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Physostigmine: Effects on Fear or Defense Responses in the Rat

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MOLLENAUER, S., M. WHITE, R. PLOTNIK AND P. B. TIFFANY. *Physostigmine: Effects on fear or defense responses in the rat.* PHARMAC. BIOCHEM. BEHAV. 11(2) 189–195, 1979.—Previous research had shown that the anticholinergic drug, scopolamine, decreased innate defensive responses of rats to a live cat or mechanical robot, and that the effects of scopolamine were attributable to actions of the drug on the central nervous system. In the present research, the anticholinesterase, physostigmine, which increases central cholinergic activity, caused an increase in the defense responses of male hooded rats. Physostigmine caused significantly more freezing and significantly more suppression of feeding and suppression of time near the aversive stimulus (ROBOT). Dose-response curves showed a positive, linear relationship between dose (0.025, 0.05, 0.1 and 0.2 mg/kg) of physostigmine and defense responses. The present results could not be attributed to general response suppression since the effects of physostigmine were situation-specific, i.e., the drug had no significant effect on behavior in the non-aversive or NO ROBOT condition. The present results were taken as further evidence of the involvement of cholinergic activity in the mediation of defense responses. The effects of cholinergic and anticholinergic drugs on the observable defense response of freezing were thought to have important implications for the large literature relating these drugs and avoidance responding.

Physostigmine Cholinergic Anticholinesterase Defense responses Fear Freezing Aversive stimulus Rat

WHETHER observed in natural environments, or the laboratory, animals appear to have innate defensive reactions, such as freezing, flight and threat, which they display in response to predators, aversive stimuli, or even novel stimuli [3]. Laboratory rats exposed to a cat for the first time show defense responses of freezing, flight [2,18] and suppression of feeding [18]. Research with the anticholinergic (antimuscarinic) drug, scopolamine, suggests that cholinergic synapses may be involved in the mediation of these defensive responses [18]. In the presence of a cat, control rats treated with saline or methyl scopolamine show a strong suppression of feeding and spend much of the trial time freezing at the extreme perimeter of the apparatus. Rats that have been treated with scopolamine show an attenuation of these defense reactions, including reduced freezing, less avoidance of the area near the cat, and less suppression of feeding. Since rats treated with methyl scopolamine fail to show these effects, it appears that the effects of scopolamine on defensive reactions are attributable to its actions on the central nervous system.

A large body of literature relating anticholinergic drugs and shock avoidance behavior also suggests a role of cholinergic activity in defense responding in the rat. Anticholinergic drugs have repeatedly been shown to impair passive avoidance [4, 10, 14] and enhance two-way active avoidance [11,20]. One explanation of this literature is that anticholinergic drugs affect avoidance behavior by decreasing the innate species-specific defense response of freezing [17]. Less research has been addressed to the effect of increased cholinergic activity on avoidance performance. As expected, however, drugs that increase cholinergic activity have had essentially the opposite effect from anticholinergic drugs [6,22]. One purpose of the present research was to assess the effects of increased cholinergic activity on the defense response of freezing.

The present research studied the effects of increased cholinergic activity on the defensive reactions of rats using the drug, physostigmine. Physostigmine is an anticholinesterase, which increases cholinergic activity by preventing the breakdown of acetylcholine by acetylcholinesterase [13]. Defense reactions were studied using the paradigm originally developed with the stimulus cat [18]. However, a mechanical robot was used as the stimulus in the present research in order to avoid possible ceiling effects. When a live cat was employed, rats displayed what may have been maximal levels of fear or defense responding. Since the cholinergic drug could be expected to have the opposite effect from an anticholinergic drug, it was important to use a stimulus situation in which animals would be capable of showing increased defense responding. The mechanical robot had the advantage that stimulus parameters (e.g., noise, speed of rotation) could be adjusted to elicit appropriate baseline levels of defense responding. Although the mechanical robot offered certain technical advantages, it did not change the essential character of the paradigm. Previous research had shown that a mechanical robot not only elicited the same pattern of defense reactions as the live cat [17]; it also resulted in the same pattern of effects from drugs [17]. As with

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the cat stimulus, rats were encountering an unconditioned aversive stimulus that did not involve pain. Using a non-pain stimulus was particularly important since drugs that increase cholinergic activity, including physostigmine, have been shown to raise the aversive threshold for shock [12].

