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Are Central Cholinergic Paths Involved in Habituation of Exploration and Distraction?

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FILE, S E. *Are central cholinergic paths involved in habituation of exploration and distraction?* PHARMAC. BIOCHEM. BEHAV. 4(6) 695–702, 1976 – Experiment 1 tested the generality of Carlton's hypothesis that central muscarinic cholinergic pathways are involved in habituation of exploration. The effects of 3 muscarinic antagonists were tested in a holeboard, under 2 test conditions, i e. with objects absent or present. Both the frequency and the duration of head-dipping were used as measures of exploration. Scopolamine prevented habituation only of the frequency of head-dipping, and only when objects were present Atropine and benzhexol did not impair the habituation of either frequency or duration of head-dipping in either test condition. The impairment of habituation seemed therefore to be specific to scopolamine, and to the more complex test condition, and thus there was little to justify the suggestion that central cholinergic paths were generally involved. Experiment 2 investigated the effects of muscarinic antagonists on habituation of distraction. None of the drugs affected the distraction to tones, nor the subsequent habituation to these stimuli. Central cholinergic paths do not therefore seem to be involved in habituation of this behavioural response

Exploration Distraction Habituation State-dependent learning Cholinergic Scopolamine Atropine Benzhexol

HABITUATION is the decrement of a response to an unreinforced stimulus, as a result of repeated presentations of that stimulus. An orienting response is elicited when an animal is exposed to novel stimuli of any modality, and both distraction and exploration have been considered as measures of orienting [2]. However, it has been suggested that habituation of different responses may involve different neuropharmacological mechanisms [22] and in order to test this hypothesis pharmacological data are needed on habituation of both exploration and distraction. Exploratory responses are elicited when an animal is exposed to a novel environment and the conceptual distinction between exploration and motor activity has been widely accepted [1, 10, 18] even though the practical separation of these 2 measures can be difficult to achieve. Thus it may be that habituation of motor activity will have a different pharmacological basis from habituation of exploration.

Carlton has suggested that a central muscarinic cholinergic system is essential for habituation of exploratory behaviour [3,4] and 2 pieces of evidence are said to support this concept. One came from a test situation in which the animals were exposed to the test environment when injected with scopolamine, but conclusions about habituation under this drug were based on a test the following day when the animals were undrugged. Scopolamine has powerful dissociative effects [16] and therefore poorer performance by animals that had been drugged on trial one, compared with animals tested undrugged on both trials, could be due to state-dependent learning and not to a failure to habituate when injected with scopolamine. The second piece of evidence comes from Grant [12] who found that scopolamine impaired short-term habituation of locomotor activity in mice, but as discussed above it is questionable whether locomotor activity can be considered an exploratory response [1,10]; and, in addition, different mechanisms may be involved in short- and long-term habituation [6,8].

The purpose of Experiment 1 was to test the generality of Carlton's hypothesis by investigating the effects on habituation of exploration of 3 muscarinic antagonist drugs, scopolamine, atropine and benzhexol The complexity of the test situation was varied in order to determine whether the impairment of habituation was related to this factor The importance of taking both frequency and duration measures of exploration has been pointed out by Robbins [19] and is emphasised by results where a decrease in the frequency of lever pressing was accompanied by an increase in duration [15].

EXPERIMENT 1

METHOD

Animals

Male hooded Lister rats 300–350 g weight, supplied by

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Olac Ltd., Bicester, were housed in groups of 6 per cage in an 11 hr light, 13 hr dark cycle at 25°C with ad lib food and water.

Male mice of CFW strain, 20–25 g weight, were housed in groups of 10 in a 10 hr light, 14 hr dark cycle at 25°C with ad lib food and water.

Apparatus

The rat holeboard was a wooden box with 46 cm high walls and a floor 66 × 56 cm. In the floor, which was 1 cm thick, were 4 equally spaced holes, 3.8 cm in dia. The floor of the holeboard was 12 cm above the base of the box and when objects were placed under the holes they came to 3 cm below the top of the hole. The objects used were a metal weight, a glass funnel containing cotton wool, an aluminium pot filled with matches, and a rubber bung. Pilot experiments had shown that these 4 objects reliably elicited equal amounts of exploration.

The mouse holeboard was a smaller version of the rat holeboard. The floor was 40 cm square and the walls were 27 cm high. The holes were 3 cm in dia. and the objects were placed 2 cm below the top of the hole. The level of illumination on the floor of the holeboards was 3.0 lumens/ft^2.

