*Pharmacology Biochemistry & Behavior*, Vol. 23, pp. 231–236, 1985. © Ankho International Inc. Printed in the U.S.A. 0091-3057/85 $3.00 + .00

# The Effects of Clozapine on Shuttle-Box Avoidance Responding in Rats: Comparisons With Haloperidol and Chlordiazepoxide

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Received 7 September 1984

SANGER, D. J. *The effects of clozapine on shuttle-box avoidance responding in rats: Comparisons with haloperidol and chlordiazepoxide.* PHARMACOL BIOCHEM BEHAV **23**(2) 231–236, 1985.—Previous studies have shown that clozapine produces effects different from those of other antipsychotic drugs on positively reinforced responding but may give rise to similar disruptions of avoidance behavior. To investigate the actions of clozapine on avoidance responding in more detail the effects of this drug were compared with those of haloperidol and chlordiazepoxide in rats trained to avoid shock in a shuttle-box. Acute administration of all three drugs reduced avoidance responses and increased escape failures although clozapine produced this latter effect only at a high dose. With repeated administration of each drug over 4 days tolerance developed rapidly to the effect of clozapine, the effect of haloperidol increased and there was no systematic change in the action of chlordiazepoxide. Disrupted avoidance responding after acute administration of clozapine does not reflect the clinical antipsychotic action of this drug.

Clozapine Haloperidol Chlordiazepoxide Avoidance Escape Rats

CLOZAPINE is a clinically effective [15,22] antipsychotic drug which differs pharmacologically from other agents used in the treatment of schizophrenia. It does not induce catalepsy in rodents nor does it produce a specific antagonism of the effects of dopamine agonists such as apomorphine [25,29]. In behavioral experiments involving responding maintained by food or electrical brain stimulation clozapine has also been found to exert effects different from those of other antipsychotic drugs such as chlorpromazine and haloperidol [1, 4, 5, 21, 23, 24, 26]. Spealman and Katz [23], for example, reported that, in mice and Squirrel monkeys, clozapine increased rates of positively reinforced responding which had been suppressed by punishment. A similar effect was observed with chlordiazepoxide but not with chlorpromazine. In a further study [24] the effects of clozapine were investigated in monkeys responding under several schedules of reinforcement. The profile of effects produced by clozapine was again more similar to that of the anxiolytic chlordiazepoxide than to effects of several other antipsychotic drugs.

In contrast to these differences between the effects of clozapine and those of other antipsychotic agents on positively reinforced behavior, studies of avoidance behavior in rats have generally reported effects of clozapine similar to those of other antipsychotics. Avoidance behavior has traditionally been considered to provide a useful means of selecting and studying antipsychotic drugs [18,20]. Such drugs have been found to disrupt the ability of animals to avoid shocks at doses which do not prevent escape from the shocks. A variety of other centrally-acting drugs, including hypnotics, sedatives and anxiolytics, however, disrupt both avoidance and escape responding at similar doses ([7,9] for review see [3,17]). Clozapine also has been shown to interfere with avoidance but not escape responding [9] and its effect on avoidance behavior in rats was apparently an important factor leading to the selection of clozapine for clinical testing in schizophrenic patients [25].

The present study, therefore, was carried out in order to investigate in more detail the effects of clozapine on the avoidance behavior of rats in a shuttle box. Both acute and repeated administration of clozapine were studied and the effects of this drug were compared with those of haloperidol. In addition, chlordiazepoxide was investigated because of the evidence, described above, that, under some circumstances, the behavioral effects of clozapine are similar to those of anxiolytic drugs.

## METHOD

### *Subjects*

Male rats weighing 230–290 g at the start of experimentation were used. The animals were Wistar (Charles-River, France) or hooded Lister (Olac, U.K.). Although these strains differed in the speed with which they acquired the avoidance response and the numbers of animals which failed to reach a high enough level of avoidance responding (Listers acquired the response more readily and very few animals failed to learn) no differences were observed in the effects of the pharmacological agents. Results obtained with the two strains have therefore been combined. The rats were individually housed under standard laboratory conditions (lights on 7.00–19.00) with food and water available at all times in the home cages.

