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Scopolamine Interacts with Reactions to Cat and Genotypic Emotional Reactivity in Rat[1]

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SATINDER, K. P. AND P. M. VALLIANT. *Scopolamine interacts with reactions to cat and genotypic emotional reactivity in rat.* PHARMAC. BIOCHEM. BEHAV. 9(4) 421–424, 1978.—In the presence or absence of a cat the effects of scopolamine hydrobromide were investigated on approach, freezing and defecation behaviors of the bidirectionally selectively bred rat strains for emotional reactivity. Compared to the baseline behavior (absence of cat and scopolamine), both the genetic lines showed decrease in approach and increase in freezing in the presence of cat. Scopolamine in the absence of cat did not affect any of the behaviors of the nonreactive strain but increased approach and decreased freezing in the reactive strain. The presence of both cat and scopolamine affected both the strains but differently, i.e., showing no significant change in the nonreactive, but increase in freezing and decrease in approach in the reactive strain, as compared to the respective baselines.

Scopolamine and emotional reactivity Reactions of rats to a cat Behavioral suppression and scopolamine

INTUITIVELY, an exposure to a potential predator is a strong stimulus and obviously a stressful situation for an organism. It is known that strong and stressful stimuli lead to a suppression of behavior, and the suppression so engendered is disrupted by anticholinergics [2]. Previous research [5,7] shows that rats differing in susceptibility to stress situation, i.e., emotional reactivity, respond differentially when confronted with a cat. Furthermore, scopolamine, an anticholinergic drug, has been shown to interact with the emotional reactivity of the rats and their reactions to a cat [5]. Unfortunately, the findings pertaining to the effects of scopolamine have been confounded by (a) differing amounts of training given to differentiate high and low emotional rats, and (b) the use of drastically unequal numbers of animals for high and low emotionality groups. These limitations are likely to make the reliability of the findings questionable. The best strategy would be to use animals selectively bred for different levels of emotional reactivity, which assures consistent and reliable differences between the groups.

Theoretical significance of this research lies in enhancing the understanding of the (a) relationship between anticholinergic drugs and avoidance behavior as referred to previously [6], (b) interaction between emotional reactivity and avoidance learning (Satinder, under preparation) and (c) more specifically the drug effects as they interact with the genetically based differences in emotional reactivity and responses to a natural predator. Hence, the purpose of present research was to investigate the interaction among scopolamine, reactions to a cat and levels of emotional reactivity by using bidirectionally selectively bred rat strains.

METHOD

Animals

The animals were 48 experimentally naive rats, 24 each from two genetic lines (MNR/Har/Lu, MR/Har/Lu) and equally represented by both the sexes. The MNR/Har/Lu and MR/Har/Lu strains have been subject to genetic selection for low and high open-field emotional reactivity, respectively. The animals were bred and reared in the laboratory, weaned at 28 days, and were 100 days of age at the start of the experiment. Before experimentation the animals were housed in same-sex pairs, with the strains on separate cage racks. During experimentation the animals were coded and housed individually to ensure that the experimenter did not know the strain of the animals. The laboratory temperature was thermostatically controlled at 22 + 1°C, and the humidity level was maintained at 40%. Fluorescent lights were on a 12 hr light-darkness cycle.

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Experimental Design

The experiment was replicated twice, once with a black cat and once with a white cat (both males, 7–8 months of age). The design for each replication was a 2 (strain)×2 (sex)×2 (cat–no cat) complete factorial with 3 animals in each factorial cell. The stimulus in the open-field arena was either an empty wire mesh cage or a cat in the same cage.

Two levels of scopolamine hydrobromide (1, 4 mg/kg) and a placebo (physiological saline) were administered IP in a volume of 2 ml/kg, approximately at the same time of the day, 30 min before testing. The order of dosages was assigned at random, and within each dosage 8 animals in each replication (for cat present and cat absent a female and a male from each of 2 strains) started their drug schedule and rotated through the remaining dosages in the order assigned. A double blind procedure was used in testing the animals. The same animals were injected and tested under the effects of 1 of the dosages on 3 successive days. No drug was administered on the last and fourth day of testing.

In order to prevent contamination of the control (absence of cat) condition with cat smell the control animals were tested first every day. At the end of each testing day, arena, stimulus cage and experimental room were thoroughly cleaned to delete any cat odor before next day's testing began.