Experiment 1 established a dose-response curve for the effect of physostigmine on defensive reactions. Rats were first food-deprived and trained to drink a sweet solution near the center of a large arena. They were then injected with saline or one of four doses of physostigmine and tested with the mechanical robot activated in the center of the apparatus. Experiment 2 compared the effects of the most effective dose of physostigmine and saline in robot and no-robot condition in order to determine whether the drug effects were situation specific.

EXPERIMENT 1

METHOD

Animals

The animals were 74 hooded Long Evans rats, weighing 250–350 g at the start of the experiment, purchased from Simonson Laboratories. Five rats were discarded for failure to meet training criteria, described in the procedure. Rats were housed individually 1 week prior to the initiation of training. During the period 1 week prior to training, rats were maintained on ad lib food and water; during training rats were given unlimited access to water, but were maintained on a 23-hour food deprivation schedule and were fed a fixed amount (approximately 12 g) at the same time each day, 30–60 min after the experimental session. Rats were tested toward the end of the light phase of their light-dark cycle; the cycle was 13 hr light, 11 hr dark.

Apparatus

The apparatus was similar to that used in previous research [17]. It consisted of a circular arena, painted black, with a hinged Plexiglas lid and a wire mesh floor. The arena was 110 cm in dia., with walls 41 cm high. A plywood board, marked off into 48 approximately equal segments, was placed under the wire mesh floor. A wire mesh enclosure, 22.5 cm in dia. and 41 cm high, was placed in the center of the arena. Four glass food cups, 3 cm in dia., were spaced at equidistant points around the center enclosure at a distance of 5 cm from the outside edge of the enclosure.

A large mirror was placed over the arena to maximize experimenter visibility and minimize distraction to the rats. White noise was used during training and test trials to mask extraneous auditory cues.

Stimulus Robot

The stimulus robot was a plastic, commercially made, battery powdered, mechanical robot, 32×17×13 cm. The head and arm sections were removed. The robot was housed within the center enclosure of the apparatus and was placed on a stand, so that it hung vertically with its feet 5 cm above the wire mesh floor. During all training trials the robot was covered by a black plastic tarp. During test trials the enclosure and tarp were removed to reveal the robot.

The robot was activated continuously throughout the test trial and always operated so that the walking cycle was just beginning at the start of the trial. When the robot was activated, it alternated between walking and rotating movements. During walking, the legs moved back and forth, one stroke per second, each leg moving a distance of approximately 2.8 cm. Every 6 sec the lower half of the robot rotated for 2 sec at approximately one rotation per second. While the robot was activated, it made a loud grinding noise; the noise was 72 db during walking and rose to about 79 db during rotation.

Drug Treatment and Design

Rats were assigned to one of five drug treatments, four doses of physostigmine sulfate (supplied by Calbiochem) (0.025, 0.05, 0.1 and 0.2 mg/kg) and one dose of saline. Physostigmine was dissolved in physiological saline and administered intraperitoneally in a volume of 1 ml/kg 30 min prior to testing. All tests were conducted blind.

At the end of training, rats were rank ordered on the basis of their cumulative feeding times; rats were then assigned to conditions with the restriction that for every five consecutive ranks, one rat had to be assigned to each of the five drug conditions. This procedure was followed because past research has indicated that emotionality of the rat, determined by cumulative feeding times, can interact differentially with drug treatment [15].

Procedure

Habituation. Previous work had shown that habituation and training in a new apparatus were greatly facilitated by the presence of rat odor. Therefore, prior to training, 10 rats (male hooded Long Evens) that had been living together were left in the apparatus overnight with water bottles and large dishes of sucrose (32% solution) available and were removed the following day. Next, the experimental rats were habituated by leaving them in the apparatus overnight in randomly selected groups of 10 or 11, with sucrose and water available. It had previously been established that this procedure did not result in fighting among rats that had been individually housed for only one week.

Training. Training was begun 24 hr after the last overnight habituation session. Each rat was given one 1-min trial per day for 5 days, with sucrose available in each of the 4 food cups. For a given trial the rat was placed (facing the center enclosure) about midway between the perimeter and the center of the apparatus; rats were always placed at the same point. From this point a rat had to move forward, toward the center enclosure, in order to reach one of the food cups. Feeding from all 4 cups would necessitate circling the enclosure. During training, some rats fed from only 1 or 2 cups; others fed from all 4. When 1 min had elapsed the rat was removed from the apparatus and returned to its home cage.

