Effects of a Tranquilizer and Two Antidepressants on Learned and Unlearned Behaviors

HARRY M. GEYER, III, NATHAN WATZMAN*, and JOSEPH P. BUCKLEY

Abstract □ The dose–response effects of chlorpromazine, imipramine, and thiazesim were investigated on unlearned behaviors (spontaneous motor activity, eating, drinking, mouse-killing, self-grooming, and forced motor activity) and learned behavior using the rat pole-climbing unit. Three or four doses of each drug were used in the study of each parameter, and ED_{50} values were calculated from the generated log dose–response line. Ratios of the forced motor activity ED_{50} divided by the ED_{50}'s of the various behavioral tests were used to determine whether the blockades of the behavioral parameters occurred at debilitating or nondebilitating doses. The tranquilizer, chlorpromazine, required a debilitating dose to block four of the five unlearned behaviors. The antidepressant, imipramine, disrupted three of these at nondebilitating doses; the antidepressant, thiazesim, blocked all unlearned behaviors at nondebilitating doses. All compounds required debilitating doses to block the learned behavior, a conditioned avoidance response. The results generally support the hypothesis that antidepressants selectively block unlearned behaviors which are not blocked by tranquilizers until debilitating doses are used.

Keyphrases □ Tranquilizer, antidepressant effects—learned, unlearned behavior □ Chlorpromazine, imipramine, thiazesim—comparative effects, learned, unlearned behavior □ Behavioral response, learned, unlearned—antidepressants, tranquilizer effects

Although two major classes of psychotherapeutic agents, tranquilizers and antidepressants, are quite different in their clinical applications, often the assignment of a compound into either of these classifications must await clinical evaluation since the difference is not readily assessible by preclinical animal testing. Tranquilizers and antidepressants have been reported to have qualitatively similar electroencephalographic effects (1–4). Herr *et al.* (5) reported the lack of qualitative differences when these compounds were compared in various toxicological and behavioral studies. Horovitz *et al.* (6) reported a possible method for a preclinical differentiation. These authors reported that antidepressants, but not tranquilizers, given to rats at nondebilitating doses had a blocking action on mouse-killing, an unlearned behavior described by Karli (7). Horovitz *et al.* (6) also reported that this difference between tranquilizers and antidepressants was not found in the comparison of drug effects on a learned avoidance response.

The present study was an attempt to determine if other nonlearned behaviors in rats show the "selective" blocking action by antidepressants.

MATERIALS AND METHODS

Subjects—The subjects were Sprague-Dawley male rats (519) and female rats (82) weighing 200–350 g. The subjects were maintained in individual wire-mesh cages on a 12-hr. light-dark cycle at ambient temperatures between 23.9 and 25.5° (75 and 78°F). Purina laboratory chow and tap water were available *ad libitum* for all subjects except those used in the studies of food and water consumption. All subjects were used only once except in the study of muricide. Maternal behavior and muricide were tested in the light portion of their light-dark cycle. All other studies were performed during the dark portion of the cycle; in the testing of self-grooming and forced motor activity, the subjects were illuminated by two 1.5-m. (5-ft.) fluorescent red light bars approximately 0.9 m. (3 ft.) from the cage and between the investigator and the subjects.

Drugs—The prototype tranquilizer selected was chlorpromazine hydrochloride, and the antidepressants were imipramine hydrochloride and thiazesim hydrochloride. All drugs were dissolved in distilled water, with concentrations adjusted to enable intraperitoneal injection in volumes of 1 ml./250 g. of body weight. The various testing procedures were performed during peak drug effect which occurred 30 min. after chlorpromazine, 40 min. after imipramine, and 30 min. after thiazesim administration (8). The saline subjects (0.9% NaCl) were tested 30 min. after saline administration. The doses used were as follows: chlorpromazine, 1, 2, and 4 mg./kg.; imipramine, 8, 16, and 32 mg./kg.; and thiazesim, 10, 20, and 40 mg./kg., i.p. In the experiments with self-grooming, mouse-killing, and conditioned avoidance response, an additional dose of chlorpromazine, 8 mg./kg., was included. In the study of water consumption, an additional dose of 5 mg./kg. of thiazesim was included, and imipramine was used in doses of 4, 8, and 16 mg./kg.