Apparatus

An open-field arena, 90 cm on each side and divided into 16 equal-area square sections marked on the floor, was used. The arena was lighted by four 90-cm-long fluorescent lights, 90 cm above the floor level, which provided an illumination of 230 ftc at the floor center of the arena. A white noise generator set at a level of 65 dB was used to mask external sources of noise (sound intensity was measured at floor level above the standard reference level of .0002 μbar by a General Radio sound level meter, Type 1551-C).

The enclosure used to place the cat in the open-field arena was a wire mesh cage measuring 38×25×20 cm.

Procedure

Both the cats were adapted, through several 1–2 hour familiarization sessions, to sit inactively in the wire mesh cage which was placed in the corner facing the center of the arena. They were also given several habituation sessions with other rats before they encountered the experimental rats. The cat made some noise mainly in shifting positions, but the small size of the cage restricted any gross movements. Since drug dosages were randomized, there was no relationship between the cat noise and dosage effect.

Animals were placed individually into the open-field arena under a Plexiglas container facing and in the vicinity of the wire mesh cage which contained either the black cat, or the white cat, or no cat. All animals were exposed in exactly the same manner and position. As the rat was released from the Plexiglas container, the experimenter activated a timer. The lights and white noise were also activated simultaneously. Each trial lasted 5 min. The open-field arena was cleaned with a disinfectant solution after every animals' testing session.

A number of measures were recorded, in accordance with the procedures developed in a previous investigation [7], in daily trials for 4 days. Approach behavior towards the cage (either cat present or absent) was defined and scored as follows: *approach* (assigned value 1), the animal moved towards the cage within 3 cm without touching it; *touch* (assigned value 2), the animal approached and touched the cage. The total values of all *approaches* and *touches* constituted the approach behavior score. The number of defecations was also recorded. The amount of time (min) the animal did not move in the arena was recorded as freezing behavior.

RESULTS

Preliminary analysis of the results showed that there were no significant differences between the 2 replications involving the black and the white cat. Hence data for the 2 replications were pooled for further analysis. The results reported were evaluated by a 2 (strain)×2 (sex)×2 (cat present or absent)×4 (test sessions) mixed analysis of variance and followed by t-tests for correlated pairs.

The means of approach behavior scores of both the genetic lines and experimental groups (cat present and cat absent) are presented in Fig. 1 according to the test sessions. In general, the reactive (MR) line scored lower on approach behavior than the nonreaction (MNR) line, $F(1,40)=17.9$, $p<0.001$, and the cat-absent group scored higher on approach behavior than the cat-present group ($p<0.003$). The ω^2 values (top of Fig. 1), reflecting the magnitude of between-strain variation, vary drastically depending upon the experimental condition. For example, under the effects of scopolamine the difference between the genetic lines disappeared in the absence of cat and amplified in the presence of cat. There was a significant interaction among strains, experimental groups (cat–no cat) and dosage levels, $F(2,80)=5.6$, $p<0.006$.

The approach behavior score of the MNR line in the absence of cat did not change significantly over 4 test sessions, however, in the presence of cat the score increased significantly (t—ratio=3.1, df=11, $p<0.009$) under the effects of 1 mg/kg scopolamine as compared to 0 mg/kg dose. In the last test session (no injection) the approach behavior score was very close to the score under 0 mg/kg dose. The approach behavior score of the MR line in the absence of cat increased significantly ($p<0.006$) under 1 mg/kg as compared to 0 mg/kg dose. The score during the last session did not return to the level of 0 mg/kg ($p<0.02$), rather remained at the level of 1 mg/kg dose. The administration of drug did not increase the score of the MR animals in the presence of cat as it happened in the MNR line. In the MNR line the differences between the cat absent and cat present groups were not significant in any of the 4 test sessions. But in the MR line the scores were significantly lower ($p<0.01$) in the cat present group as compared to the cat absent group in all the four sessions. It is interesting to observe (Fig. 1) that the effects of scopolamine decreased differences between the groups in the MNR and increased in the MR genetic line.

Means of freezing time of both the genetic lines and experimental groups are presented in Fig. 2 according to the test sessions. The MR strain showed significantly ($p<0.01$) more freezing behavior than the MNR line and the cat group more than the no-cat group ($p<0.001$). As the ω^2 values indicate, there were hardly any differences between the lines in freezing time in the absence of cat. However, in the presence of a cat and drug the differences between the strains became significant (the ω^2 value of 0.22 yields a F value to be significant at $p<0.01$ level).