On Day 4 all rats that had not fed at least 15 sec of the 1 min trial were given extra trials until they had met this criterion, or until 5 extra trials had been given. For these extra trials, rats were not removed from the apparatus between trials. No extra trials were administered on Day 5. Any rat that did not feed for at least 15 sec of any 1 min trial on Day 4, or during the 1 min trial on Day 5 was discarded; 5 rats were discarded for failure to meet this criterion.

Testing. On Day 6, rats were injected with one of the 5 treatments and tested with the robot. The procedure was essentially the same as in training except that the center enclosure was removed exposing the robot and the robot was

activated. For each trial the rat was placed in the apparatus as in training and the robot was immediately activated in its walk cycle. The robot then remained activated throughout the 1-min trial, alternating between walking and rotation cycles. At no time during testing did any of the rats come in physical contact with the robot.

Measures

Four measures were taken on the last day of training and on test day. Feeding time was also taken throughout training trials.

Feeding was the cumulative time during the 1-min trial that the rat spent feeding from any one or all of the food cups near the robot.

Center time was the cumulative time the rat spent with its two front feet within the concentric circle nearest the enclosure, 30 cm from the edge of the enclosure.

Freezing was the cumulative time the animal spent rigidly immobile. The experimenter was trained to record freezing using video tapes; as previously reported [18], freezing is a highly stereotyped response that can be scored with considerable interobserver reliability.

Lines was the total number of lines crossed or recrossed, irrespective of proximity to the robot. This measure was included to provide an indication of the drug's effects on motor function and general activity.

Results

Defense reactions. The data from Experiment 1 are summarized in Fig. 1. As the figure shows, the dose-response curves for defensive reactions to the robot were essentially linear. Increasing doses of physostigmine produced increasing defense reactions: more freezing, greater suppression of center time and greater suppression of feeding. Trend analyses were performed across the five treatments, using a correction for unequal intervals between doses as described by Winer [24] and Robson [21]. These analyses confirmed the impression of linearity. For freezing a significant linear trend accounted for 98.89% of the variance, $F(1,64)=32.15$, $p<0.001$. For center time a significant linear trend accounted for 87.0% of the variance, $F(1,64)=13.64$, $p<0.001$; and for feeding a significant linear trend accounted for 85.95% of the variance, $F(1,64)=8.67$, $p<0.005$.

Analyses of variance performed on these data showed a significant effect of drug treatment on all three measures, freezing, $F(4,64)=8.13$, $p<0.005$; center time, $F(4,64)=4.04$, $p<0.01$; and feeding, $F(4,64)=2.52$, $p<0.05$. In Newman Keuls' comparisons of individual means the 0.2 mg/kg dose of physostigmine differed from saline on all three measures: freezing, feeding, and center time ($p<0.05$). This dose also differed from all other doses on freezing and center time ($p<0.05$). No other individual comparisons were significant.

Activity. The data for activity, number of lines crossed, are also shown in Fig. 1. In contrast to defensive reactions, which varied in a linear fashion with dose, the number of lines crossed was virtually the same for all four doses of physostigmine. There was no significant trend in these data and no significant effect of drug treatment in the analysis of variance. It seems clear from these data that the dose-response curves for defensive reactions could not be attributed to either dose-related activation or suppression of activity.