Forced Motor Activity (FMA)—Forced motor activity was evaluated by the use of a revolving wooden rod, 5.08 cm. (2 in.) in diameter, as described by Watzman *et al.* (9). The rod first revolved at 7.8 r.p.m., and the speed was increased by 4.5 r.p.m. every 30 sec. The amount of time the subjects remained on the rod was used as the measure of motor activity. The subjects, 81 male rats, were given five consecutive training trials both in the morning and afternoon of the 1st day, four consecutive trials in both the morning and afternoon of the 2nd day, and three consecutive trials on the morning of the 3rd day. The rotarod performance was found in preliminary trials to be stable by the 3rd day. The drugs and saline were administered in the afternoon of the 3rd day, and the subjects were given three consecutive test trials at the time of peak drug effect. The test trials were averaged for each subject, and the groups receiving the experimental compounds were compared with the saline controls.

Spontaneous Motor Activity (SMA)—Spontaneous motor activity was tested in four circular, 6-beam photocell activity cages (Actophotometer, Metro Industries, Inc., New York, N. Y.), using a single subject per cage. The drugs, doses, saline controls, and cages were arranged in a modified factorial design. The testing was performed on 3 consecutive days. There were three experimental subjects and one saline control at each testing session, and each drug–dose group was compared with its appropriate saline controls. The subjects, 95 male rats, were introduced at the time of peak drug effect; their activity counts were recorded at the end of 30 min.

Food Consumption—In this study, 68 male rats were deprived of food for 24 hr. but water was available *ad libitum*. At the end of the deprivation period, the subjects were treated with drugs or saline and, at the time of the peak effect, were given a preweighed amount of Purina laboratory pellets. At the end of 1 hr., the remaining pellets and spillage were collected and weighed. The recorded data were both the weight eaten and the weight of the subject just prior to testing.

Water Consumption—The subjects used in the study of drinking (75 male rats) were deprived of water for 3 days and then placed at the time of peak drug effect in a compartment, 25.4 × 26.6 × 27.9 cm. (10 × 10.5 × 11 in.), which had a drinking spout attached to a drinkometer apparatus. The number of licks recorded in 30 min. was used as the measure of water consumption.

Maternal Behavior—Maternal behavior was studied 1 to 4 days postparturition. The subjects (82 female rats) were removed from their home cages momentarily, the wood floors were cleared, and a new supply of the nesting material and all pups were deposited on

