Pharmacology Biochemistry & Behavior, Vol. 14, pp. 121–124. Printed in the U.S.A.

Interaction Among *d*-Amphetamine, Scopolamine and Genotype in Avoidance Behavior of Rats[1]

K. PAUL SATINDER[2]

Department of Psychology, Lakehead University, Thunder Bay, Ontario, Canada

Received 22 December 1978

SATINDER, K. P. *Interaction among d-amphetamine, scopolamine and genotype in avoidance behavior of rats.* PHARMAC. BIOCHEM. BEHAV. 14(1) 121–124, 1981.—Effects of *d*-amphetamine and scopolamine were investigated on either-way avoidance in two genetic lines. In the either-way task, the animal has the option to respond in either of the two directions available. Differences between the genetic lines were statistically significant under the effects of scopolamine, but were absent under the effects of *d*-amphetamine. It seems that scopolamine is more likely to disrupt responsiveness to visual stimuli in the low-avoidance line and this difference in responsiveness is influenced by bidirectional genetic selection.

Scopolamine and genetic lines | *d*-Amphetamine | Scopolamine and avoidance behavior | Genetic lines | CS modality and avoidance

IN a genetic line selected for low 2-way avoidance, under the effects of scopolamine, an auditory conditioned stimulus (CS) produced higher facilitation of either-way avoidance than a visual CS [8]. No such differences were found in the genetic line selected for high-avoidance. in the same investigation [8], significant differences found during training between the high-(RHA) and low-avoidance (RLA) lines disappeared under the effects of scopolamine, however this only occurred when CS was auditory and not when CS was visual. This lack of significant differences between the lines under the effects of scopolamine with auditory CS were in line with previous findings [7] relating to the effects of *d*-amphetamine with auditory CS. The question arises whether the genetic differences in response to scopolamine with visual CS are specific to this drug or generalizable to *d*-amphetamine, because, earlier investigations [3,5] indicate the similarity of effects between these drugs, especially on the learning of aversively-motivated responses [1,2]. Hence, the purpose of this research was to investigate the effects of *d*-amphetamine and scopolamine on either-way avoidance with visual CS in the genetically selected lines of rats.

METHOD

Subjects

The animals were 64 experimentally naive rats, 32 each from two genetic lines (RHA/Lu, RLA/Lu) and equally represented by both the sexes. The RHA/Lu and RLA/Lu strains have been subject to genetic selection for high and low rates of two-way active avoidance learning, respectively. The animals were bred and reared in the laboratory, weaned at 28 days, were 100 days of age at the start of the experiment, and were from 37–38th generations of the respective strains. Before the experiment the animals were housed in same-sex pairs, with the strains on separate cage racks. Just prior to the start of the experiment, 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.

Apparatus

The apparatus consisted of a circular Plexiglas runway (12 cm wide and 15 cm high, with an outside circumference of 220 cm), which could be divided into four equal compartments by guillotine doors. The runway floor was constructed of stainless steel rods, 0.25 cm diameter, spaced 1 cm apart (center to center). A scrambled shock could be delivered to the grids. The shock unit contained a primary 115-V AC power source and a step-up transformer with a secondary rating of 3,000 V (Hammond Mode 216-60). The shock was delivered through a 2.7×10^6 Ω resistor. Calibra-

[1]This research was supported in part by an Operating Research Grant A0321 from the Natural Sciences and Engineering Research Council of Canada to the author.

[2]Requests for reprints should be sent to K. Paul Satinder, Professor of Psychology, Lakehead University, Thunder Bay, Ontario, Canada, P7B 5E1.