The MNR animals in the absence of cat showed no significant differences in freezing behavior under the effects of

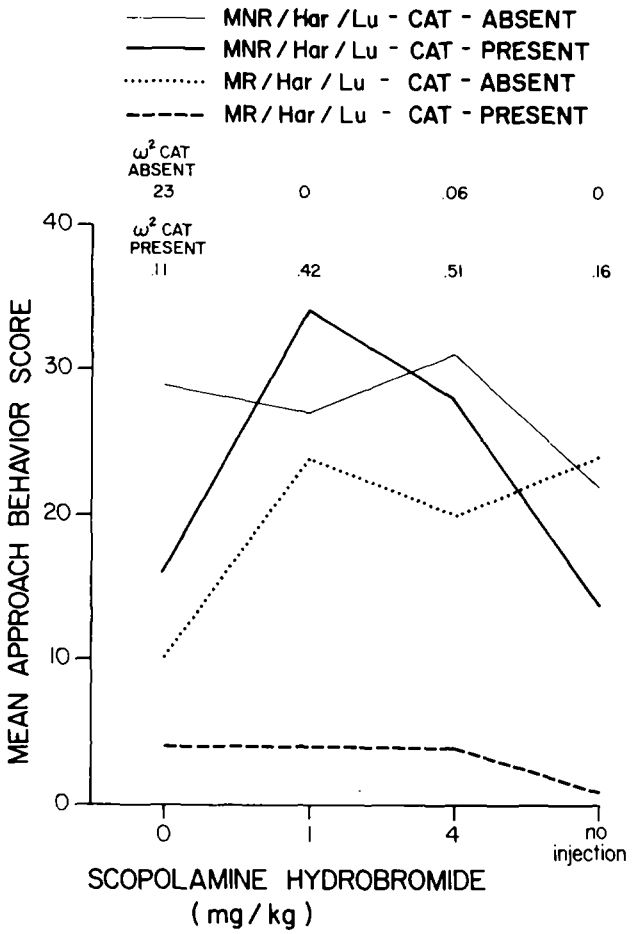

FIG. 1. Means of approach behavior scores of MNR (Nonreactive) and MR (Reactive) genetic lines in the presence or absence of cat under the effects of scopolamine hydrobromide.

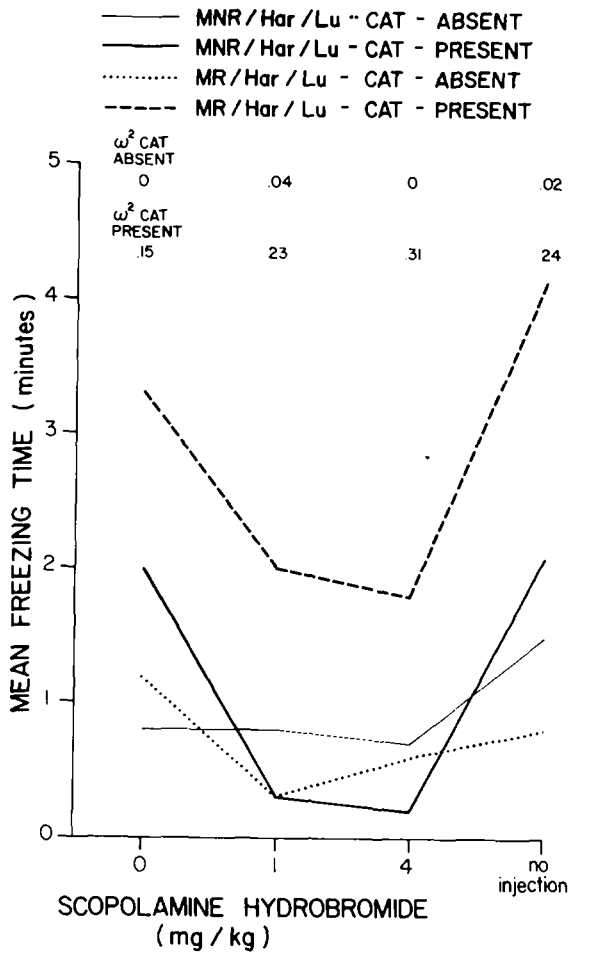

FIG. 2. Means of freezing time of the MNR (Nonreactive) and MR (Reactive) genetic lines in the presence or absence of cat under the effects of scopolamine hydrobromide.

scopolamine, however, in the presence of cat there was a significant reduction ($p<0.003$) in freezing under the effects of scopolamine as compared to no drug sessions. In the MR line there was a reduction in the freezing time under the effects of the drug both when a cat was present and absent but these differences were not significant. The difference in freezing time between the cat present and cat absent groups were not significant in the MNR line in any of the sessions, but the corresponding difference in the MR line were significant ($p<0.01$) except 4 mg/kg dose.