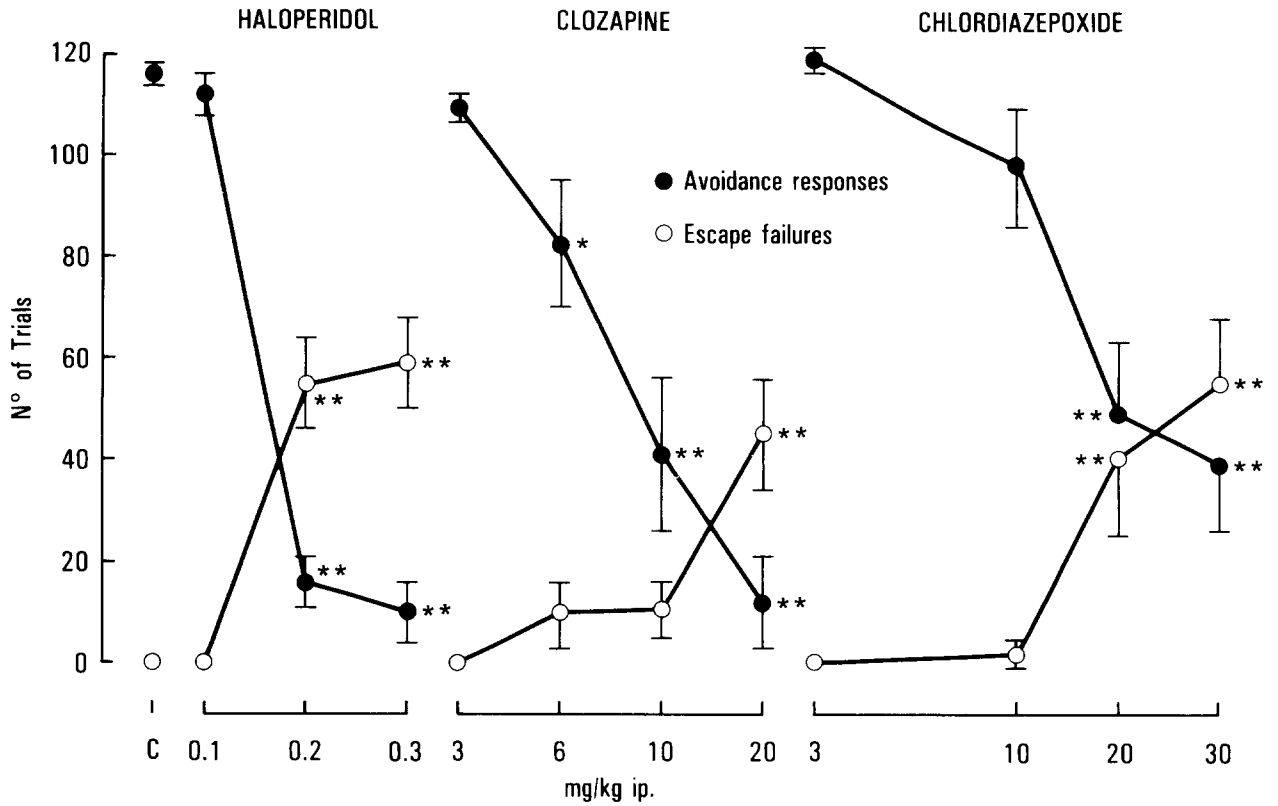


FIG. 1. Dose-response curves of the effects of haloperidol, clozapine and chlordiazepoxide on the number of trials on which the rats avoided shock and the number of trials on which they failed to escape the shock. Each value is the mean±SEM for the 10 rats. The values at C were obtained from all sessions immediately preceding drug sessions (11 sessions for each of the 10 rats). *$p<0.05$; **$p<0.01$ difference from control.

*Apparatus*

The study was carried out in two identical shuttle-boxes (Ugo, Basile). Each box was of dimensions 50×28×28 cm high and was divided into two, equally sized compartments by a partition with a hole 7 cm wide by 8 cm high through which a rat could pass. Movements between the two compartments were detected by a microswitch which was operated by the movement of the pivoted grid floor of the cage. Each shuttle-box was housed in a sound- and light-attenuating outer cubicle.

