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Scopolamine Effects Dependent Upon Pretreatment Level of Emotionality in the Rat[1]

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MOLLENAUER, S., R. PLOTNIK AND E. SNYDER. *Scopolamine effects dependent upon pretreatment level of emotionality in the rat.* PHARMAC. BIOCHEM. BEHAV. 1(5) 509–514, 1973.– Rats were classified low or high emotional (E) depending upon the initiation of consummatory behavior in an open field. High E included those that failed to begin drinking before the fifth training day. By the last day of training, drinking time did not differ for Low and High E; except for a slight, but nonsignificant, depression in activity, High E animals had become behaviorally indistinguishable from Low E. When both groups were confronted with a cat, differences in emotionality reemerged. High E rats showed significantly more behavioral suppression – more freezing and less ambulation. Moreover, pretreatment differences in emotionality resulted in radically different drug effects. For Low E animals, scopolamine (Sco) reduced fear responses: Sco animals showed significantly more approach into the area near the cat and significantly more drinking near the cat than controls. For High E animals, there was no significant difference on either measure between Sco and control. These data indicate that pretreatment level of emotionality is an important predictor for drug effects and that differences in emotionality can be masked but not eliminated by training.

Emotionality Scopolamine effect Drug effects

IN THE study of drug effects on emotional or fear-motivated behaviors, an important source of variance is the animal's pretreatment or initial level of emotionality. Following the work of Hall [7], emotionality has been assessed from responses of the rat in an open field; high emotionality has been attributed to animals that defecate or urinate across many days or show little ambulation or take many trials before eating, or show some combination of these responses. When the pretreatment level of emotionality has been controlled, investigators have found repeatedly that drugs have very different effects depending upon initial emotionality. For example, significant interactions between emotionality and drug effects on avoidance responding have been reported for the Tryon S1 and S3 animals treated with amobarbital [9]. On the basis of open field performance, the Tryon S1 rats were described as high emotional and Tryon S3 rats as low emotional. A moderate dose of amobarbital had very different effects on these two strains, producing an increase of conditioned avoidance responding in the S1 rats, but not the S3 rats. Interactions between initial or pretreatment emotionality and drug effects have also been reported for the Maudsley strain, selectively bred for high defecation (high emotional) or low defecation (low emotional). Broadhurst [1] reported that for the Maudsley strain, high doses of reserpine increased avoidance responding in high emotionals and decreased this response in the low emotionals. More recently, Gupta and Holland [5] reported complex interactions between emotionality and drug effects when Maudsley high and low emotional animals were treated with amphetamine, methylpentynol or their combination.

Even when animals have not been selectively bred for emotionality, initial differences in level of emotionality have been important for predicting drug effects. When Wistar albinos were divided into high and low emotionals on the basis of open field performance (defecation-urination and ambulation), amobarbital facilitated avoidance in high emotionals and decreased avoidance in low emotionals [8].

The anticholinergic drug, scopolamine, has also been shown to have different effects on shuttle-box avoidance of Wistar albinos, depending upon the initial level of avoidance

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responding [3]. Assuming that differences in baseline levels of avoidance responding were related to differences in emotionality [2], these data offer further evidence that drugs affect avoidance behavior differently depending upon initial emotionality. Thus, emotionality has been important whether animals were selectively bred along some measure assumed to measure emotionality or were taken from some strain (mixed) and screened for responses related to high and low emotionality.

The present study was designed to investigate the relationship between emotionality and the effects of an anticholinergic drug on avoidance of a natural predator. It was previously reported that the anticholinergic drug, scopolamine, significantly decreased fear responses in rats confronted with a cat (Plotnik, Mollenauer and Snyder, in press). Compared with controls, rats treated with scopolamine showed significantly less freezing, more approach into the area near the cat and more drinking in close proximity to the cat. The present study asked whether scopolamine would have differential effects on animals' responses to the cat depending on the pretreatment level of emotionality; whether the drug and pretreatment level of emotionality interaction would be found in avoidance situations that did not involve shock as used in previous studies; and whether the interaction would be generalized to another strain of rats such as the Long-Evans hooded.