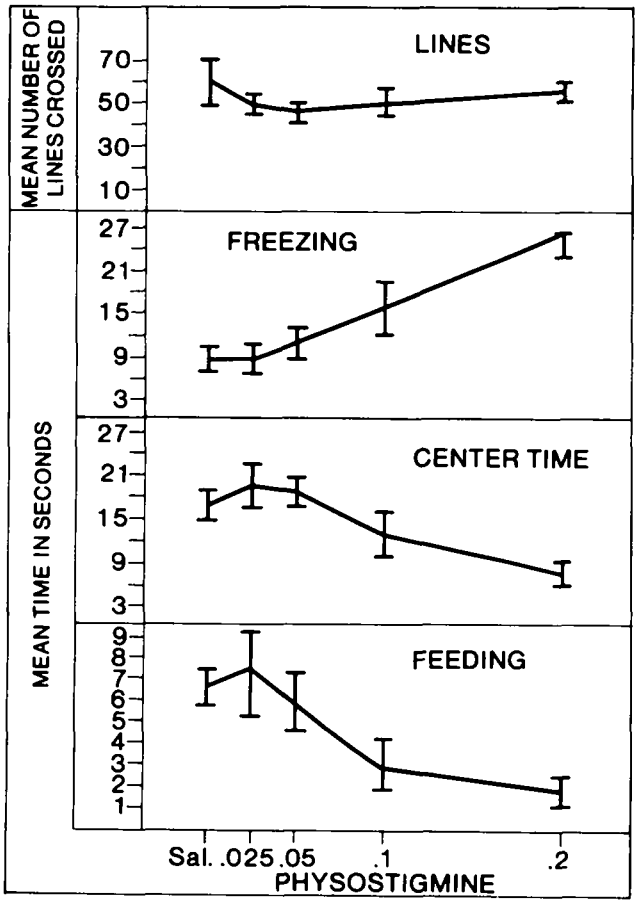

FIG. 1. Mean Freezing, Center, and Feeding times in seconds (± SEM) and mean number of lines crossed (± SEM) as a function of drug treatment during ROBOT test.

EXPERIMENT 2

In Experiment 1, the defensive reactions of rats were found to vary in a linear fashion with increasing doses of physostigmine. The higher the dose of physostigmine the more freezing and the greater the suppression of feeding and center time. In Experiment 1 all animals were tested in a situation (robot condition) designed to elicit defensive responding. Experiment 2 was designed to determine whether the effects of physostigmine were specific to defensive responding.

In this experiment physostigmine's effects on defensive responding were studied in robot and no-robot conditions. Rats were treated either with saline or the dose of physostigmine (0.2 mg/kg) that had differed from saline in Experiment 1. If the effects of physostigmine observed in Experiment 1 were specific to defense responding then drug treatment and test condition would be expected to interact. Thus, physostigmine would affect behavior differently in a situation designed to elicit defense responses (robot) than in a situation not designed to elicit defense responses (no robot).

METHOD

Animals

The animals were 62 male hooded Long Evans rats, weighing 250–350 g at the start of the experiment, purchased from Simonson Laboratories. Eight rats were discarded for failure to meet training criteria, described in Experiment 1, and one rat was discarded after being struck by the robot during testing. Rats were housed, habituated, and fed in the same manner as in Experiment 1.

Apparatus

The apparatus used in this experiment was the same as that used in Experiment 1.

Stimulus Robot

The stimulus robot used in this experiment was constructed for research purposes and was modeled after the commercially-made robot used in Experiment 1. It was constructed from sheet metal and was electrically operated. The robot was suspended from a tripod, such that it hung approximately 1 cm above the wire mesh floor.

The robot alternated between walking and turning cycles. During the walk cycle, the legs moved two stroks per second, approximately 2 cm per stroke for 2.5 sec. During the spin cycle, the lower section of the robot turned at a rate of three revolutions per second for approximately 4.5 sec. The robot was activated in its walking cycle at the beginning of the test trial and remained on throughout the trial. While the robot was activated, it made a loud grinding noise; during the walk cycle, the noise level was 83 db, rising to 94 db during the rotation cycle.

As in Experiment 1, the stimulus parameters of the robot were adjusted in pilot work to produce appropriate baseline defense responding.

Design

Rats were assigned to one of two drug conditions, either saline or 0.2 mg/kg physostigmine, and tested under one of two conditions (ROBOT or NO ROBOT). Rats were randomly assigned to these 4 groups with the same procedures and restrictions as described in Experiment 1.

Procedure

The training procedures for Experiment 2 were the same as those used in Experiment 1, except that 2 additional day of training were administered in order to maximize the homogeneity of feeding times among animals. Training criteria were still implemented on Days 4 and 5.

On the test day, procedures were essentially the same as in training for rats tested in the NO ROBOT condition. For rats tested in the ROBOT condition the enclosure was removed exposing the robot, which was immediately activated beginning in a walk cycle.

The same measures were recorded as in Experiment 1.