*Procedure*

Rats were trained to avoid electric footshock by passing between the two compartments of the shuttle-box (two-way active avoidance) according to the following procedure. Each rat was placed in the shuttle box daily and presented with a series of 120 trials consisting of an inter-trial interval of 7 sec followed by a conditioned stimulus (CS=tone+light) of 4 sec and then by a period of CS presentation with footshock (65 V scrambled) for 3 sec. A passage across the box during the inter-trial interval had no effect whereas shock could be avoided during the CS or escaped during the shock+CS by passing from one compartment to the other. Daily sessions of 120 trials required just over 30 min. Most rats required between 4 and 10 training sessions to reach a high level of efficiency at avoiding shock presentation. Control levels of avoidance averaged over 110 shocks avoided during the 120 trials. Animals which did not avoid at least 100 shocks each session were not used in the study.

The study consisted of two experiments. The first used 10 rats. After training in the avoidance task these animals were given injections of clozapine (3, 6, 10, 20 mg/kg), haloperidol (0.1, 0.2, 0.3 mg/kg) and chlordiazepoxide hydrochloride (3, 10, 20, 30 mg/kg). Different drugs and doses were given in a mixed order with the constraint that two injections of the same drug were not given during the same week. At least two non-drug days intervened between successive drug injections. Drugs were prepared as solutions or suspensions in deionized water to which two drops of Tween 80 had been added. Vehicle injections were given on all non-drug days. Injections were given by the intraperitoneal route 30 min before the start of a session and injection volume was 5 ml/kg.

In the second experiment the effects of repeated administration of one dose of clozapine (20 mg/kg), haloperidol (0.2 mg/kg) or chlordiazepoxide (30 mg/kg) were studied. These doses were chosen on the basis of the results of the first experiment. After training on the avoidance task, as described above, rats were given four daily injections of one of these drugs. Separate groups of rats were injected 30 min before each of the four daily sessions or immediately after the first three sessions and 30 min before the fourth session. This latter procedure was used to investigate whether any tolerance observed during repeated administration was due to pharmacological or psychological factors [8]. Eight

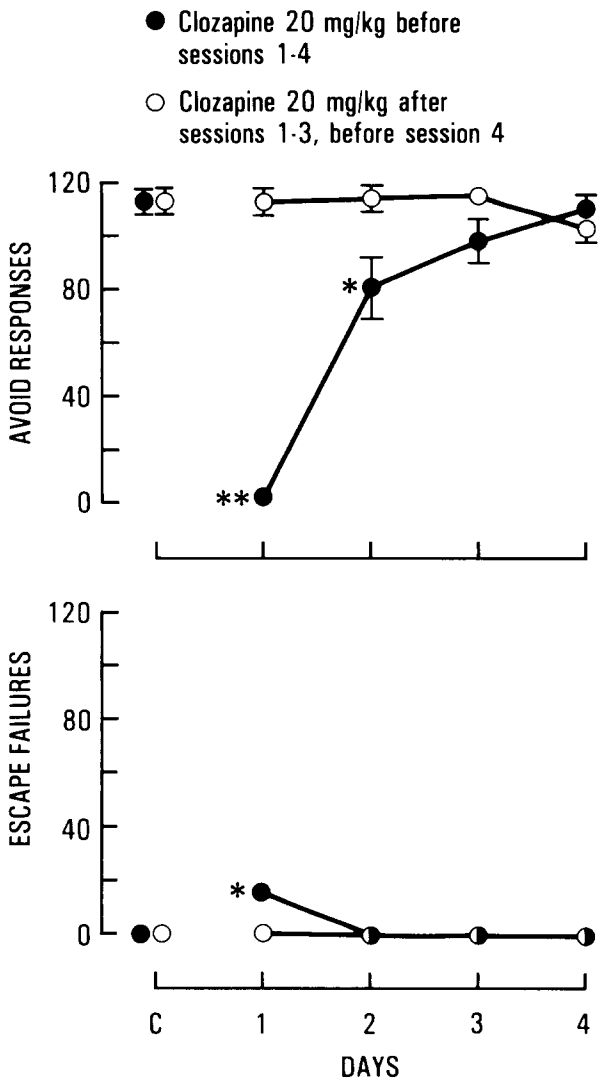


FIG. 2. The effects of repeated administration of clozapine (20 mg/kg) on avoidance responses and escape failures. Each value is the mean±SEM for 8 rats. The values at C are the control levels of responding obtained on the day immediately preceding the first drug day. *$p<0.05$; **$p<0.01$ difference from day C.