METHOD

Animals

The animals were 83 male, hooded, Long-Evans rats 300–500 g at start of experiment, purchased from Simonsen Laboratory (Gilroy, Cal.). One animal was found to be completely unmanageable and was discarded prior to the second day of training. From the beginning of the experiment, animals were housed individually with unlimited access to water and maintained on a 23-hr food deprivation schedule. They were fed a fixed amount of food (approximately 12 g) at the same time each day, 30–60 min after the experimental session.

Stimulus Cat

The stimulus cat was a 4.5 kg male house cat. During testing the cat usually sat quietly in its wire mesh enclosure. The animal sometimes shifted positions, but the small size of the enclosure restricted any gross movements. Since drug treatments were randomized, there was no relationship between activity of the cat in the enclosure and drug treatment.

Apparatus

The apparatus was a circular arena, 108 cm in dia. with 31 cm walls and a wire mesh floor. During test sessions with the cat, wall height was increased to 68 cm to prevent rats from jumping out of the arena. A sheet of black plastic was placed under the wire mesh floor; this sheet was marked off in concentric circles 11 cm apart, and each circle was divided spoke-fashion to form a total or 48 equal sigments. In the center of the apparatus was a cylindrical wire mesh enclosure, 22.5 cm in dia. used to restrain the cat during test sessions. Four plastic drinking cups, 2.25 cm in dia., were equally spaced around the enclosure at a distance of 5 cm from the edge of the cat enclosure.

If training trials and exposure-to-the-cat trials were administered on the same day, training trials always preceded exposure trials. Following exposure trials, the apparatus was steam cleaned to remove any cat odor.

Above the arena was a large mirror used to observe the rats. The mirror was used for better visibility and to minimize distraction to the animals.

White noise (db = 70) was present during all trials. In addition, on the cat test trial, recorded cat sounds were played during the trial. This procedure was followed because the cat would infrequently meow during a trial. With the recorded cat sounds, all rats were exposed approximately equally to this cue.

Drug Treatments

There were six drug treatments; three doses of scopolamine hydrobromide (Supplied by Penick and Co., 100 Church St., N.Y., N.Y., 10007) (SCO 1.2, 0.8 and 0.4 mg/kg), two doses of scopolamine methylbromide (METH 1.2 and 0.8 mg/kg) and physiological saline (SAL). All drugs were dissolved in physiological saline and administered intraperitoneally in a volume of 1 ml/kg approximately 30 min before testing. Scopolamine methylbromide mimics all of the peripheral effects of scopolamine but has little, if any, effect centrally [4].

The assignment of animals to these drug treatments was random, with certain restrictions described in *Procedure.* All behavioral tests were conducted blind; one investigator administered drugs and a second investigator, who had no knowledge of which subject received which treatment, tested the animals.

Procedure

The experiment was run in three replications with treatment groups represented proportionately in each group.

Training. On Day 1 of training, rats were placed in the apparatus in groups of two, for periods of 10 min with no reinforcer present. Beginning on Day 2 and thereafter, each animal was given one 5-min trial per day with reinforcement (32% solution of sucrose) available in the four drinking cups. In order to expedite training, those rats that failed to drink on Days 2 and 3 were left in the apparatus overnight on Day 3 with sucrose and water available. On subsequent training days, animals that failed to drink for at least 60 of the 300 sec were given an extra trial. All animals were given a minimum of six training trials to learn drinking. For any animal that had not accumulated 300 sec of drinking time in six trials, training trials were continued until this criterion of 300 sec drinking time was met.

Determination of emotionality. A variety of approaches have been used to assess emotionality or timidity in the rat. The measure used in the present study was trials to initiation of consummatory behavior in an open field. Hall [6] has reported that for male rats, trials to initiation of consummatory behavior had a correlation of 0.96 with days defecating in an open field. Moreover, pilot work had suggested that the effects of drug treatments differed depending on how fast the animal had begun drinking in the arena. This preliminary work showed that animals that failed to drink in the apparatus until the fifth or sixth day of training responded very differently to drug treatment. Accordingly, animals were divided into two groups: those that began drinking before the fifth day of training (n = 48) were designated low emotional (Low E), and those that failed to drink before the fifth day (n = 34) were designated

high emotional (High E).

