION

Central muscarinic synapses have been implicated in the inhibitory control of defensive responding in cats and rodents. Most of this evidence is based upon the induction of affective defense by intracerebral injection of muscarinic agonists (1,32) and the effects of agonists and antagonists on footshock-elicited defensive fighting (4, 23, 24, 30, 31). While intracerebral injection has been reported to produce potent effects, notably on defensive threat and attack ["affective defense"; (32)], these vary

TABLE 6

Parameter	Scopolamine HBr 0.0	0.6	1.2	Scopolamine MBr 0.0	0.6	1.2 mg/kg
Location:						
Zone 1	42.72 ± 8.10	31.00 ± 6.32	45.83 ± 7.74	30.11 ± 11.25	31.89 ± 11.89	13.56 ± 7.26
Zone 6	56.33 ± 9.40	62.67 ± 7.50	51.28 ± 9.07	66.56 ± 11.74	70.22 ± 11.80	93.33 ± 9.28
Rearing	3.17 ± 0.45	4.67 ± 0.69	3.78 ± 0.88	3.56 ± 0.93	3.00 ± 0.86	2.00 ± 0.59
Grooming	0.11 ± 0.07	0.11 ± 0.07	0.06 ± 0.05	0.22 ± 0.14	0.33 ± 0.22	0.56 ± 0.32

Effects of scopolamine hydrobromide and scopolamine methylbromide, 0.6–1.2 mg/kg (IP) on location (duration in sec), rearing (frequency) and grooming (frequency) during the pre-cat period. Data are presented as means (±s.e.m.). Although ANOVA revealed no treatment effects, a tendency to prefer zone 6 was apparent in both studies (see text).

TABLE 7

	mg/kg					
	0.0		0.6		1.2	
Period	1–5	6–10	1–5	6–10	1–5	6–10
SCOP HBr						
Location:						
Zone 1	6.44 ± 3.61	0.72 ± 0.70	29.83 ± 11.85*	9.78 ± 5.60*	48.94 ± 20.60*	35.72 ± 18.07§
Zone 6	264.00 ± 16.82	278.67 ± 16.21	257.44 ± 16.04	280.17 ± 9.36	235.33 ± 22.88	250.94 ± 18.31‡
Scanning	5.06 ± 1.26	4.27 ± 1.00	11.00 ± 2.92*	15.22 ± 4.70†	13.00 ± 5.11*	13.72 ± 4.60*
Rearing	0.83 ± 0.34	0.83 ± 0.37	2.56 ± 0.98*	5.56 ± 1.62‡	2.83 ± 1.35	2.11 ± 0.79*
Grooming	0.33 ± 0.16	1.06 ± 0.51	0.11 ± 0.11‡	0.67 ± 0.33	0.28 ± 0.17	0.44 ± 0.26*
SCOP MBr						
Location:						
Zone 1	4.67 ± 1.99	0.00 ± 0.00	3.33 ± 2.69	0.00 ± 0.00	3.22 ± 2.19	0.00 ± 0.00
Zone 6	275.67 ± 10.36	296.44 ± 1.71	265.22 ± 23.26	300.00 ± 0.00	287.33 ± 8.29	299.56 ± 0.42
Scanning	5.44 ± 2.58	9.22 ± 2.70	3.67 ± 1.28	4.33 ± 1.99	4.44 ± 1.52	5.22 ± 1.33
Rearing	0.22 ± 0.14	1.56 ± 0.63	0.33 ± 0.22	0.22 ± 0.21†	0.44 ± 0.32	0.33 ± 0.22*
Grooming	0.11 ± 0.10	0.33 ± 0.22	0.11 ± 0.10	0.11 ± 0.10	0.11 ± 0.10	0.22 ± 0.21

Effects of scopolamine hydrobromide and scopolamine methylbromide, 0.6–1.2 mg/kg (IP) on defensive behaviours during the two 5-min cat periods. Data are given as mean (±s.e.m.) duration (location, sec) or frequency (scanning, rearing, grooming). *$p<0.05$, †$p<0.01$, ‡$p<0.005$, §$p<0.001$ versus saline control.

with injection locus as well as dose level and test situation, making it difficult to evaluate their relationship to the effects of systemic injection of muscarinic agonists or antagonists. Present results, however, indicate that central muscarinic blockade produces rather limited effects on antipredator defensive reactions in wild and laboratory rats.

In wild *Rattus rattus*, tested in the Fear/Defense Test Battery (Experiment 1), the only significant behavioural effect of central muscarinic receptor antagonism over the dose range studied was a dose-dependent increase in flight distance. As other defensive elements, such as avoidance, freezing, defensive threat and attack, all remained intact, it is unlikely that the effect of scopolamine on flight distance was due to general behavioural disinhibition (15). This conclusion is further supported by the absence of significant changes in locomotor activity during the initial 5-minute pretest. Furthermore, as methyl scopolamine did not affect flight distance, the rather selective effect of the parent compound on this parameter cannot be attributed to peripheral muscarinic blockade. It should perhaps be noted that methyl scopolamine was not totally devoid of behavioural activity in that all doses of this compound significantly inhibited auditory startle responses. As this action was not seen with the parent drug, this inhibitory effect on auditory startle may either be due to a purely peripheral muscarinic blockade (which implies an opposing action of central muscarinic antagonism) or to a noncholinergic action of the compound. Nevertheless, the general failure of methyl scopolamine to alter defensive responding agrees with earlier findings (24, 30, 32).