animals were tested in each condition except that an additional 5 rats were included in the group treated with chlordiazepoxide before each of the four sessions (i.e., a total of 13 rats in this condition). This was done because of the variability of the results initially observed with this drug.

## RESULTS

Acute administration of clozapine, haloperidol and chlordiazepoxide produced dose-related disruptions of avoidance responding. Figure 1 shows the effects of these drugs expressed as the number of trials on which rats successfully avoided shock and the number of trials on which they failed both to avoid and to escape the shock (maximum number=120). These results were analyzed separately for each drug and each of the two measures using analysis of variance followed by paired comparisons. It can be seen from Fig. 1 that under control conditions the rats avoided almost all the shocks and those few shocks which were not avoided were escaped. The figure also shows that all three drugs both decreased avoidance responding and increased escape failures. Clozapine appeared to be less active than haloperidol and chlordiazepoxide in increasing escape failures as this occurred only at the highest dose (20 mg/kg) which almost completely suppressed avoidance responding. Levels of statistical significance are given in Fig. 1.

Figure 2 shows the effects of repeated administration of 20 mg/kg of clozapine on avoidance behavior. After the first pre-session injection, there was a very marked reduction in the number of avoidance responses and a small increase in escape failures. This effect is equivalent to that seen in the first experiment although the increase in escape failures was slightly smaller. Figure 2 shows clearly that with repeated pre-session administration of clozapine the initial effect declined so that by the fourth administration there was no disruption of avoidance responding. Additionally, rats injected after the first three sessions showed little disruption of avoidance responding when the drug was given before testing on session 4. Statistical analysis of these data made use of repeated measures analysis of variance followed by individual comparisons. The results of these analyses are shown in the figure.

The effects of repeated administration of haloperidol (0.2 mg/kg) are shown in Fig. 3. As in the first experiment, pre-session injection of haloperidol reduced avoidance responses and increased escape failures. In contrast to the effects seen with clozapine, however, tolerance did not occur but rather these effects increased with repeated injection. This effect was confirmed by statistical analysis using analysis of variance and paired comparisons, the results of which are shown in Fig. 4. Furthermore, for these animals behavior on day 1 differed significantly from that on day 4 ($p<0.05$ for avoidance, $p<0.01$ for escape). When the rats injected after sessions 1–3 were injected before session 4 avoidance and escape showed significant changes from control values. A comparison between the performance of the two groups on day 4 showed a significant difference in numbers of escape failures ($p<0.01$) although the difference between numbers of avoidance responses did not reach an acceptable level of statistical significance ($p>0.05$).

Figure 4 shows the effects of repeated administration of chlordiazepoxide (30 mg/kg) on avoidance responses and escape failures. It is clear that chlordiazepoxide reduced numbers of avoidance responses and increased escape failures and no systematic changes in these effects occurred over the course of the 4 days of drug administration. Rats injected after sessions 1–3 showed a significant disruption in responding when tested after drug administration on day 4 ($p<0.01$ for avoidance and escape) and the differences between the two groups on day 4 were not statistically significant ($p>0.05$ for avoidance and escape).

## DISCUSSION

Avoidance responding of rats has been considered a particularly useful technique for the preclinical evaluation of antipsychotic drugs. In an early report Cook and Weidley [7] found that chlorpromazine, reserpine and morphine disrupted the pole jump response of rats during trials when a stimulus previously paired with shock was presented alone but not during trials when the stimulus was presented with an electric footshock. In contrast, meprobamate, pentobarbital and barbital disrupted responding under both conditions.