Results

Defensive reactions. The data from Experiment 2 are summarized in Fig. 2. A reciprocal transformation was performed on the data for feeding and center time to correct for heterogeneity of variance [24]. These means are plotted as

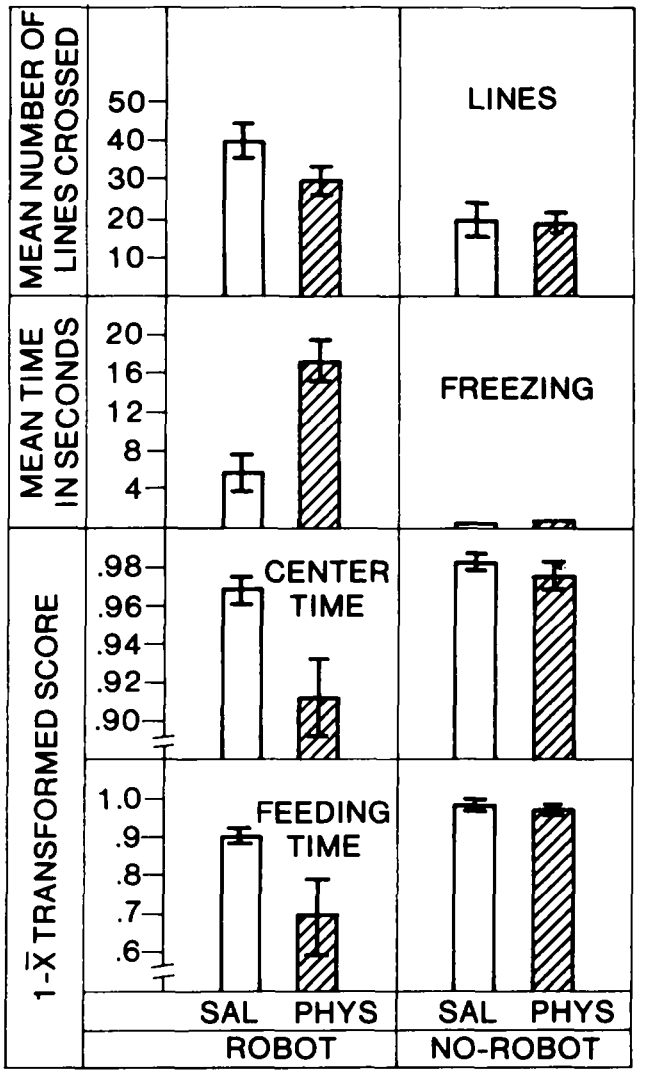

FIG. 2. Mean Freezing, Center, and Feeding times (± SEM) and mean number of lines crossed (± SEM) in ROBOT and NO ROBOT conditions. Freezing time is plotted in seconds; Center and Feeding times are plotted as 1 − the mean reciprocal score in order to reflect the direction of effect; i.e., low scores indicate low times and high scores indicate high times.

1–$\bar{X}$ transformed scores in order to show the direction of the effect.

As Fig. 2 shows, 0.2 mg/kg of physostigmine had virtually no effect on defense response or activity in the NO ROBOT condition. However, in the ROBOT condition, the drug caused an increase in defense responding: greater suppression of feeding and center time and increased freezing. These data were analyzed using unweighted means analyses of variance [24]. The main effect for test condition (ROBOT-NO ROBOT) was significant for freezing, $F(1,49)=30.27$, $p<0.001$; center time, $F(1,49)=10.0$, $p<0.01$; and feeding, $F(1,49)=11.67$, $p<0.01$. The main effect for drug condition (physostigmine vs saline) was also significant for freezing, $F(1,49)=8.11$, $p<0.01$; center time, $F(1,49)=5.38$, $p<0.05$;

and feeding, $F(1,49)=6.25$, $p<0.05$. The fact that the drug affected defense responding in the ROBOT condition but not the NO ROBOT condition was reflected in significant interactions for freezing, $F(1,49)=7.19$, $p<0.01$; center time, $F(1,49)=6.74$, $p<0.05$; and feeding, $F(1,49)=4.58$, $p<0.05$. Newman Keuls' comparisons of individual means showed that physostigmine and saline rats differed significantly on freezing, center time and feeding in the ROBOT condition ($p<0.05$), but did not differ significantly on any measure in the NO ROBOT condition. All ROBOT–NO ROBOT comparisons were significant.