The behaviourally specific effect of central muscarinic inhibition on flight distance in wild rats contrasts markedly with previous F/DTB effects reported for traditional and atypical fear- or anxiety-reducing agents, using the same subject species. Thus, low doses of ethanol enhanced defensive threat and attack, whereas higher doses tended to depress all aspects of the defensive repertoire (13). Diazepam and related benzodiazepines consistently inhibited defensive threat and attack elements (8), an effect shared by 5-HT_{1A} agonists such as buspirone and gepirone which additionally increased the percentage of animals that could be approached to the point of physical contact (9). Against these F/DTB profiles for other putative anxiolytics, and given the absence of a scopolamine effect on other defensive elements in this test battery, it would seem difficult to argue that the observed *increase* in flight distance is indicative of reduced fear or anxiety. Rather, present findings would be more consistent with a situation- and response-dependent alteration in mechanisms of selective attention (33) whereby, having taken flight, scopolamine-treated subjects are less attentive to stimulus factors that normally terminate or inhibit such responses. Importantly, the hippocampus has long been implicated in mechanisms of selective attention and in the behavioural effects of cholinergic agents (34). In this context, it may be pertinent to note that, in a previous study in laboratory rats, we found that hippocampal lesions produce hyperactivity and increased avoidance only after the introduction of a cat to the test chamber (11). Thus, one interpretation of present data is that the observed increase in flight distance may be a function of a specific deficit in selective attention resulting from blockade of muscarinic synapses in the hippocampal formation.

The present F/DTB effects contrast with the reported fear-reducing effects of scopolamine hydrobromide on the reactions of laboratory rats to the presence of a cat. Plotnik and colleagues (22,25) found that scopolamine, but not its methyl derivative, increased the number of approaches made towards the cat enclosure, as well as reducing freezing and enhancing drinking in the presence of the cat. Present results, using the wild rat F/DTB, may offer an alternate interpretation to the fear reduction hypothesis. Thus, in the studies by Plotnik *et al.* (22,25), the stimulus cat was presented in a wire mesh enclosure situated centrally in a circular arena into which the rat subject was placed. If, as present data suggest, the principal effect of scopolamine treatment is to

produce hyperactivity in the presence of a threat stimulus, increased approaches to the cat, reduced freezing and increased drinking may simply be a reflection of such hyperactivity. To further assess the nature of scopolamine effects on defensive responding, a second study was conducted in which the effects of the drug on responses to a cat stimulus were assessed in Long-Evans rats.

The method employed in Experiment 2 derives from a battery of tests recently developed to examine anxiety, as opposed to fear, reactions in laboratory rat subjects (7). The test used involved exposure to a cat behind a wire mesh screen located at one end of a test enclosure, and hence represents a less threatening situation than 'unprotected' exposure to a cat (12) or human (14) stimulus. Although rather similar to that used by Plotnik and colleagues (22,25), the method differed both in terms of the topographical relationship between the rat and cat enclosures and with respect to the behavioural parameters recorded. When tested with the cat present, control subjects in both studies (scopolamine hydrobromide and methylbromide) spent approximately 90% of the test period at the end of the apparatus farthest from the cat chamber (vs. approximately 20% in the pre-cat period), and showed reduced rearing and increasing grooming. Our data show that, compared to saline control and peripheral muscarinic blockade (scopolamine methylbromide), central muscarinic antagonism resulted in a significant increase in time spent in close proximity to the cat chamber. In addition, scopolamine-treated subjects showed increased scanning and rearing, together with reduced grooming. It is notable that these effects, although statistically reliable, were not strong and were most pronounced during the second half of the cat exposure period. In particular, there was little evidence that scopolamine treatment reduced the amount of time spent by subjects in zone 6, farthest from the cat. As such, the main effect of drug treatment was to produce a redistribution of the relatively small amount of time spent in other zones. These data, together with the findings of Experiment 1, suggest that at these doses scopolamine effects on antipredator defense are relatively weak and limited to less intense threat situations. It is therefore interesting to note that Mollenauer *et al.* (22) reported inhibitory effects of scopolamine on defensive reactions to a cat stimulus *only* in animals prerated as low in emotionality.

In summary, the sole effect of scopolamine in wild rats was to increase (not decrease) flight distance, suggesting interference with mechanisms of selective attention. A similar action may account for the very mild effects of the compound in laboratory rats confronted with, but protected from, a live predator. Together, these findings fail to support a significant role for muscarinic cholinergic mechanisms in defensive behaviour.

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