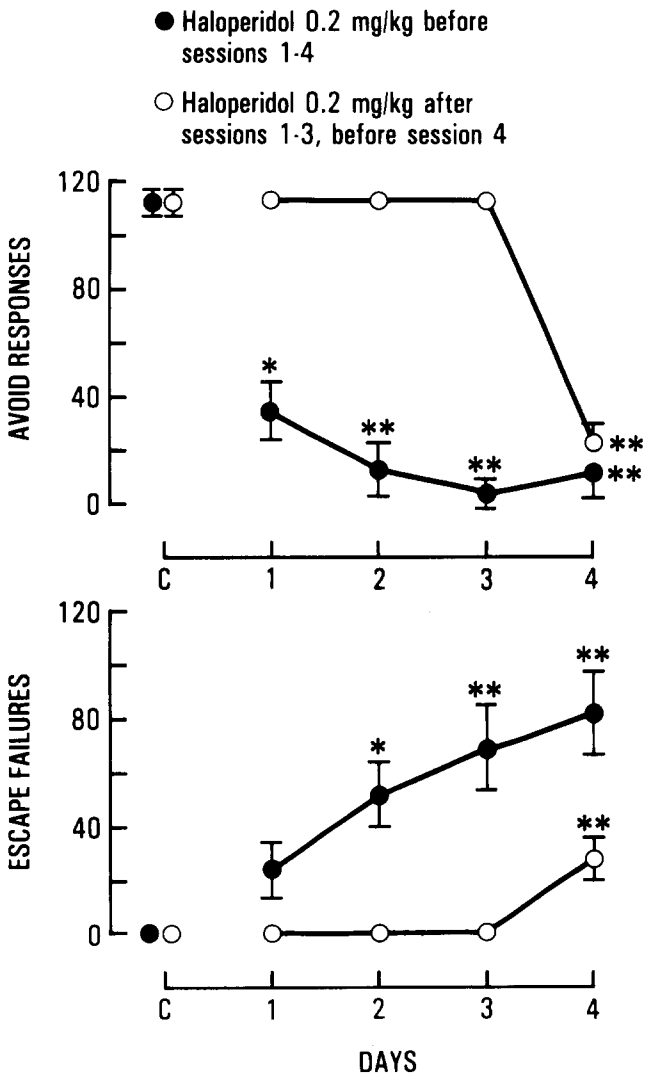


FIG. 3. The effects of repeated administration of a dose of haloperidol (0.2 mg/kg) on avoidance responses and escape failures. Each value is the mean±SEM for 8 rats. The values at C are the control levels of responding taken from the day immediately preceding the first day of drug administration. *$p<0.05$; **$p<0.01$ difference from day C.