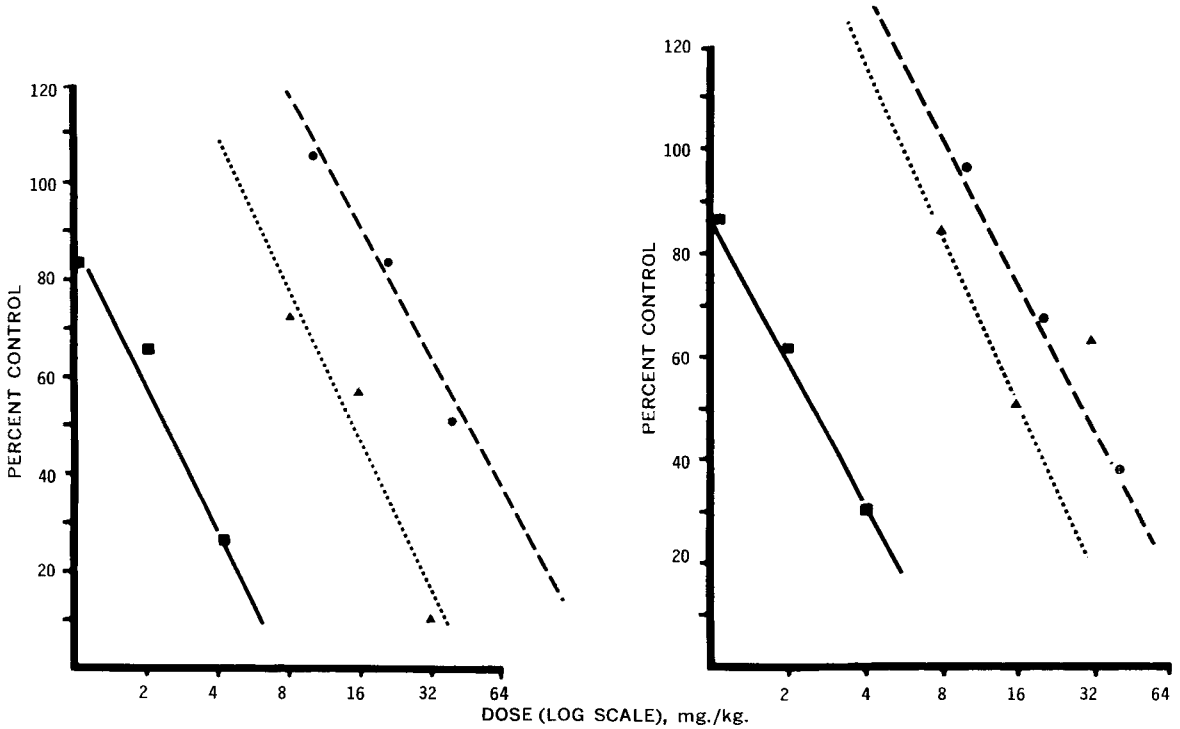

Figure 1—*Effects of chlorpromazine, imipramine, and thiazesim on spontaneous (right side) and forced motor activity (left side). Key:* ■—, *chlorpromazine;* ▲. . ., *imipramine; and* ●- - -, *thiazesim.*

the side of the cage opposite the previously noted nesting site. The subjects were then returned to their cages and rated on the 5-point scale of 0, 1, 2, 3, and 4 according to approximate percents of the nesting material retrieved to the nesting site: 0, 25, 50, 75, and 100%. Simultaneously, the subjects were scored according to the percent of young retrieved. Each session was 8 min. in duration; 24 hr. later the testing procedure was repeated at the time of peak drug action. The experimental subjects were compared with saline controls, and the preliminary trial (saline injection) was used to eliminate the few subjects not completing both nest-building and young-retrieval within 4 min.

Mouse-Killing—An albino mouse, 25 to 30 g., was introduced in the rat's home cage for 5 min.; if the rat killed the mouse, it was retested at least once a day for 5 days. The latter tests were 30 min. after saline injection, and a 60-sec. time limit was imposed. Only those rats (36 males) that killed in all of the predrug trials were used in this study. The animals received drug injections and were tested on four to six occasions. There were always at least 1 week and one saline test between drug administrations. The total number killing within 60 sec. was recorded for each drug and dose.

Self-Grooming—The 84 male rats used in this study were immersed in a sample of rat urine to wet the forepaws, hind paws, and undersides. They were immediately returned to their home cages, and the total time spent grooming was measured over a 5-min. period. Twenty-four hours later, the subjects were weighed and injected and the same procedure was repeated. The time spent grooming each day was recorded.

Conditioned Avoidance Response (CAR)—This was studied in automated pole-climbing units (25.4 × 26.6 × 35.5 cm.) (10 × 10.5 × 14 in.) (10). A 15-sec. tone was followed by an electrical shock (300 v., 2 mamp.) from the grid floor with a maximum duration of 45 sec. The tone was continued through the shock period, and this was followed by a 2-min. intertrial period. Pole-climb latencies during tone alone (shock avoidance) and during the shock cycle (shock escape) were recorded on a pen polygraph (Lehigh Valley Electronics No. 1321-4).