Assignment to treatments. After animals had been classified high or low emotional, they were assigned to drug treatments. These assignments were random with the following restrictions. In the low emotional group, animals were distributed evenly across the six drug treatments with any extra animals (over a multiple of six) being assigned to the central scopolamine treatments. For the low emotional animals, the n's for each treatment were as follows: 1.2 mg/kg SCO, n = 8; 0.8 mg/kg SCO, n = 10; 0.4 mg/kg SCO, n = 10; 1.2 mg/kg METH, n = 9; 0.8 mg/kg METH, n = 9; SAL, n = 10. Approximately the same assignment procedure was followed for high emotional animals. However, there was some problem with cell size, since the percentage of these animals in the population was rather low (10–20%). Therefore, a slightly higher proportion of high emotional animals was assigned to 1.2 mg/kg SCO, to insure that at least one central scopolamine dosage would have a reasonable cell size. The n's for the high emotional treatment groups were as follows: 1.2 mg/kg SCO, n = 7; 0.8 mg/kg SCO, n = 4; 0.4 mg/kg SCO, n = 4; 1.2 mg/kg METH, n = 3; 0.8 mg/kg METH, n = 4; SAL, n = 4.

Cat test. On the test day, animals were placed individually in the apparatus for a 5-min trial, 30 min after injection. The procedure for testing was exactly the same as on training days. The only difference was that a large male house cat was confined in the wire mesh enclosure in the center of the apparatus.

Measures. On the training day immediately preceding the test day and on the test day itself, four measures were recorded for each animal. (1) Drinking: cumulative time during 5-min trial that animal spent drinking from cups placed near the cat enclosure; (2) Approach: cumulative time animal spent, when not drinking, with two front feet in the concentric circle nearest the cat (farthest point was 23 cm from edge of cat's enclosure); (3) Number of lines: total number of lines crossed or recrossed irrespective of proximity to cat; (4) Freezing: cumulative time animal remained rigidly immobile.

The above measures were not independent. Rather, they represent a constellation of responses that, together, serve to operationally define the level of fear. Thus, high fear would be indicated by suppression of drinking, increased freezing and avoidance of the cat. A low level of fear would be indicated by more drinking, less freezing and more approach to the cat.

RESULTS

Analyses paralleling all analyses on test data, were performed on baseline data to check for sampling error. Comparisons of prospective drug groups revealed no differences even approaching significance.

Comparisons between Low E and High E groups on this last day of training showed that there was no significant difference in drinking time, freezing or approach. The High E group crossed fewer lines than the Low E group; this difference approached significance ($F = 3.76$, $df = 1/78$, $p<0.056$). These data indicate that after sufficient training the high emotional animals had become behaviorally indistinguishable from low emotional animals on every measure except number of lines crossed.

Fear Test.

The two groups receiving methyl scopolamine (METH 1.2 mg/kg and 0.8 mg/kg) did not differ significantly from each other or from the group receiving saline (SAL) on any measure. Therefore, the data from these groups were pooled to form a single control group (CONT) within each level of emotionality. The fact that METH and SAL groups did not differ on any measure indicates that the results described below for scopolamine can not be attributed to peripheral actions of that drug.

The data from the three doses of scopolamine (1.2 mg/kg, 0.8 mg/kg, and 0.4 mg/kg) were also pooled to form one group (SCO) within each level of emotionality. These data were pooled because there were no differences attributable to dose on any measure for High E animals, and dosage differences were observed only on selected measures for Low E animals.