The MR line defecated significantly more ($p<0.001$) than the MNR line. The presence or absence of cat did not affect defecation in either of the strains significantly, however, both MNR ($p>0.1$) and MR ($p<0.003$) lines defecated less under the effects of scopolamine which deleted significant differences between the lines. The differences between the genetic lines were significant ($p<0.001$) both under 0 mg/kg does and no injection condition. The lower defecation score under the drug dosages may be due to the inhibitory effects of anticholinergics on the motor activity of duodenum, jejunum, ileum and colon [4].

There were no significant difference between the effects of 1 and 4 mg/kg dosages of scopolamine in any of the behavioral measures, or genetic lines, or experimental groups or sexes. In the MNR line there was an interaction between drug dosages and experimental groups (cat–no cat) in both freezing, $F(2,40)=4.7$, $p<0.02$, and approach behavior ($p<0.03$).

DISCUSSION

It is evident from Figs. 1 and 2 that the mere presence of cat (scores under 0 mg/kg) produced lower approach behavior score and increased freezing in both the genetic lines as compared to the baseline scores (scores of cat-absent groups under 0 mg/kg) of the respective strains. This suggests that the presence of a cat is effective in increasing behavioral suppression in rats irrespective of the differences in the genetic background of animals. This finding supports previous results [7] in which 5 genetic lines reacted similarly to the presence of a cat on a wide variety of behaviors.

The effects of scopolamine in the absence of cat producing no significant change in the behavior of MNR, increase in approach and decrease in freezing in the MR animals as compared to the corresponding baseline scores, would indicate genotype specific effects of scopolamine. The fact that the effects of scopolamine in the presence of cat produced

diagonally opposed changes between the genetic lines as compared to the respective baseline behaviors would suggest an interaction among scopolamine, genotype and reactions to a cat and in fact it was statistically significant for approach behavior score.

The fact that scopolamine did not disrupt the behavioral suppression produced by the presence of cat would suggest that in the MR line the presence of cat had a dominant effect. Whereas in the MNR line there was a significant interaction between the effects of scopolamine and presence or absence of cat.

The present findings extend a partial support to the previous results [5], in which administration of scopolamine in the presence of cat reduced freezing behavior in both high and low emotional animals, as compared to their behavior in the presence of cat without scopolamine. Correspondingly approach behavior increased in the low emotional animals but decreased to some degree in the high emotional animals.

In light of the previous findings referred to above [6] regarding the relationship between anticholinergic drugs and avoidance behavior, the present set up is comparable to the passive avoidance paradigm, which involves withholding responses from approaching aversive stimuli and both approach and freezing behaviors studied in the present research qualify to be considered as passive avoidance responses. Previous research has shown that scopolamine impairs passive avoidance [1,3] and decreases the latency to approach the stimulus [8].

The pattern of effects of scopolamine seen in the present findings must qualify its support to the suggestion that anticholinergics interfere with the animal's tendency to suppress [2]. The qualification being that the effects of scopolamine in the present findings are dependent upon the genetic background of the animals, the behavior under investigation, and possibly the nature and extent of the stress producing suppression of behavior.

In consideration of the genetic background of the animals, it is clear from the results (Figs. 1 and 2) that the nonreactive (MNR) genetic line, which has been selectively bred for low levels of emotional reactivity, shows disinhibiting effects of scopolamine on both approach and freezing behavior only when cat is present in the cage and not when stimulus is only an empty cage. Whereas the reactive (MR) genetic line, selectively bred for high emotional reactivity, shows disinhibiting effects of an anticholingergic drug on both the behaviors investigated, when the stimulus is an empty cage. But when the cat is in the cage the disinhibiting effect is only seen on the freezing and not in approach behavior. The absence of any disinhibiting effects of scopolamine on the approach behavior of MR line in the presence of cat may be construed to indicate that the stress produced by the mere presence of cat is so massive that the present dosages of scopolamine were not effective enough to counteract the suppressed approach behavior of the reactive animals in the presence of cat. It has been previously suggested that "the effects of the anticholinergics is presumably related to the degree of initial stress. It is reasonable to suppose that such stress could be so massive that there would be no effect of the drug at nontoxic doses", ([2] p. 313).

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