The subjects, 80 male rats, were trained to a minimum of 75% avoidance in three to five 1-hr. sessions. The training sessions were once daily on consecutive days, and the animals were retested at peak drug effect 24 hr. after the training session in which they reached the 75% avoidance criterion. The number of avoidances made by the drugged subjects were compared with the appropriate saline controls.

RESULTS

Forced Motor Activity (FMA)—All drugs produced dose-dependent decrease in motor performance, and chlorpromazine and imipramine produced a decrement exceeding 50%. Thiazesim did not quite reach the 50% level at the highest dose, 40 mg./kg. (Fig. 1). This was the maximum dose used because pilot studies indicated that both 50 and 60 mg./kg. could produce convulsions. The data were calculated and presented as the percent of saline scores.

Spontaneous Motor Activity (SMA)—The SMA data (Fig. 1) depict the drug actions as dose-dependent decreases in activity and are reasonably straight lines for chlorpromazine and thiazesim. The imipramine effects appear biphasic in this and in several other measures in this study. This biphasic action will be discussed later. The ED_{50} value for imipramine was calculated with only the low and medium doses because the latter approaches the 50% level (51.8%).

Food Consumption—The data on this parameter produced a fairly linear dose-dependent decrease in food consumption by each drug (Fig. 2). The results depicted are percent control values and are calculated as the amount eaten, divided by the subject's weight. The subject's weights did not differ significantly between groups; however, the within-group weights varied up to 100 g. For this reason the results were weighted in relation to subject's size. This weighting of scores assumed that larger subjects would eat more, even under the experimental conditions. This was substantiated by the overall mean Pearson correlation coefficient of +0.48 for the weight of food eaten per gram of body weight.

Water Consumption—The inhibitory effects on water consumption produced by the three compounds are summarized in Fig. 2. Although the linearity of chlorpromazine and thiazesim results is clear, imipramine again shows a biphasic response. The two low doses, one on either side of the 50% level, were used to calculate the ED_{50} value.

Maternal Behavior—The results summarized in Fig. 3 failed to show a clear log dose–response relationship in most cases. Although rough approximations of the respective ED_{50} values do agree with the proposed hypothesis, the lack of clear dose–response relationships is considered ample reason for deletion of these parameters from further considerations. The cause of this discrepancy is not clear, although there is the possibility that the home cages [20.3 × 21.6 × 27.9 cm. (8 × 8.5 × 11 in.)] may not have been of sufficient size to stimulate the maternal behaviors tested. That is, pups,