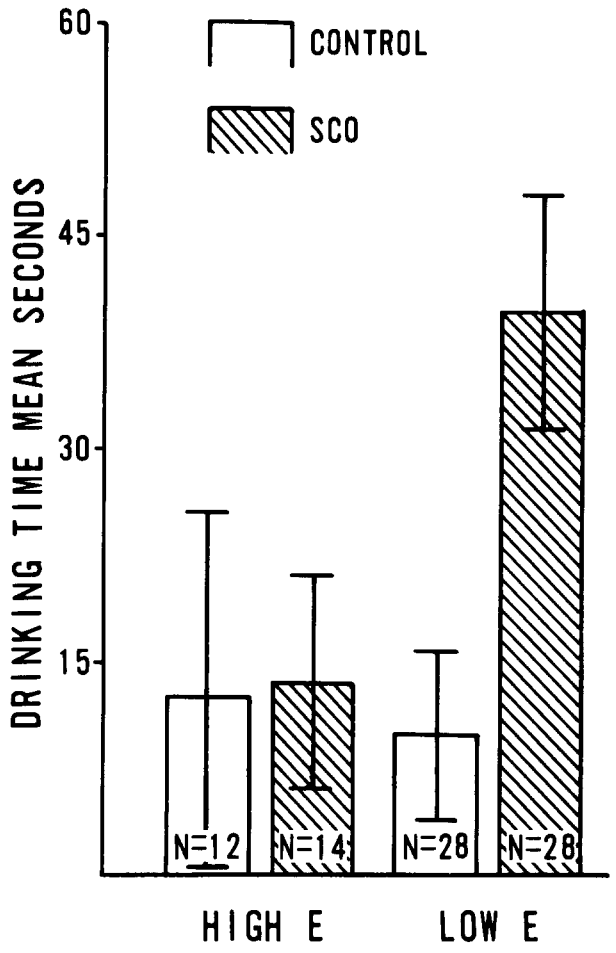

FIG. 1. Mean drinking time ($\pm SE_m$) during cat test. Data show a drug by emotionality interaction: Scopolamine (SCO) increased drinking for Low E, but not High E.

The results of the cat test showed a reemergence of the difference between high emotional and low emotional animals. The data for drinking time (Fig. 1) and approach (Fig. 2) show an interaction between emotionality and drug treatment. Under the drug treatment, Low E animals showed significantly more drinking time ($F = 4.93$, $df = 1/41$, $p<0.05$) and significantly more approach ($F = 6.57$, $df = 1/41$, $p<0.01$) than High E animals. In the control

treatment, there was no difference between High and Low E animals on either drinking ($F<1$) or approach ($F<1$). The interaction between drug treatment and emotionality was significant for approach ($F = 4.05$, $df = 1/78$, $p<0.05$) and approached significance for drinking time ($F = 3.10$, $df = 1/78$, $p<0.08$).

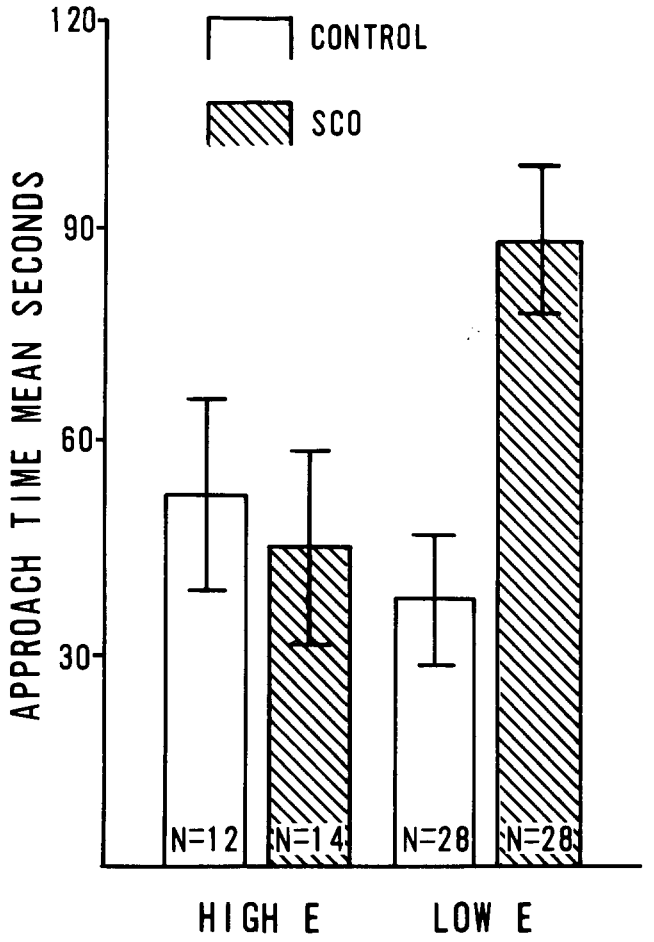

FIG. 2. Mean approach, time rat spent in area nearest the cat while not drinking ($\pm SE_m$). Data show a drug by emotionality interaction: Scopolamine (SCO) increased approach for Low E, but not High E.