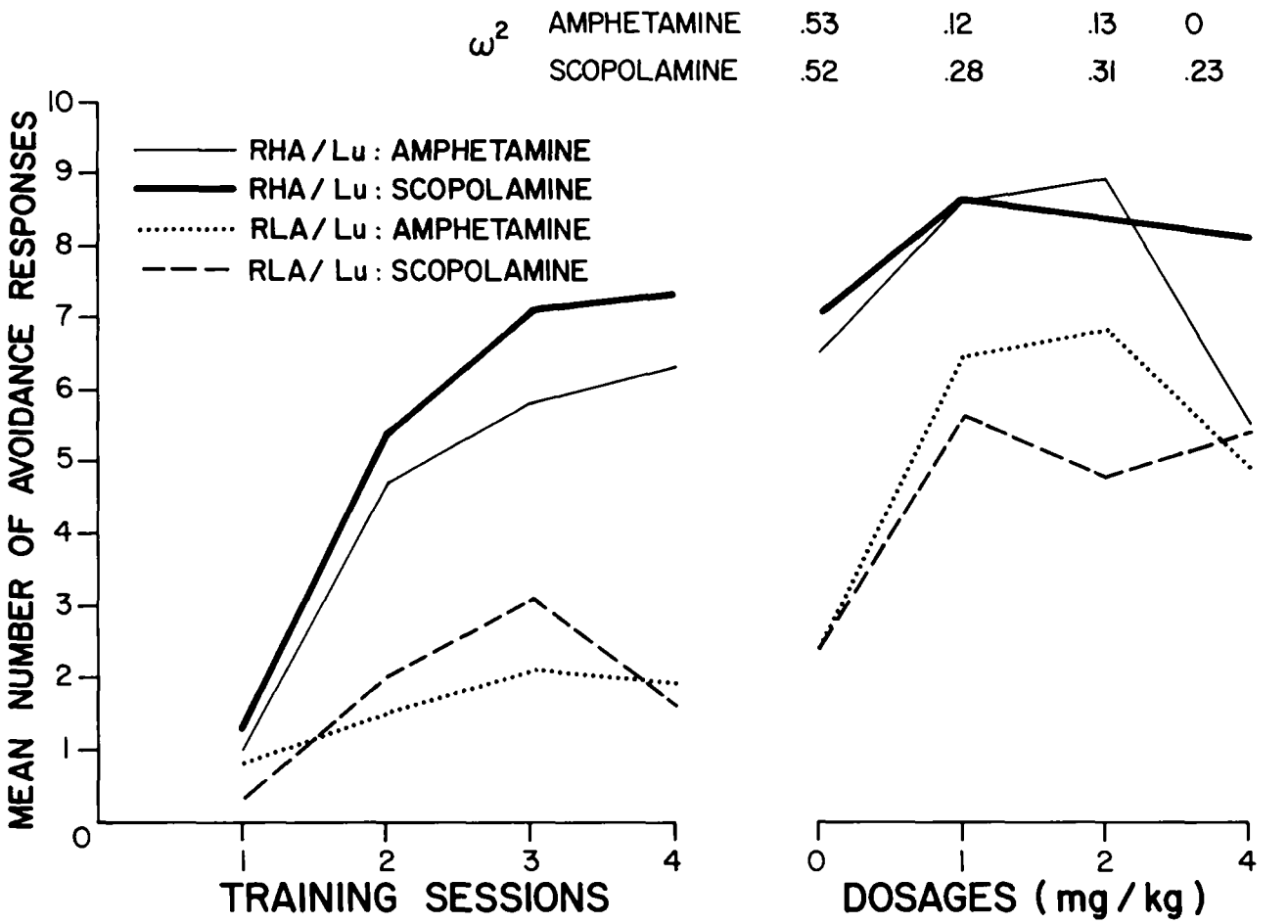

FIG. 1. Mean number of avoidance responses of two genetic lines of rats during training and under the effects of drugs. (Note: No drugs were administered during training but animals were assigned to drug groups before the training started, see text).

tion of shock intensity was based on the assumption that the animal contributed an additional 47,000 Ω resistance. This circuit, with a high-voltage-high-resistance source, provided relatively constant electric current through the rat. There was a 6-W clear transparent light bulb, 3 cm above the grid floor situated in the center of each compartment of the runway, emitting illumination of 100 ft-c. (1,076 lux) at the source and varying from 2–20 ft-c (21.5–215 lux) at various points in the compartment. Automatic timers were used to control the durations of electric shock and light. A digital clock was used to record response latencies.