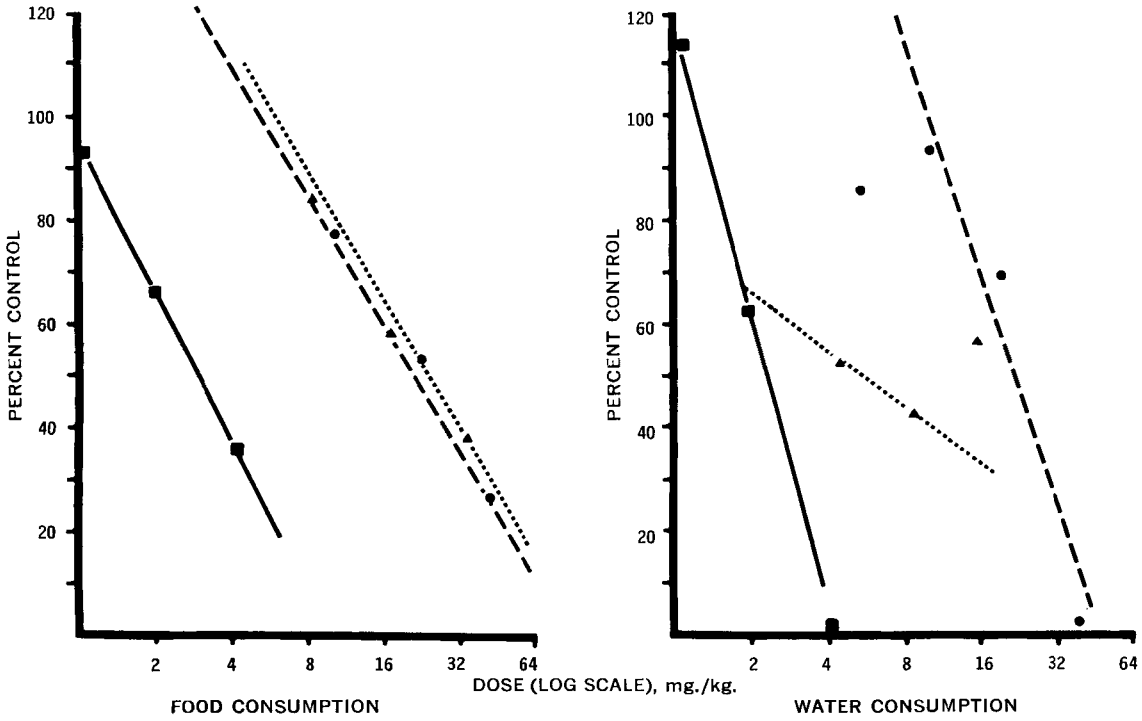

Figure 2—*Effects of chlorpromazine, imipramine, and thiazesim on food and water consumption. Key:* ■—, *chlorpromazine;* ▲. . ., *imipramine; and* ●- - -, *thiazesim.*

8 to 10 in. away in the home cage, may have been an inadequate stimulus to induce retrieval behavior in some of the animals.

Mouse-Killing—The data summarized in Fig. 4 indicate that the drugs produced a linear log dose–response relationship. The ability of chlorpromazine to block muricide, even at the high dose (8 mg./kg.), is, however, open to question. The test sequences were only 60 sec. and, at the highest dose, chlorpromazine appeared to induce the expected sedation. To clarify this, after the end of their test sequence, some sedated rats were manually jostled and the mice were immediately killed by the aroused rat. This was found in eight of the nine subjects receiving 8 mg./kg. of chlorpromazine, although the same procedure did not induce killing when used with six of the subjects receiving 32 mg./kg. of imipramine or six of the subjects receiving 40 mg./kg. of thiazesim. The tranquilizer, therefore, appeared to block mouse-killing mainly due to its sedative action.

Self-Grooming—The results depicted in Fig. 4 show the log dose–response relationship for the compounds investigated. Of interest is the evident increase in grooming induced by 2 mg./kg. of chlorpromazine, although this difference was short of statistical sig-