Drugs

The drugs used were scopolamine hydrobromide and atropine sulphate, from Sigma Chemical Co., and benzhexol hydrochloride from Winthrop laboratories. All the drugs were dissolved in 0.9% saline and the doses are expressed in terms of the salt The drugs were administered by intraperitoneal injection and the volume of injection never exceeded 2 ml for rats and 0.5 ml for mice.

Procedure

Since the level of exploration varies throughout the day [8] the rats were tested only between 10.00 and 12.00 hr, and the mice were tested only between 14.00 and 16.00 hr, with the order of testing randomised between drug and saline groups

The first batch of rats was randomly allocated to groups which received saline, scopolamine (1 mg/kg) or scopolamine (2 mg/kg) The second batch of rats was randomly assigned to saline, atropine (10 mg/kg) or atropine (20 mg/kg) groups, and the third batch to saline and benzhexol (40 mg/kg) groups. All groups were subdivided into 2, with 1 subgroup tested in the presence of objects and the other in the absence of objects. Each subgroup contained 10 rats. The mice were randomly allocated to a saline or scopolamine (1 mg/kg) group and the groups subdivided into the objects present or absent condition, to give 10 mice per subgroup. All animals were injected intraperitoneally 20 min before testing.

Each animal was placed singly in the centre of the board, facing away from the observer, and its behaviour was recorded for 10 min. The number of times the animal put its head down each hole and the duration of each head-dip (to the nearest second) were recorded. A head-dip was scored if the animal's head entered the hole at least as far as both eyes being below the floor of the box. The number of rears was also counted, including both free-standing rears and those against the sides of the box. After each trial the floor of the apparatus was washed and dried to remove traces of the animal's path.

Animals received one 10 min trial per day for 3 days, except for the rats tested with scopolamine, which received trials for 4 days.

RESULTS

Scopolamine

Rats. From Fig. 1 it can be seen that in all groups of rats the duration of head-dipping was significantly increased when there were objects present ($F(1,54) = 30\ 5$, $p<0\ 001$). Scopolamine produced a dose-related increase in this measure ($F(2,54) = 6.3$, $p<0.01$), but in spite of this all groups showed significant habituation ($F(3,162) = 12.8$, $p<0.001$), and there was no interaction between the rate of habituation and scopolamine ($F(6,162) = 0.95$). There was no interaction between the rate of habituation and the presence or absence of objects ($F(3,162) = 1.1$) nor was the interaction between all 3 factors significant ($F(6,162) = 0.26$). The frequency of head-dips also increased with the drug dose ($F(3,162) = 24.2$, $p<0\ 001$). However, when there were objects present the frequency of head-dips did not show habituation over trials in the scopolamine groups, although the control group did habituate, this led to a significant drug × trial × objects interaction ($F(6,162) = 2\ 4$, $p<0.05$).

Rats showed more rears when objects were absent ($F(1,54) = 5.4$, $p<0.025$), scopolamine increased rearing in rats ($F(2,54) = 13.3$, $p<0.001$), although as can be seen from Fig 3 this was due to the effect of the 2 mg/kg dose. All the rats showed habituation over trials ($F(3,162) = 8.7$, $p<0\ 001$) and none of the interactions was significant

Mice The results from the group of mice tested in the hole-board are essentially the same as the results from rats (see Fig. 2). Both the drug (1 mg/kg scopolamine) and saline groups showed habituation of the duration of head-dipping ($F(2,36) = 17\ 4$, $p<0.001$) when there were objects present, but the drug group did habituate more slowly than the control ($F(2,36) = 12.7$, $p<0.001$) There was a significant effect of scopolamine on the frequency of head-dipping ($F(1,18) = 16.5$, $p<0.001$), owing to the failure of the drug group to habituate. As was the case with rats, when objects were absent from the situation both measures showed significant habituation ($F(2,36) = 11.3$ and 13.7, $p<0.001$, for frequency and duration respectively).

It can be seen from Fig. 3 that all mice showed habituation of rears over trials ($F(2,72) = 61\ 0$, $p<0.001$), and more rearing when there were no objects under the holes ($F(1,36) = 7.4$, $p<0.01$), the trials × drug interaction was not significant ($F(2,72) = 0\ 32$). However, habituation was more rapid when the objects were absent ($F(2,72) = 24$, $p<0.001$) and this was particularly marked for the mice injected with scopolamine (trials × drugs × objects interaction $F(2,72) = 7.9$, $p<0\ 001$) Scopolamine reduced the number of rears made by mice ($F(1,36) = 10.5$, $p<0.001$).