Experimental Design

The design was a 2 (genetic line) × 2 (sex) × 2 (drug) complete factorial with 8 animals in each factorial cell.

An avoidance trial included a maximum of 10 sec of conditioned stimulus (CS) alone, followed by 10 sec of both CS and electric foot shock as unconditioned stimulus (US) and a 40 sec intertrial interval (ITI). The CS was a light as described in the apparatus section and US was equivalent to the UER (see *Procedure*) of the respective animal. The CS was followed by the US if no avoidance response occurred within the CS duration. The CS terminated immediately after an avoidance response, and both CS and US terminated immediately after an escape response. The very first training trial was an escape trial for each animal. Each animal was given four training sessions of 10 trials each on four successive days.

Three levels of both *d*-amphetamine sulfate and scopolamine hydrobromide (1, 2, and 4 mg/kg) and a placebo (physiological saline) were administered to respective animals on subsequent four successive days. The drugs were administered intraperitoneally in physiological saline 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 then, at each dosage, an equal number of animals started their drug schedule and rotated through the remaining dosages in the order assigned.

The procedure for testing under the effects of drug was the same as during training. A double-blind technique was used. The person recording the responses did not know the strain, drug or dosage.

Procedure

Unconditioned escape response (UER). All animals were tested for UER (2 days before avoidance training) because UERs of these genetic lines are known to differ and this difference affects 2-way active avoidance [6]. Each animal was individually adapted in the circular runway for a 1-minute period prior to receiving foot shock. Electric shock was administered until the animal escaped within 5 sec by

TABLE 1

MEAN NUMBER OF AVOIDANCE RESPONSES ACCORDING TO GENETIC LINES, DRUGS, SEX AND DOSAGE LEVELS

Genetic Lines	Drugs	Sex	Dosages (mg/kg)			
			0	1	2	4
RHA/Lu	*d*-amphetamine	Female	7.1	9.4	9.1	6.3
		Male	5.9	7.8	8.6	4.8
	scopolamine	Female	6.4	8.9	8.6	8.8
		Male	7.8	8.3	8.1	7.5
RLA/Lu	*d*-amphetamine	Female	2.4	8.0	9.1	5.1
		Male	2.5	4.8	4.4	4.8
	scopolamine	Female	2.3	3.9	4.1	4.9
		Male	2.6	7.3	5.5	5.9

running at least a distance equivalent to a quarter length of the runway (all doors open) in either direction, and this was defined as UER. Each animal was given 10 trials of the ascending series, by using the method of limits, with an inter-trial interval of approximately 5 sec. Shock intensities ranged between 0.27–0.97 mA in 22 steps. The highest electric shock intensity to elicit UER for each animal was determined, and according to UER, animals of each genetic line and sex were divided into two matched groups. The matched groups were assigned at random to one of the two drugs before avoidance training started.

Avoidance acquisition. Each animal was exposed in one of the quarters of the circular runway with both ends closed with guillotine doors for 1 min. At the start of the first trial the door leading to the next quarter was raised from the grid. On subsequent trials both doors of the compartment occupied by the animal were raised, permitting the animal to return to the compartment previously occupied or to run away from that compartment, i.e., allowing the animal to respond either-way. The height of the door opening was adjusted according to the size of each animal such that the rat had to squeeze under the door. This was done to make the "crossing response" very distinctive to the rat.

RESULTS AND DISCUSSION

Mean number of avoidance responses of each genetic line are presented in Fig. 1 for both training and drug (*d*-amphetamine and scopolamine) sessions. The results were evaluated by a 2 (genetic line)×2 (drug) ×2 (sex) ×4 (session) mixed analysis of variance separately for training and drug. Sessions factor referred to successive daily training and dosage levels during testing phase.