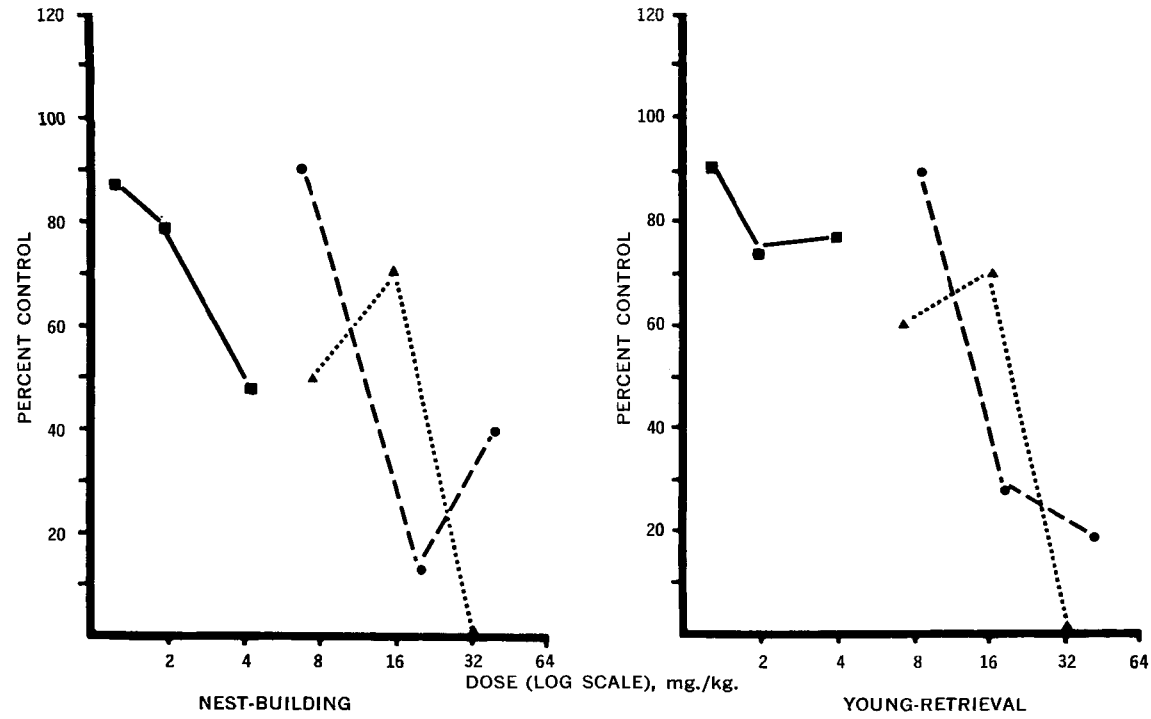

Figure 3—*Effects of chlorpromazine, imipramine, and thiazesim on nest-building and young-retrieval. Key:* ■—, *chlorpromazine;* ▲. . ., *imipramine; and* ●- - -, *thiazesim.*