Atropine

The effects of atropine on habituation of exploration are shown in Table 1. From this table it can be seen that there was no effect on the frequency of head-dipping of the 2 doses (10 and 20 mg/kg) of atropine ($F(2,27) = 0.52$ and 0.04, for the objects present and absent conditions respectively), and all groups showed significant habituation even

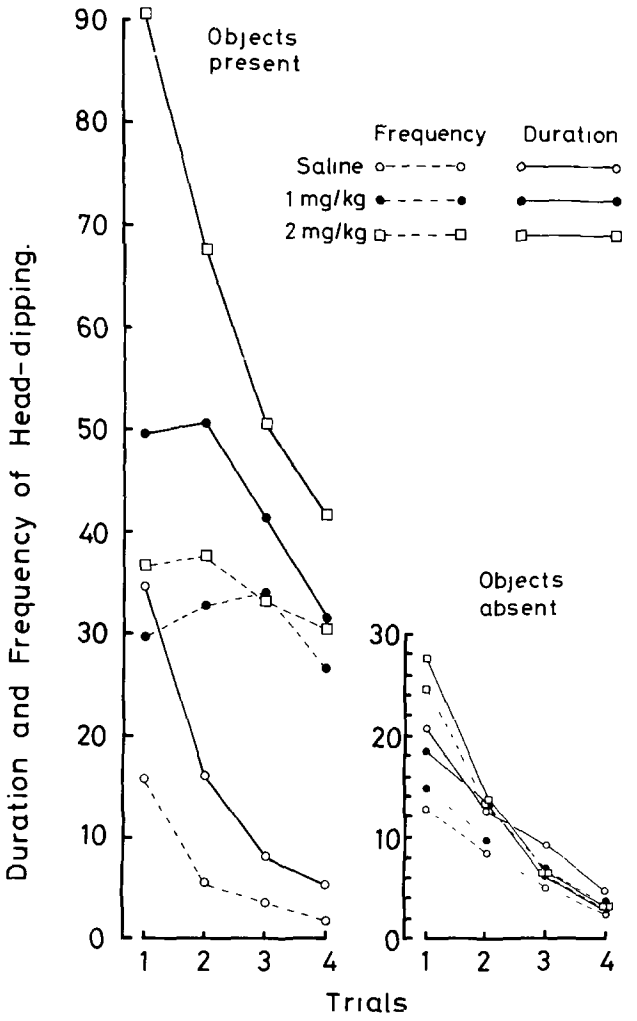

FIG 1. The duration (s) and frequency (number/10 min) of head-dipping over successive trials in rats injected with saline, 1 or 2 mg/kg scopolamine and tested in the presence or absence of objects