An important implication of this interaction was that the drug had very different effects depending upon the initial emotionality of the animals. As Figs. 1 and 2 show, scopolamine attenuated fear reactions for Low E but not High E animals. In the Low E group, animals treated with scopolamine showed significantly more drinking in the presence of the cat ($F = 9.21$, $df = 1/54$, $p<0.005$) and significantly more approach into the area near the cat ($F = 14.15$, $df = 1/54$, $p<0.001$) than animals treated with methylscopolamine or saline. In the High E group, there was no significant difference between animals given scopolamine and those given control treatments for drinking ($F<1$) or approach ($F<1$).

The data for number of lines crossed (Fig. 3) and freezing (Fig. 4) indicate that the presence of the cat produced a greater suppression of behavior in High E animals than Low E. Analysis of the data for number of lines revealed a significant main effect of emotionality: High E animals crossed significantly fewer lines than Low E ($F = 6.76$, $df = 1/78$, $p<0.01$). Analyses of the simple effects showed that this difference between high and low emotional animals was attributable to the control treatment. High E animals given control drugs crossed significantly fewer lines than the Low E animals given control drugs ($F = 8.63$, $df = 1/37$, $p<0.01$). When the comparison was between High E and Low E animals given scopolamine, the difference was in the same direction but was not significant ($F = 1.65$, $df = 1/41$, $p<0.2$).

A greater behavioral suppression for High E animals was also found in freezing (Fig. 4). On freezing time, the main effect of emotionality approached significance with High E animals showing more freezing than Low E ($F = 3.73$, $df = 1/78$, $p<0.058$). Analyses of the simple effects showed that this difference was attributable to animals in the drug groups. In the control treatment, both groups showed a high level of freezing and there was no difference between Low and High E animals ($F<1$), but in the scopolamine treatment Low E animals showed less freezing than High E, with the difference approaching significance ($F = 3.65$, $df = 1/41$, $p<0.06$).

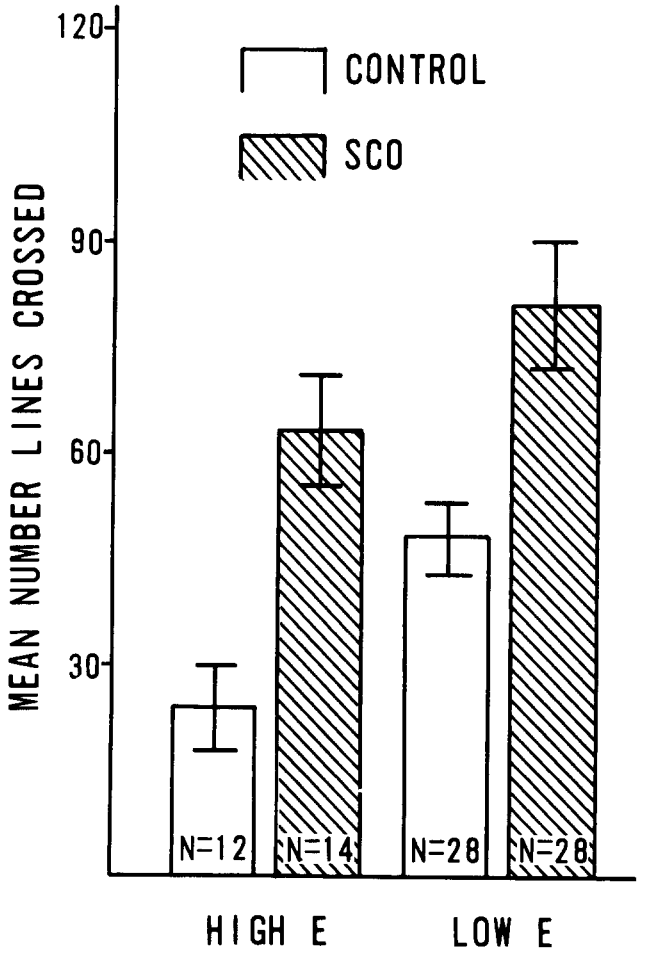

FIG. 3. Mean number of lines crossed ($\pm SE_m$) during cat test. High E animals showed fewer lines crossed than Low E. Scopolamine (SCO) increased lines crossed for both groups.