Both during training and under the effects of drugs the RHA line showed higher avoidance than did the RLA line: training $F(1,56)=111.8$; drug, $F=38.2$, $p<0.00001$. (For dosages 1, 2, and 4 mg/kg only the F ratio was 22.1, $p<0.00002$.) During training avoidance increased over the four sessions, $F(3,168)=75.4$, $p<0.001$, and the genetic lines showed different acquisition rates (RHA: $F(3,84)=69.4$, $p<0.001$; RLA: $F=13.1$, $p<0.001$), thus showing an interaction between genetic lines and training sessions, $F(3,168)=23.6$, $p<0.001$. Under the effects of drugs there were changes ($p<0.00001$) among dosage levels showing mainly facilitative effects of 1 and 2 mg/kg dosages as compared to the respective placebo (0 mg/kg) condition. The facilitative effects of 1 mg/kg dosage (RHA-*d*-amphetamine: $t(15)=4.0$, $p<0.001$; RHA-scopolamine: $p<0.006$; RLA-*d*-amphetamine: $p<0.001$, RLA-scopolamine: $p<0.0001$) were higher than the 2 mg/kg dosage ($p<0.0001$, $p<0.03$, $p<0.0001$, $p<0.02$, respectively). In addition, the 4 mg/kg dosage also showed facilitative effects in the RLA line (*d*-amphetamine: $p<0.004$, scopolamine: $p<0.0001$) only, but in the RHA line the 4 mg/kg dosage of *d*-amphetamine showed suppression as compared to the 1 or 2 mg/kg dosage ($p<0.001$).

Analysis of the data based on 4 dosage levels, i.e., including placebo (physiological saline), revealed an interaction between genetic lines and dosages ($p<0.001$), however, analysis of drug dosages (1, 2, and 4 mg/kg) data, showed no such significant interaction. This clearly indicates that this interaction was attributable to the apparent difference between 0 mg/kg dosage and the remaining drug dosages. It is evident from Fig. 1 and Table 1 that increase in avoidance response from 0 mg/kg to 1 mg/kg dosage of both the drugs was higher in the RLA than the RHA line. Interactions between drugs and dosages ($p<0.0005$), and sex and drugs ($p<0.02$) were present irrespective of the fact whether four or three dosages data were considered. The drugs × dosages interaction was mainly present in the RHA ($p<0.0009$) and not the RLA ($p>0.2$) line, and among female ($p<0.0003$) rather than male groups ($p>0.4$) as evident from Table 1. The interaction between drugs and sex was significant due to the fact that females under the effects of *d*-amphetamine and males under the effects of scopolamine avoided at a higher rate. As evident from Table 1, this interaction was mainly present in the RLA ($p<0.006$) and not the RHA ($p>0.8$) line. Furthermore, sex differences in the RLA line were more pronounced under the effects of *d*-amphetamine (1 mg/kg: $F(1,14)=5.7$, $p<0.04$; 2 mg/kg: $p<0.0003$) than scopolamine (1 mg/kg: $p<0.02$). These two-way interactions led to a four-way interaction among genetic lines, sex, drugs and dosages ($p<0.004$). However, this interaction disappeared in the analysis pertaining to 3 dosages of drugs (1, 2, and 4 mg/kg) and was reduced to three-way interaction between genetic lines, sex and drugs ($p<0.04$), the details of which are evident in Table 1.

The finding that the differences between the genetic lines are of much higher magnitude under the effects of scopolamine than *d*-amphetamine (Fig. 1, the ω^2 value of 0.17 yields a F value to be significant at $p<0.01$ level) is of theoretical significance. The lack of statistically significant differences between the genetic line in response to *d*-amphetamine support previous results ([7]; Experiments 1 and 2, Fig. 1) with regard to the either-way avoidance, but highly significant differences between the lines in response to scopolamine require further examination. The differences between the genetic lines under the effects of scopolamine, (refer Table 1) are mainly due to the differences between the female groups ($p<0.0009$) than the male groups ($p<0.03$). Furthermore, differences between female groups under the effects of scopolamine were significant under all the four dosage levels, where as between the male groups, significant differences were restricted to 0 mg/kg dose.