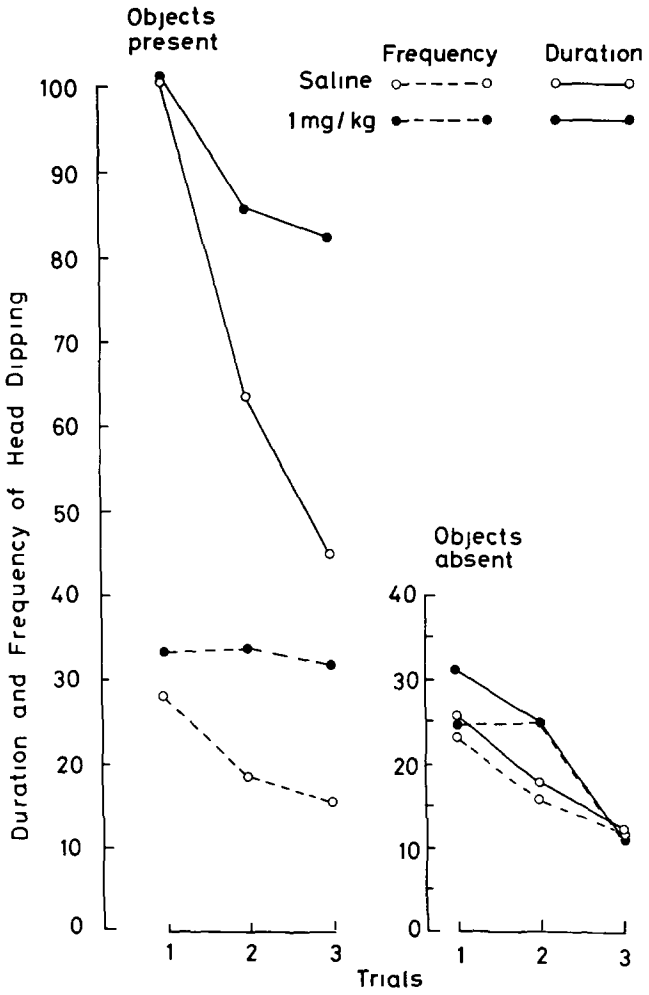

FIG. 2. The duration (s) and frequency (number/10 min) of head-dipping over successive trials in mice injected with saline or 1 mg/kg scopolamine and tested in the presence or absence of objects

over the first 2 trials ($F(1,127) = 110.8$ and 69.5, $p<0.001$, for the objects present and absent conditions), and there was no drug x trials interaction ($F(2,27) = 0.38$ and 1.7, for objects present and absent conditions). Similarly, atropine did not affect the duration of head-dipping ($F(2,27) = 0.69$ and 0.10 for objects present and absent) and all groups showed significant habituation even over the first 2 trials ($F(1,27) = 44.1$ and 52.4, $p<0.001$, for objects present and absent).

Atropine reduced the number of rears ($F(2,154) = 4.5$, $p<0\,025$) and all the rats showed habituation over trials ($F(2,72) = 129.8$, $p<0.001$); there were no significant interactions (see Table 2).

Benzhexol

Benzhexol (40 mg/kg) did not alter the frequency or duration of head-dipping when objects were present ($F(1,18) = 1.3$ and 0.83 respectively) or absent ($F = 0.04$ and 0.54 respectively), see Table 1. Rats injected with benzhexol showed significant habituation of exploration when the objects were present ($F(2,36) = 36.9$ and 10.6, for number of head-dips and duration of head-dipping respectively, $p<0.001$) and when they were absent ($F(2,36 = 18.0$ and 44.3, for frequency and duration of head-dipping, $p<0.001$). Benzhexol had no overall effect on the number of rears ($F(1,36) = 0.43$), all rats habituated over trials ($F(2,72) = 110.9$, $p<0.001$), but those injected with benzhexol showed a more rapid decrease over trials ($F(2,72)) = 8.3$, $p<0.001$).

DISCUSSION

Scopolamine increased both the frequency and the duration of head-dips which agrees with other reports of increased response to novel stimuli [13,14]. This increased exploration was not seen after injections of atropine or benzhexol, which agrees with the effects of atropine (10 mg/kg) on head-dipping in mice, although an extremely high dose (32 mg/kg) did increase the frequency of head-dipping [20].

Both mice and rats injected with scopolamine failed to show between-trial habituation of the number of head-dips