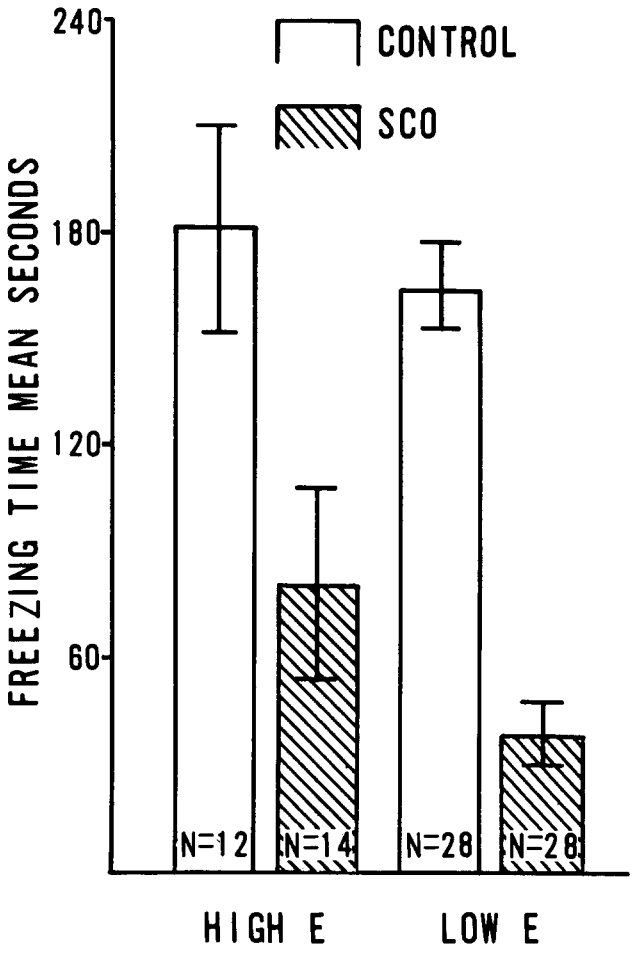

FIG. 4. Mean freezing ($\pm SE_m$) during cat test. High E animals showed more freezing than Low E. Scopolamine (SCO) reduced freezing for both groups.

The data in Figs. 3 and 4 show that scopolamine reduced the degree of behavioral suppression elicited by the cat. Thus, there were significant main effects, showing that animals given scopolamine crossed significantly more lines ($F = 18.17$, $df = 1/78$, $p<0.001$) and spent significantly less time freezing ($F = 36.67$, $df = 1/78$, $p<0.001$) than animals given control treatments. Analyses of simple effects showed that scopolamine reduced freezing for both Low E ($F = 37.06$, $df = 1/54$, $p<0.0001$) and High E ($F = 5.6$, $df = 1/24$, $p<0.05$) animals, and increased the number of lines crossed for both Low E ($F = 10.02$, $df = 1/53$, $p<0.01$) and High E ($F = 15.03$, $df = 1/24$, $p<0.01$).

In most of the comparisons, there were no significant differences attributable to dosage in either main effects or interactions. However, the fact that high emotional animals constitute a smaller percentage of the population resulted in very small cell sizes (e.g., n = 4) for doses within the High E group. Thus, further work will be needed to define the dose response relationship for High E animals. For the Low E animals there was a significant effect of dose on freezing ($F = 5.12$, $df = 2/25$, $p<0.01$) and on approach ($F = 6.88$, $df = 2/25$, $p<0.05$). Subsequent t-tests suggested that, for Low E animals, 0.4 mg/kg SCO was a less effective dose than 0.8 mg/kg on both freezing and approach. The mean freezing under 0.8 mg/kg SCO was 11 sec, and under 0.4 mg/kg SCO was 75 sec ($t = 2.9$, $df = 18$, $p<0.01$). The mean approach under 0.8 mg/kg SCO was 128 sec, and under 0.4 mg/kg SCO was 54 sec ($t = 2.86$, $df = 18$, $p<0.05$). The 0.4 mg/kg dose of SCO was also less effective than the 1.2 mg/kg dose of SCO on freezing: the mean freezing times were 75 sec and 27 sec, respectively ($t = 2.37$, $df = 16$, $p<0.05$). Since there were no dosage differences on any of the other measures, it would be premature to suggest any dose–response relationship.

DISCUSSION

Animals differed markedly in initiation of consummatory behavior in an open field. Some animals began drinking a sucrose solution on the first or second day of training; others required five or six days of training before they began drinking. Following Hall [7], these differences were used to divide animals into high and low emotional groups.