Activity. The data for activity, number of lines crossed, are also shown in Fig. 2. In contrast to the results for defensive reactions, physostigmine and saline did not appear to have different effects on activity in the two conditions. Instead, all animals, including those treated with physostigmine, showed an increase in activity in the ROBOT condition. These impressions are confirmed by the ANOVA in these data. The main effect of test condition was significant, $F(1,49)=16.65$, $p<0.001$, but the main effect of drug and the interaction terms were not. Newman Keuls' comparisons of individual means confirmed that the increased activity in the ROBOT condition was shown by both physostigmine and saline animals ($p<0.05$). No other comparisons were significant. These data on activity rule out the possibility that increased defensive reactions resulted from different effects on activity.

GENERAL DISCUSSION

In the present experiments, the anticholinesterase, physostigmine caused an increase in the defensive reactions of rats. Physostigmine increased freezing and caused greater suppression of time feeding and time near the aversive stimulus (robot). The dose-response curves for these effects were linear, the higher the dose, the greater the effect on defense responses. The fact that the curves were linear ruled out the possibility that the high doses of physostigmine were causing a blockade. The effects of physostigmine were also situation-specific. That is, physostigmine caused an increase in defensive reactions in the presence of the robot, but had no effect when animals were tested in the absence of the robot. Thus, the actions of physostigmine in this instance, appeared to be specific to a situation that elicited defensive responding. Whether the effects of physostigmine would be the same for other threatening stimuli, such as a cat, can not be answered from the present data. However, previous research had shown that a mechanical robot not only elicited the same pattern of defense reactions as the live cat [17]; it also resulted in the same pattern of effects from drugs [17] and brain lesions [16]. Thus, there is some basis for expecting the present results to generalize to other defense-appropriate situations.

The results of physostigmine treatment were the opposite of results previously obtained with the anticholinergic drug, scopolamine [15, 17, 18]. Scopolamine was shown to reduce the defensive reactions of rats to a natural predator, cat [18], and to a mechanical robot [17]. Rats treated with scopolamine showed less freezing, less suppression of feeding and less suppression of time near the stimulus, and methyl scopolamine, the peripheral counterpart of scopolamine, had virtually no effect on these defensive reactions [18]. These earlier results were taken as evidence of central cholinergic involvement in the mediation of defensive reactions of the rat. The fact that physostigmine produced exactly the opposite pattern of results lends strong support to the view that cholinergic synapses are involved in the mediation of defensive reactions.

Anticholinesterases, such as physostigmine, have been shown to have a depressant effect on behavior in a wide variety of situations [1]. Physostigmine had also been shown to increase general activity at low doses and have the opposite effect at high doses [8]. Thus, an important question for the present research is whether the effects of physostigmine on defense reactions could be attributed to a general suppression of behavior or to changes in activity. Several factors argue against this interpretation. In both of the present experiments, physostigmine failed to have any significant effect on activity as indicated by number of lines crossed. In Experiment 1, dose-response curves for defensive reactions showed a strongly linear increase with increasing dose, while the dose-response curve for activity was essentially flat, with number of lines crossed being about the same for all doses of physostigmine. In Experiment 2 in the NO ROBOT condition, physostigmine- and saline-treated rats showed virtually identical levels of activity. Thus, physostigmine did not cause any change in general activity in the non-threat condition. In the ROBOT condition, physostigmine-treated rats showed somewhat more variability in activity, but again, the mean number of lines crossed was almost the same as for saline-treated rats. Not only did physostigmine fail to produce either a suppression or elevation of general activity, but it did not significantly alter the stimulus-induced changes in activity. Thus, the data on activity indicate that the effects of physostigmine on defensive reactions could not be attributed to changes in general activity level.

Also arguing against an explanation based on general behavior depression is the fact that physostigmine's effects on defensive responding were situation-specific. In the NO ROBOT condition the feeding times and center times of physostigmine-treated rats were indistinguishable from those of saline animals. Thus, the suppression of feeding and center times occurred only in the context of defensive responding. Nor did the physostigmine animals show freezing in the NO ROBOT condition. Freezing occurred only in the ROBOT condition, where it was potentiated by physostigmine treatment. Thus, physostigmine did not cause a suppression of behaviors except in the context of defensive responding.