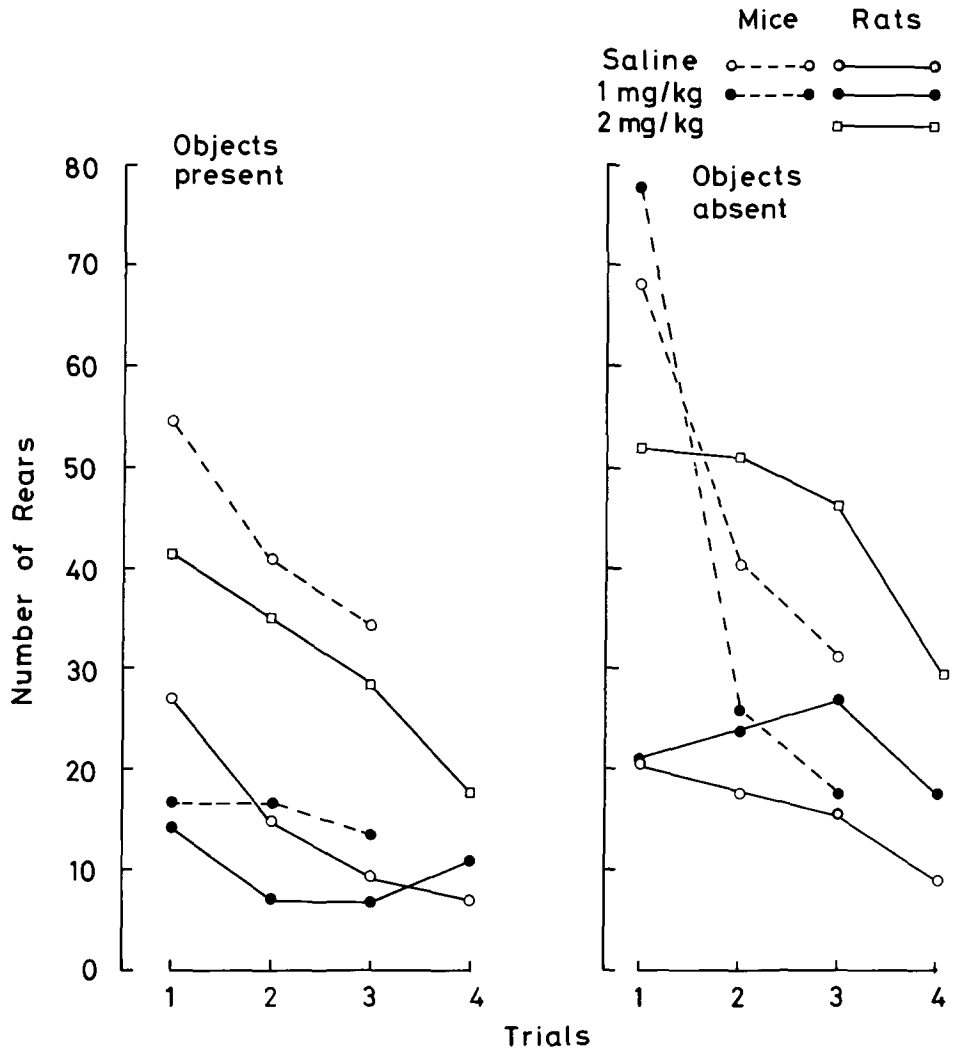

FIG. 3. The number of rears over successive trials made by mice and rats injected with saline or scopolamine and tested in the presence or absence of objects

made when objects were present, although habituation was normal in the absence of objects. This supports the suggestion [5] that scopolamine has a greater effect as the complexity of the task is increased. But why are different results obtained with the frequency and duration of head-dipping? It has been shown [9] that the duration of head-dipping is a more valid measure of exploration than the number of head-dips, and thus scopolamine may impair habituation of activity [12], but not habituation of exploration. The importance of distinguishing between motor activity and exploration has been stressed by many authors [1, 9, 18]. It is unlikely that these results are due to the peripheral effects of scopolamine, since different results were obtained with the other anticholinergics, and the quaternary derivatives methylscopolamine and methylatropine do not effect habituation [3,12] One of the reasons for obtaining different results with scopolamine is that it is a CNS depressant, whereas atropine is a CNS stimulant.

The present results do not support a general involvement of a central muscarinic cholinergic system in between-trial habituation of exploration. The relative potencies of scopolamine and atropine are approximately 10·1 so the failure to find an impairment of habituation with atropine cannot be due to using too low a dose. Similarly the dose of benzhexol was chosen on the basis of its potency when compared with atropine, and was roughly equivalent to the 20 mg/kg dose of atropine. The effects reported by Carlton and by Grant seem therefore to be primarily restricted to scopolamine and to certain test situations. A similarly restricted result has been reported for the habituation of the flexor withdrawal reflex [17], the acquisition of which is impaired by atropine (30 mg/kg) but not by scopolamine (up to 0 5 mg/kg).

TABLE 1

EFFECTS OF ATROPINE AND BENZHEXOL ON EXPLORATION IN THE HOLEBOARD

Trials	1		2		3	
Head-Dips	Freq	Duration	Freq	Duration	Freq	Duration
(A) Objects Present						
Saline	16 4	30 3	6 0	12 8	3 1	8 3
10 mg/kg Atropine	15 3	33 2	6 9	17 1	3 4	9 5
20 mg/kg Atropine	18 2	37 9	8 2	18 7	3 5	10 4
Saline	17 6	43 8	6 0	11 8	2 8	6 6
40 mg/kg Benzhexol	19 9	40 8	9 0	26 3	7 5	15 0
(B) Objects Absent						
Saline	12 3	24 9	7 2	11 8	2 8	5 0
10 mg/kg Atropine	11 7	26 4	7 6	12 1	2 6	4 6
20 mg/kg Atropine	13 5	31 0	6 5	9 6	2 5	3 8
Saline	12 1	22 8	5 9	10 7	2 4	5 1
40 mg/kg Benzhexol	11 7	20 5	5 8	9 0	3 2	3 2