In spite of a significant difference in amount of drinking across training trials, the high and low emotional animals no longer differed by the last day of training, on which baseline data were collected. On this baseline trial, high emotional animals were behaviorally indistinguishable from low emotional animals on all measures except for a slight, but not significant, difference in ambulation. Thus, at this point in training, it appeared that the initial differences in emotionality had been compensated by extra training trials. However, the differences in emotionality reemerged dramatically in the fear test. The high emotional animals showed a greater suppression of behavior; they showed significantly more freezing and less ambulation than low emotional animals.

Even more important, the initial differences in emotionality, which had been masked at the end of training, interacted significantly with drug treatment. Low emotional animals given scopolamine showed a reduction of fear on all measures, but high emotional animals given scopolamine showed only a partial reduction. The Low E rats given scopolamine not only showed significantly less freezing than Low E rats given control drugs, they also showed significantly more approach to the cat and significantly more consummatory behavior in its presence. In marked contrast, the high emotional animals given scopolamine showed a reduction on only the locomotor measures. The differential responses of the High E and Low E animals to the scopolamine treatment resulted in a significant drug by emotionality interaction. These results demonstrate that pretreatment level of emotionality is a variable equal in importance to drug treatment in predicting behavioral outcome.

The interaction between scopolamine and emotionality can be attributed to actions of the drug on the central nervous system. Animals treated with methyl scopolamine, regardless of emotionality, showed the full constellation of fear responses and did not differe from saline controls on any measure. Since methyl scopolamine mimics all of the peripheral actions of scopolamine, it can be assumed that these peripheral actions do not account for the reduction of fear following scopolamine treatment.

The present finding of an interaction between drug and emotionality is in agreement with previous investigators

who have reported that drug effects on avoidance behavior were related to pretreatment emotionality of the animal [1, 8, 9]. In addition, the present data indicate that training may not be able to compensate for differences in pretreatment level of emotionality.

The present study shows further that a drug by emotionality interaction was present in avoidance situations that did not employ shock, but, instead, used a cat. Thus, the drug by emotionality interaction is not unique to a shock escape-avoidance paradigm. Finally, a drug by emotionality interaction has been shown not only for Maudsley [1] and Tryon [9] animals, but also for Wistar albinos [8] and now for Long-Evans hooded rats. This indicates that pretreatment level of emotionality is an important variable in research with commonly used strains of rats.

REFERENCES

1. Broadhurst, P. L. The hereditary base for the action of drugs on animal behaviour. In: *Animal Behavior and Drug Action,* edited by H. Steinberg. Boston: Little Brown, 1964, pp. 224–37.
2. Broadhurst, P. L. and S. Levine. Behavioural consistency in strains of rats selectively bred for emotional elimination. *Br. J. Psychol.* **54:** 121–125, 1963.
3. Carro-Ciampi, G. and G. B. Bignami. Effects of scopolamine on shuttle-box avoidance and go-no go discrimination: Response-stimulus relationships, pretreatment baselines, and repeated exposure to drug. *Psychopharmacologia* **13:** 89–105, 1968.
4. Goodman, L. S. and A. Gilman (Eds.). *The Pharmacological Basis of Therapeutics.* New York: Macmillan Co., 1968.
5. Gupta, B. D. and H. C. Holland. Emotion as a determinant of the effects of drugs and their combination on different components of behavior in rats. *Neuropharmacology* **11:** 31–38, 1972.
6. Hall, C. S. Emotional behavior in the rat: I. Defecation and urination as measures of individual differences in emotionality. *J. comp. Psychol.* **18:** 385–403, 1934.
7. Hall, C. S. Temperament: A survey of animal studies. *Psychol. Bull.* **38:** 909–934, 1941.
8. Martin, L. K., B. J. Powell and D. K. Kamano. Effects of amobarbital sodium on avoidance performances of rats differing in emotionality. *Proc. 74th Am. Psychol. Convention (New York),* 125–126, 1966.
9. Powell, B. J., L. K. Martin and D. K. Kamano. Relationship between emotionality, drug effects, and avoidance responses in Tryon S_1 and S_3 strains. *Can. J. Psychol.* **21:** 294–300, 1967.
10. Winer, B. J. *Statistical Principles in Experimental Design* (2nd ed.). New York: McGraw-Hill, 1971.