The present results take on added importance in view of the fact that there is a large literature relating cholinergic activity and avoidance behavior. It is well established that anticholinergic drugs impair passive avoidance [4, 10, 14] and enhance two-way active avoidance [11,20]. Drugs that increase cholinergic activity have had the opposite effect [6,22]. Carlton [5] proposed a theory that would explain this literature in terms of cholinergic mediation of behavioral suppression; in this case, suppression refers to an induced or stimulus-elicited condition as opposed to overall, nonspecific, suppression. He postulated that shock induces behavioral suppression and that anticholinergic drugs have their effect by reducing this suppression. Drugs that increase cholinergic activity would be expected to have the opposite effect and, thus, potentiate induced behavioral suppression. The results of the present research and earlier work using an anticholinergic drug [17] are generally compatible with Carlton's view. However, an interpretation in terms of innate species-specific defense responses [3] would seem to enjoy a number of advantages over the behavior suppression view.

Considering behavior in the context of species-specific defense responses [3], it would not be accurate to characterize freezing simply as behavioral suppression or lack of activity. When the rat is freezing, whether in response to a robot, cat or electric shock, it is not merely inactive; it is rigidly immobile. From the experimenter's perspective this animal may seem to show a behavior suppression since it is not performing the conditioned responses demanded by the particular paradigm. However, from a defense-response perspective, the animal's behavior has become restricted to a species-specific defense response, the performance of which precludes other behaviors [3]. Viewed in this way, the effect of physostigmine is not to suppress behavior but, rather, to increase or potentiate the innate defense response of freezing. This perspective has the advantage that freezing is an observable, measurable phenomenon. Thus, the effects of cholinergic and anticholinergic drugs on avoidance behavior can be explained by their actions on the observable defense response of freezing. Decreased freezing caused by an anticholinergic drug would account for impaired passive avoidance and enhanced active avoidance; increased freezing caused by a cholinomimetic drug would account for the opposite pattern of results.

The fact that the present paradigm employs an unconditioned, nonpainful stimulus also offers several advantages for the possible explanation of avoidance literature. Most attempts to explain cholinergic mediation of avoidance behavior are complicated by two factors, the question of learning/memory and the question of pain sensitivity. Cholinergic activity, and physostigmine in particular, has been shown to be involved in both the storage and retrieval of memory [7, 9, 19]. Since avoidance conditioning involves a learned response, it is difficult to disentangle the effects on emotional or defensive responses from possible effects on learning/memory. With the present paradigm the results of physostigmine and scopolamine [18] could not be attributed to alterations in learning/memory. A second difficulty in evaluating the role of cholinergic activity in avoidance behavior is the question of aversive threshold. Anticholinesterases, including physostigmine, have been shown to induce a centrally-mediated analgesia [12]. The relationship between anticholinergic drugs and shock sensitivity is less clear. It has been reported that anticholinergic drugs do not affect shock sensitivity [23]. However, anticholinergic drugs do antagonize the analgesia induced by a cholinomimetic [12]. Hence, some involvement in aversive threshold can not be ruled out. The fact that cholinergic and anticholinergic drugs may actually alter sensitivity to shock makes it difficult to evaluate drug effects on emotional or defensive responding. The present paradigm avoids this problem by using an aversive stimulus that does not involve physical contact. With the present paradigm, it has been possible to conclude that cholinergic and anticholinergic drugs affect the defensive reactions of the rat, apart from any possible effects on aversive threshold.

In summary, previous research had shown that the anticholinergic drug, scopolamine, caused a decrease in the innate defensive reactions of rats to a live cat [18] or mechanical robot [17]. In the present research, increased cholinergic activity had just the opposite effect. It caused an increase in the defensive reactions of freezing, suppression of feeding and suppression of time near the stimulus robot. The effects of the drug were specific to the threat situation and, hence, could not be attributed to either general suppression or activation. The actions of cholinergic and anticholinergic drugs on the species-specific defense response of freezing can explain many of the effects of these drugs on avoidance conditioning.