TABLE 2

MEAN NUMBER OF REARS MADE IN THE HOLEBOARD ON SUCCESSIVE TRIALS

Drug	Trials 1	2	3
(A) Objects present			
Saline	23 6	16 1	11 5
Atropine 10 mg/kg	20 5	16 2	11 7
Atropine 20 mg/kg	21 0	11 0	8 5
Saline	19 3	6 7	6 2
Benzhexol 40 mg/kg	30 0	5 9	6 9
(B) Objects Absent			
Saline	20 4	15 9	10 6
Atropine 10 mg/kg	24 9	18 0	12 6
Atropine 20 mg/kg	19 1	9 5	6 8
Saline	24 1	15 0	7 5
Benzhexol 40 mg/kg	29 0	11 7	2 5

EXPERIMENT 2

Although Carlton has implicated a cholinergic system in habituation of exploration this system is not essential for the habituation of startle responses [21,22], and he suggests that at the neuropharmacological level different mechanisms may be involved in different types of habituation. A third type of behavioural habituation that has been studied is the habituation of orienting responses, measured in a distraction task [7]. The habituation in this situation is long-term since it is retained for several days [7], and the rate of habituation is constant for stimulus presentation rates of 1½ min to 24 hr [8]. The purpose of Experiment 2 was to examine the effects of muscarinic antagonists on this type of habituation The distraction produced in rats by tones was measured by the duration of interruption in their baseline licking activity. Since muscarinic antagonists produce peripheral changes, e.g. dryness of the mouth, which might affect behaviour in this task, two additional control groups were tested. These groups received methylatropine and methylscopolamine, drugs that produce the same peripheral changes but do not readily pass the blood-brain barrier.

A second purpose in this experiment was to examine the extent to which anticholinergic drugs produced state-dependent habituation [16] This would mean that a rat would be able to habituate after injection with a muscarinic antagonist, and would also show retention of habituation if retested after the same drug injection. However, if the rat was retested when it was undrugged then it would show less or no habituation Similarly, rats habituated undrugged would show good retention when tested undrugged, but would show less retention if tested after an injection of an anticholinergic drug. If state-dependent habituation is found this would mean that the habituation was specific to the drug state in which it was acquired, but this is quite different from saying that anticholinergics prevent the acquisition of habituation [4].

METHOD

Animals

Male hooded Lister rats, 300–350 g weight, were housed as described in Experiment 1. These rats were initially deprived of water for 48 hr and thereafter only received water in the test chamber, or immediately following their test, in sufficient quantity to maintain a steady body weight Food was available ad lib

Apparatus

The test chamber was 19 cm high with a grid floor 19 × 26 5 cm and was enclosed in an acoustically insulated box. A slit in the end wall gave access to a water spout, and a drinkometer recorded the rat's licking. Experimental events were automatically programmed and the tones were delivered via a loudspeaker positioned in the lid of the chamber at the water spout end. The tone used was 9 kHz 75 dB (re. 0.0002 dynes/cm^2) for 9 sec

Drugs

The drugs used were the same as in Experiment 1. In addition scopolamine methylbromide and atropine methylbromide were used These drugs were supplied by Sigma Chemical Co., and dissolved in 0.9% saline.

Procedure

Rats were randomly allocated to saline, scopolamine (1, 2 and 4 mg/kg), methyl-scopolamine (1 and 4 mg/kg), atropine (10, 15 and 20 mg/kg) methylatropine (15 and 20 mg/kg) and benzhexol (40 mg/kg) groups, to give 12 rats per group. Injections were given intraperitoneally 20 min before testing, from the second day onwards

On the first day following the initial water deprivation no injections were given and the rats were left in the test chamber for 15 min or until they had made 2,000 licks. On the second day the rat's 200th lick switched on a control period of 9 sec in which the number of licks made (A) was counted. Following this period the rat's next 200th lick switched on a 9 sec period during which the tone stimulus

was delivered, and the number of licks during this period (B) was counted. Control and tone periods alternated with an average interval between tones of 75 sec. A ratio of $\frac{A-B}{A}$ was used as a measure of distraction, complete distraction to the tone giving a ratio of one and complete habituation a ratio of zero. A criterion of habituation was adopted of 3 successive stimulus presentations to which the distraction ratio was ≤ 0.10. For each rat the distraction ratio to the first stimulus presentation was calculated and the number of trials, or stimulus presentations, needed to reach habituation was counted.