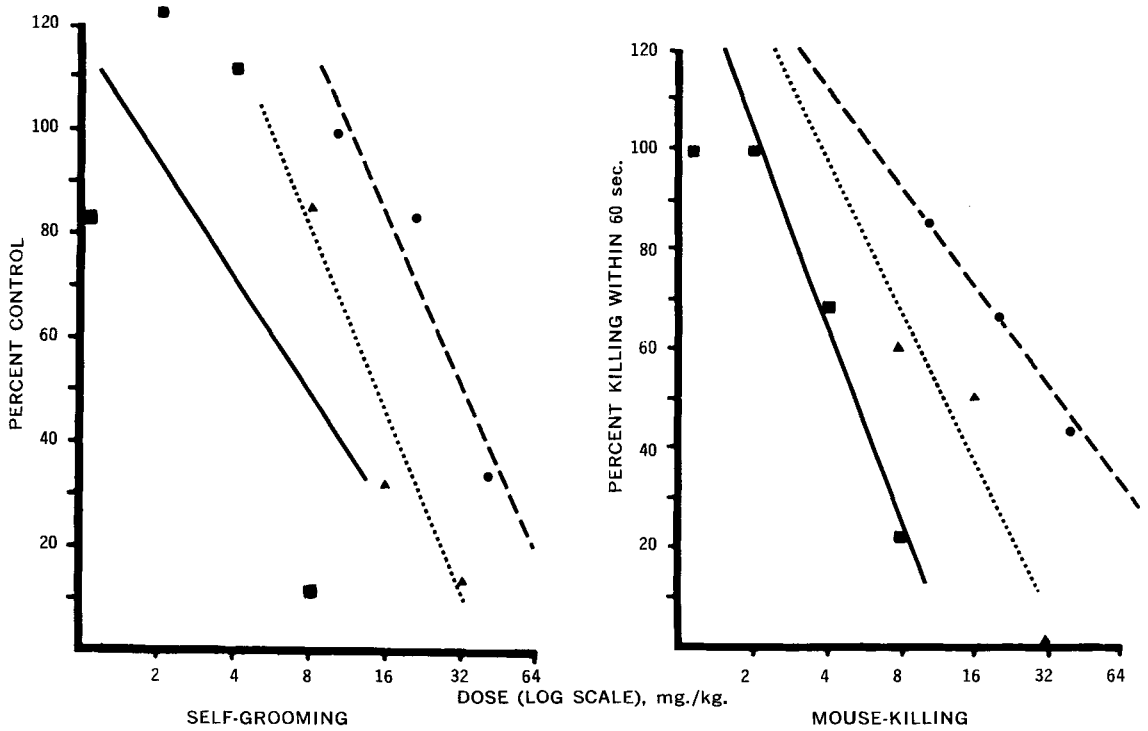

Figure 4—*Effects of chlorpromazine, imipramine, and thiazesim on self-grooming and mouse-killing in the rat. Key:* ■—, *chlorpromazine;* ▲. . ., *imipramine; and* ●- - -, *thiazesim.*

nificance ($p > 0.05$). These results are in agreement with the report of Silverman (11), which gave data indicating that low doses of chlorpromazine significantly increase unstimulated self-washing of the face and forepaws. An increase in any behavior after chlorpromazine administration is of interest because of this compound's general depressant action.

Conditioned Avoidance Response (CAR)—The results shown in Fig. 5 are for the most part in agreement with those reported by Horovitz *et al.* (6), although these investigators found imipramine to be more potent in CAR disruption. This causes no change in direction of the ratio values employed by these investigators, only an insignificant change in magnitude.

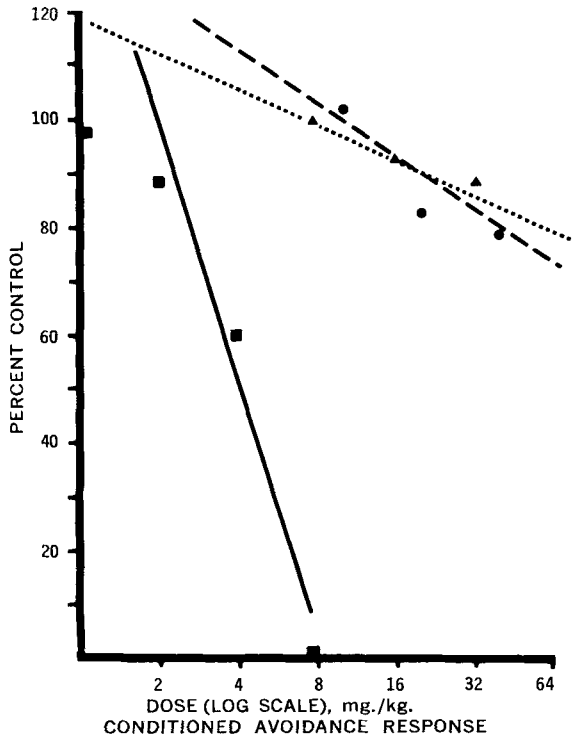

Figure 5—*Effects of chlorpromazine, imipramine, and thiazesim on the rat pole-climbing conditioned avoidance response. Key:* ■—, *chlorpromazine;* ▲. . ., *imipramine; and* ●- - -, *thiazesim.*

ED_{50} Values—The ED_{50} values were calculated from the best fitting straight lines as established by the method of least squares (Table I). Exceptions to this method of analysis were the aforementioned cases involving the biphasic imipramine action in SMA and water consumption. In these cases, the line between the lower doses transversed or approached the 50% level, and the ED_{50} was calculated from this line. The ED_{50} for the thiazesim effect on drinking was calculated after deletion of the lowest of the four doses.

Ratio Values—The ratio values presented in Table II are the ED_{50} values of the experimental compounds in the FMA divided by their respective ED_{50} values in the behavioral parameters investigated. The ratio values for CAR are listed as <1.0, since the ED_{50}'s for impramine and thiazesim were greater than the maximum doses used. Ratio values greater than unity are, therefore, indicative of a "selective" blocking action, a disruption of specific behaviors at nondebilitating doses. Ratio values less than 1 show that the behavior was blocked by debilitating doses.