REFERENCES

1. Bignami, G. and G. L. Gatti. Neurotoxicity of anticholinesterase agents. Antagonistic action of various centrally acting drugs. In: *Neurotoxicity of Drugs*. Amsterdam: Excerpta Medica Foundation, 1967, pp. 93–123.
2. Blanchard, R. J. and D. C. Blanchard. Effects of hippocampal lesions on the rat's reaction to a cat. *J. comp. physiol. Psychol.* **78:** 77–82, 1972.
3. Bolles, R. C. Species-specific defense reactions and avoidance learning. *Psychol. Rev.* **77:** 32–48, 1970.
4. Calhoun, W. H. and A. A. Smith. Effects of scopolamine on acquisition of passive avoidance. *Psychopharmacologia* **13:** 201–209, 1968.
5. Carlton, P. L. Brain acetylcholine and inhibition. In: *Reinforcement and Behavior*, edited by J. T. Tapp. New York: Academic Press, 1969.
6. Cox, T. The effects of physostigmine during the acquisition of avoidance behavior as a function of intersession interval. *Q. J. exp. Psychol.* **26:** 387–394, 1974.
7. Davis, K. L., R. C. Mohs, J. R. Tinklenberg, A. Pfefferbaum, L. E. Hollister and B. S. Kopell. Physostigmine: Improvement of long-term memory processes in normal humans. *Science* **201:** 272–274, 1978.
8. Egbe, P. and S. R. Wray. Differential attenuation by atropine and d-amphetamine on hyperactivity: Possible clinical implications. *Psychopharmacologia* **54:** 25–30, 1977.
9. Ellison, G., M. S. Eison, H. S. Huberman and F. Daniel. Human serial learning: Enhancement with arecholine and ocholine and impairment with scopolamine. *Science* **201:** 274–278, 1978.
10. Gruber, R. P., G. C. Stone and D. R. Reed. Scopolamine-induced anterograde amnesia. *Int. J. Neuropharmac.* **6:** 187–190, 1967.
11. Hamilton, L. W. and S. P. Grossman. Behavioral changes following disruption of central cholinergic pathways. *J. comp. physiol. Psychol.* **69:** 76–82, 1969.
12. Houser, V. P. and D. A. Van Hart. Modulation of cholinergic activity and the aversive threshold in the rat. *Pharmac. Biochem. Behav.* **2:** 631–637, 1974.
13. McLennan, H. *Synaptic Transmission*. Philadelphia: W. B. Saunders, 1963.
14. Meyers, B., K. H. Roberts, R. H. Riciputi and E. F. Domino. Some effects of muscarinic cholinergic blockers on behavior and the electrocorticogram. *Psychopharmacologia* **5:** 289–300, 1964.
15. Mollenauer, S. O., R. Plotnik and E. F. Snyder. Scopolamine effects dependent upon the pretreatment level of emotionality in the rat. *Pharmac. Biochem. Behav.* **1:** 509–514, 1973.
16. Mollenauer, S., R. Plotnik and E. F. Snyder. Effects of olfactory bulb removal on fear responses and passive avoidance in the rat. *Physiol. Behav.* **12:** 141–144, 1974.
17. Mollenauer, S. O., R. Plotnik and P. Southwick. Scopolamine: Effects on fear or defense response in the rat. *Pharmac. Biochem. Behav.* **5:** 157–163, 1976.
18. Plotnik, R., S. O. Mollenauer and E. F. Snyder. Fear reduction in the rat following central cholinergic blockade. *J. comp. physiol. Psychol.* **86:** 1074–1082, 1974.

19. Puerto, A., F. Molina, J. Rogers and D. E. Moss. Physostigmine-induced amnesia for an escape response 12 to 72 hours after training. *Behav. Biol.* **16:** 85–90, 1976.
20. Rech, R. H. Effects of cholinergic drugs on poor performance of rats in a shuttle box. *Psychopharmacologia* **12:** 371–383, 1968.
21. Robson, D. S. A simple method for constructing orthogonal polynomials when the independent variable is unequally spaced. *Biometrics* **15:** 187–191, 1959.
22. Rosic, N. and G. Bignami. Depression of two-way avoidance learning and enhancement of passive avoidance learning by small doses of physostigmine. *Neuropharmacology* **9:** 311–316, 1970.
23. Smith, R. F. Scopolamine does not affect footshock sensitivity in the rat. *Pharmac. Biochem. Behav.* **8:** 31–34, 1978.
24. Winer, B. J. *Statistical Principles in Experimental Design* (2nd edition). New York: McGraw-Hill, 1971.