When each rat reached criterion it was assigned to a state-constant or a state-change condition thus, on the next day animals in the drug groups received either their usual drug injection before being tested (a state-constant group) or they were tested undrugged (a state-change group). The saline groups were similarly divided into those tested again undrugged (state-constant) and those tested drugged (state-change). The doses chosen for this test were scopolamine (4 mg/kg) methylscopolamine (4 mg/kg), atropine (15 mg/kg), methylatropine (15 mg/kg) and benzhexol (40 mg/kg) Once again the initial distraction to the tone and the trials to habituate were scored

RESULTS

Since the baseline activity in this experiment was licking it was thought that the peripheral effects of the muscarinic antagonists (e.g. dryness of the mouth) might interact with any central effects of the drugs on the distraction, and/or subsequent habituation, to tone stimuli. This did not seem to be the case because neither methylscopolamine nor methylatropine affected distraction or habituation. Indeed, the rate of licking did not vary between any of the drug and saline groups, although the muscarinic antagonists did increase the latency with which the rats started to lick, and decreased the time for which steady licking was obtained. These effects are probably due to a central action of the drugs and agree with reports of a reduced water intake following injection of scopolamine [11].

Table 3 shows the mean distraction ratios to the first tone presentation for the saline and drug groups From this it can be seen that the mean distraction ratios for the 3 saline groups showed little variation, but nor was the distraction ratio of any of the drug groups significantly different from the saline controls ($p>0$ 05). Similarly, none of the drug groups took significantly longer or shorter to habituate to the tone stimulus than did the controls ($p>0.05$).

Table 4 gives the data for the retention test 24 hr after the rats had reached habituation criterion. In the state-constant groups the animals were tested in the same state in which they had been habituated. From the left-hand columns in Table 4 it can be seen that all the state-constant groups remained habituated and did not distract to the tone. Thus, the rats were not only capable of habituating when dosed with anticholinergic drugs but retained this habituation when tested the following day, after the same drug injection.

In contrast, the right hand columns in Table 4 show the distraction to the test tone for rats tested in a changed drug state, and the trials to rehabituate. All groups showed an increase in distraction compared with their criterion level the previous day, the animals habituated under 1, 2 or 4 mg/kg scopolamine showed a significant (at least $p<0.05$) return of orienting when tested undrugged (t = 4.17, 5.67, 3.0, df = 5) and, similarly, the rats habituated when undrugged showed a significant return of orienting when tested after 4 mg/kg scopolamine (t = 4 11, df = 5). However this state-dependent habituation cannot be entirely due to central effects because rats habituated under 1 and 4 mg/kg methylscopolamine also showed a significant increase in orienting if they were tested undrugged (t = 5 06 and t = 2 78, df = 5) as did those habituated undrugged but tested after 4 mg/kg methylscopolamine (t = 3.67, df = 5) Atropine produced less pronounced state-dependent effects, for whilst rats habituated under the higher doses, 15 and 20 mg/kg, showed an increased distraction when tested undrugged (t = 11.06, 4.82, df = 5, $p<0.01$) those habituated under 10 mg/kg showed no significant loss when tested undrugged (t = 2.21, df = 5, $p>0.05$) and those habituated undrugged did not show a significant return of orienting when tested after injection with 15 mg/kg atropine (t = 2.45, df = 5, $p>0$ 05). Once more the state-dependent effects at the higher doses might have been due to peripheral changes produced by atropine because rats habituated under 15 and 20 mg/kg methyl-atropine showed a significant return of orienting when tested undrugged (t = 3.34, 3.80, df = 5, $p<0.05$), and those habituated undrugged but tested under 15 mg/kg methylatropine also showed increased orienting (t = 3 11, df = 5, $p<0.05$). Finally, benzhexol produces state-dependent habituation with rats transferring from a drugged to an undrugged state showing increased orienting (t = 3.79, df = 5, $p<0.05$) and vice versa (t = 4.24, df = 5, $p<0.05$).