DISCUSSION

The biphasic aspects of the imipramine action were troublesome in analyzing the data. The possibility of multiple experimental

Table I—Drug Effects on Learned and Unlearned Behavior

Behavioral Parameter	ED_{50} Values, mg./kg., i.p.		
	Chlorpromazine	Imipramine	Thiazesim
Forced motor activity	2.48	15.10	43.89
Spontaneous motor activity	2.51	16.68	29.88
Food consumption	2.88	21.52	20.56
Water consumption	2.26	5.09	21.33
Self-grooming	7.71	14.13	32.10
Mouse-killing	5.10	11.76	33.30
Conditioned avoidance response	4.08	>32.00	>40.00

Table II—Ratio Values for Experimental Compounds[a]

Behavioral Parameter	Chlorpromazine	Imipramine	Thiazesim
Spontaneous motor activity	0.98	0.92	1.47
Food consumption	0.86	0.70	2.14
Water consumption	1.09	2.96	2.06
Grooming	0.32	1.07	1.37
Mouse-killing	0.49	1.28	1.32
Conditioned avoidance response	<1.0	<1.0	<1.0

[a] ED_{50} forced motor activity/ED_{50} behavior parameter.

errors was not considered likely; as in the study of SMA, eight subjects were used at each dose, including saline controls, and a prior pilot study measuring SMA with two subjects per activity cage yielded similar results. A close examination of the literature revealed that this biphasic effect is not uncommon. Furgiuele *et al.* (12) reported a biphasic imipramine response in SMA testing that was parallel to that observed in the present experiment. The biphasic imipramine action has also been reported on bulbocapnine and paraldehyde depression of motor control (13, 14). Osborne and Sigg (15) and Schaeppi (16) reported that the pressor responses to epinephrine and norepinephrine injections were potentiated by low doses of imipramine and blocked by high doses. This biphasic imipramine action in the adrenergic system may be related to its effect on drinking, since Grossman (17) and Hutchinson and Renfrew (18) have reported that adrenergic stimulation in several areas of the brain can greatly modify drinking behavior. In addition, Furgiuele *et al.* (12) also reported a biphasic imipramine effect on SMA increased by a compound with adrenergic activity. The work of Thoenen *et al.* (19) may well explain the biphasic imipramine action. These investigators studied the perfused cat spleen and induced contractions by stimulation of the postganglionic splenic nerve. Their data indicate that imipramine, in low doses, augments adrenergic responses by inhibiting reuptake of the transmitter; in high doses the adrenergic response is inhibited in a manner resembling α-adrenergic blockage. Although extrapolation of data from actions on isolated tissue to the behavioral responses of the whole organism is hazardous at best, the wealth of information relating imipramine to adrenergic activity, the reports of biphasic imipramine actions, and the desire for explanations of obtained results all make the extrapolation possible.

The present study provides evidence that there is a qualitative difference between the effects of a tranquilizer and antidepressant compounds on unlearned behaviors. The antidepressants appear to block selectively unlearned behaviors which were blocked by chlorpromazine only at debilitating doses. The ratio values (Table II) show that 12 of the 15 pairings of the three experimental compounds could have been predicted on this basis. As discussed previously, the biphasic imipramine action in drinking and SMA did create some problem in the determination of the ED_{50} values. It should be noted that these two parameters are the only ones that did not show an imipramine block at nondebilitating doses. The use of only one tranquilizer and two clinically classified antidepressants does make generalizing the results to include the two classes quite speculative. However, Horovitz (20) reported at least six tranquilizers and six antidepressants had the ratio values of FMA ED_{50}/muricide ED_{50} characteristic of their respective classes, and this suggests that the generalization may be warranted.

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* Present address: Division of Research Grants, National Institutes of Health, Bethesda, MD 20014