The CNS stimulant drug *d*-amphetamine is a known anorectic drug [4], hence differences between the body weights of the first and the last days of the experiment were evaluated. In general, the last day mean body weight was significantly lower than the first day mean body weight, $F(1,56)=22.9$, $p<0.0001$, but this difference was larger in the

d-amphetamine groups than the scopolamine groups thus producing a significant ($p<0.03$) interaction between drugs and pre-post differences in body weights. Males showed larger decreases than females in both the genetic lines and drugs, hence producing an interaction ($p<0.0003$) between drugs and sexes.

The RHA line (females=0.276 mA, males=0.281 mA) showed higher sensitivity for the unconditioned escape response to electric footshock than the RLA line (females=0.389 mA, males=0.745 mA), $F(1,56)=82.5$, $p<0.001$, thus supporting previous findings [6,7]. Similarly, in general, females showed higher sensitivity to footshock than the males ($p<0.001$) but the lack of significant differences between the sexes in the RHA line and the presence of such differences in the RLA line produced an interaction between the lines and sex of the animals ($p<0.001$). As expected (matched groups) there were no significant differences between the groups assigned to the two drugs.

Summarizing, the results of the present investigation in light of the previous findings [8] revealed that (a) the effects of *d*-amphetamine on either-way avoidance learning are minimally affected by the genetic background of the animals and the nature of the conditioned stimulus. This is evident from the finding that avoidance of these genetically selected rats (RHA and RLA) were not much different under the effects of *d*-amphetamine either with a visual (Fig. 1) or an auditory ([7]; Experiments 1 and 2, Figure 1) CS. (b) Avoidance response of the RHA (high avoidance) line is not differentially affected by either of the drugs (Fig. 1) or by the nature of the conditioned stimuli (Fig. 1 and [7]). This finding is in line with previous observations [3,5] that the effects of *d*-amphetamine and scopolamine are similar and this similarity of effect is generalizable to either-way avoidance paradigm. However, one has to be aware of the fact that this particular congruity is applicable only to one of the two genetic lines investigated in the present study. (c) The genetic differences in response to scopolamine with visual CS are specific to this drug and are not generalizable to *d*-amphetamine. (d) The fact that differences between the visual (Fig. 1) and auditory [7,8] CS exist only in the RLA and not the RHA line suggest that responsiveness to visual stimuli is influenced by the bidirectional genetic selection.

REFERENCES

1. Barrett, R. J., N. J. Leith and O. S. Ray. A behavioral and pharmacological analysis of variables mediating active-avoidance behavior in rats. *J. comp. physiol. Psychol.* **82**: 489–500, 1973.
2. Bignami, G., L. Amorico, M. Frontali and N. Rosic. Central cholinergic blockade and two-way avoidance acquisition: The role of response disinhibition. *Physiol. Behav.* **7**: 461–470, 1971.
3. Carlton, P. L. Brain-acetylocholine and inhibition. In: *Reinforcement and Behavior*, edited by J. T. Tapp. New York: Academic Press, 1969.
4. Innes, I. R. and M. Nickerson. Norepinephrine, epinephrine, and the sympathomimetic amines. In: *The Pharmacological Basis of Therapeutics*, edited by L. S. Goodman and A. Gilman. New York: Macmillan Publishing Co., Inc., 1975.
5. Khavari, K. A. Adrenergic-cholinergic involvement in modulation of learned behavior. *J. comp. physiol. Psychol.* **74**: 281–291, 1971.
6. Satinder, K. P. Sensory responsiveness and avoidance learning. *J. comp. physiol. Psychol.* **90**: 946–957, 1976.
7. Satinder, K. P. Arousal explains difference in avoidance learning of genetically selected rat strains. *J. comp. physiol. Psychol.* **91**: 1326–1336, 1977.
8. Satinder, K. P. Interaction among scopolamine, conditioned stimulus modality, genotype and either-way avoidance behavior of rats. *Psychopharmacology* **67**: 97–99, 1980.