For those animals showing state-dependent habituation,

TABLE 3

EFFECTS OF ANTICHOLINERGIC DRUGS ON DISTRACTION AND HABITUATION TO TONE STIMULI

	Distraction Ratio	Trials to Habituate
Saline	0 52	14.0
1 mg/kg Scopolamine	0 54	13 9
2 mg/kg Scopolamine	0 56	14.4
4 mg/kg Scopolamine	0 57	13 6
Saline	0 52	13 2
1 mg/kg Methyl-scopolamine	0 56	12 9
4 mg/kg Methyl-scopolamine	0 59	14 0
Saline	0 50	13 8
10 mg/kg Atropine	0 50	15 9
15 mg/kg Atropine	0 51	15 0
20 mg/kg Atropine	0 52	15 3
Saline	0 52	13 5
15 mg/kg Methyl-atropine	0 63	14 0
20 mg/kg Methyl-atropine	0 71	13 4
Saline	0 50	13 8
40 mg/kg Benzhexol	0 53	10 3

TABLE 4

TRANSFER OF HABITUATION FOR STATE-CONSTANT AND STATE-CHANGE GROUPS

State-Constant Groups	Ratio*	Trials†	State-Change Groups	Ratio*	Trials†
Scopolamine			Scopolamine		
			From		
saline	0 04	0	1 mg/kg	0 47	17 5
1 mg/kg	0 05	0 2	2 mg/kg	0 33	12 0
2 mg/kg	0 05	0 2	4 mg/kg	0 26	10 8
4 mg/kg	0 06	0	To		
			4 mg/kg	0 41	15 8
Methyl-scopolamine			Methyl-scopolamine		
			From		
saline	0 04	0	1 mg/kg	0 34	11 8
1 mg/kg	0 06	0	4 mg/kg	0 34	13 5
4 mg/kg	0 04	0	To.		
			4 mg/kg	0 59	7 0
Atropine			Atropine		
			From.		
saline	0 05	0	10 mg/kg	0 24	4 5
10 mg/kg	0 04	0	15 mg/kg	0 48	15 3
15 mg/kg	0 04	0	20 mg/kg	0 49	14 3
20 mg/kg	0 06	0	To		
			15 mg/kg	0 41	8 0
Methyl-atropine			Methyl-atropine		
			From		
saline	0 03	0	15 mg/kg	0 36	7 3
15 mg/kg	0 04	0	20 mg/kg	0 39	7 6
20 mg/kg	0 05	0	To		
			15 mg/kg	0 21	6 3
Benzhexol			Benzhexol		
			From		
saline	0 04	0	40 mg/kg	0 31	9 0
			To		
40 mg/kg	0 07	0	40 mg/kg	0 35	11 1

*Ratio = mean distraction to the first test tone
†Trials = mean number of trials to rehabituate

as reflected in a significant return of orienting, the number of trials needed to rehabituate gives a measure of the extent to which the habituation was state-dependent Complete dissociation would mean that rats transferring, say, from 1 mg/kg of scopolamine to an undrugged state would need as many trials to rehabituate as animals had taken that had originally been habituated undrugged. If a significant saving in regabituation occurs then this is evidence that there is some transfer between states. None of the rats in the scopolamine groups showed significant savings on the trials to rehabituate, indicating that with this drug the dissociation is complete. Similarly, the only atropine group showing significant savings was the one transferring from 10 mg/kg (t = 2 48, df = 16, $p<0.05$), which had already shown transfer on the distraction measure. Benzhexol produced a similar result with no significant savings. The only evidence that this dissociated, or state-dependent, habituation is due to central effects is that whilst it was total for the centrally acting muscarinic antagonists, for the drugs with primarily peripheral effects there was some savings in rehabituation, indicating partial transfer between states. However, only in the case of rats habituated undrugged and tested with 15 mg/kg methyl-atropine was this savings significant at the 5% level (t = 2.26, df = 16) for the other groups the savings was only significant at the 10% level.

DISCUSSION

The results of Experiment 2 show that muscarinic antagonists do not prevent habituation of distraction, and that there is 24 hr retention of this habituation, provided that the rats are tested in the same drug state in which they were habituated.

The results from the rats tested in a different drug state from the one in which they were habituated indicated that all the anticholinergics produced powerful dissociation, i.e. the habituation was state-dependent. Thus an animal habituated under scopolamine but tested

undrugged will fail to demonstrate habituation – not because scopolamine had prevented habituation, but because this habituation does not transfer to an undrugged state. This reinforces the suggestion made earlier in this paper that the poor performance of the scopolamine animals in the water-approach task [4] could well have been due to state-dependent learning, and not to a failure to habituate when drugged.

GENERAL DISCUSSION

A central muscarinic system does not seem to be critically involved in behavioural habituation in the rat. There is no evidence for a cholinergic role in the habituation of startle [21,22], none of the antimuscarinic drugs affected habituation of distraction; and only scopolamine affected habituation of exploration, and then only in certain test conditions.

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