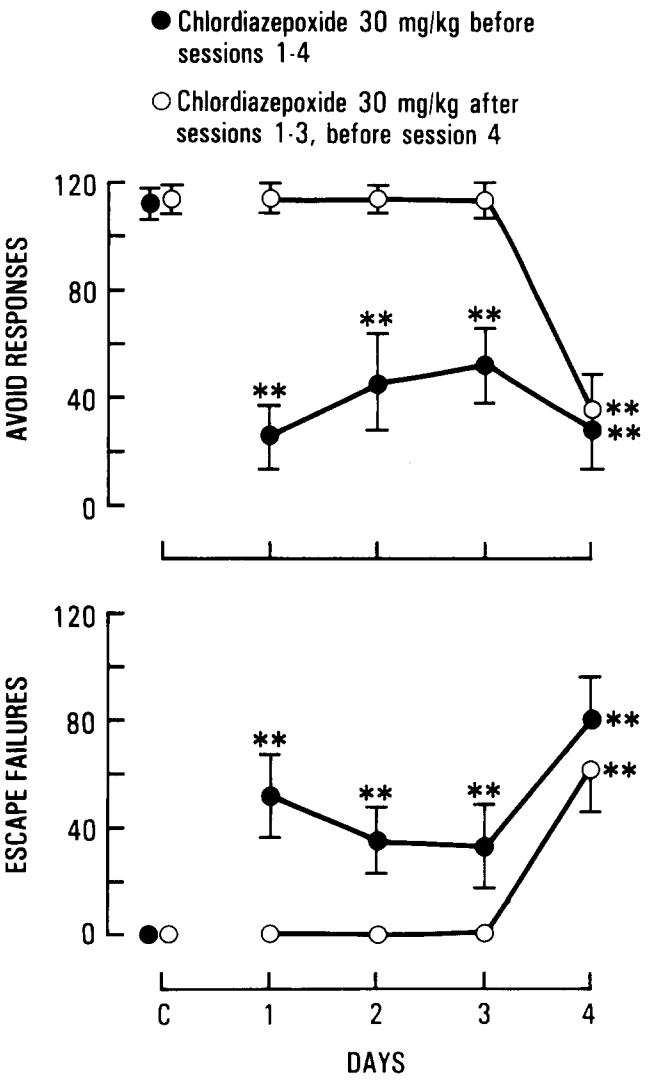


FIG. 4. The effects of repeated administration of a dose of chlordiazepoxide (30 mg/kg) on avoidance responses and escape failures. Values are means±SEM of 13 rats given the drug before each of the 4 tests and 8 rats given the drug after session 1–3 and before session 4. The values at C are the control levels taken from the day immediately preceding the first day of drug administration. **$p<0.01$ difference from day C.

More recently, Davidson and Weidley [9] compared the effects of a variety of antipsychotic drugs, including haloperidol and clozapine, on the acquisition of avoidance and escape responding of rats and found that considerably higher doses were necessary to disrupt escape responding than the doses which interfered with avoidance. Several barbiturates, benzodiazepines and antidepressants were also tested and found to disrupt both avoidance and escape at similar doses.

It is apparent from the results of the present experiment that clozapine, haloperidol and chlordiazepoxide reduced avoidance responding but also disrupted the ability of the animals to escape from the shock. Thus the present procedure did not provide a clear distinction between the effects of the antipsychotic drugs and those of the anxiolytic. It is not clear why this was so although it is probably related to the many procedural differences between this and other studies. For example, in the present study the durations of the CS and of the shock were relatively short (4 sec and 3 sec) whereas in the study of Davidson and Weidley [9] the corresponding durations were 10 sec and 15 sec. It is possible, that, had longer durations been selected for use in the present study, a distinction between the effects of the different drugs may have emerged. Another possibility is that the use of a two-way shuttle avoidance response in the present study may have led to the lack of differentiation between the different drugs. Previous studies have made use of one-way shuttle [2,6], pole-jump [7] or lever press [9] avoidance responses. Experiments are presently being carried out in the author's laboratory to compare the effects of drugs on avoidance responding with different response requirements.

Repeated administration of clozapine, haloperidol and chlordiazepoxide showed clear differences between the ef-

fects of the three drugs. Complete tolerance occurred to the avoidance-disrupting effect of clozapine after three or four daily injections. Similar tolerance has been reported to the effects of clozapine on locomotor activity in mice [25] and to the disruption of positively reinforced responding in rats [10,11]. Taken together, these results suggest that the effects of clozapine in these different behavioral situations are due to a common effect, perhaps muscle relaxation or sedation, to which tolerance occurs rapidly. This further suggests that the disruption of avoidance responding produced by clozapine cannot be an indication of its clinical antipsychotic efficacy.

The effects of repeated administration of haloperidol were quite different from those of clozapine. With daily injections of haloperidol the disruption of escape/avoidance responding became gradually more pronounced. Inclusion of a group of rats injected after the first three daily tests and before the test on the fourth day showed that the increasing drug effect was not due to accumulation of the drug and required rats to be repeatedly tested in the drugged state. Similar results have been reported by Hayashi and his coworkers [16] who found that the disruption of avoidance behavior produced by haloperidol increased with repeated dosing but only if the rats were tested after each drug administration. Furthermore, these researchers found that the increasing potency of haloperidol after repeated administration was not associated with changes in the serum levels of the drug. Similarly, it has been shown that repeated administration of pimozide produces an increase in the disruption of one-way avoidance responding and that this effect is not due to drug accumulation [2].

These effects of repeated administration of dopamine antagonist drugs are particularly interesting in relation to the theoretical account of neuroleptic action proposed by Wise [27]. Wise and his colleagues [14,28] have shown that when rats are trained to make responses reinforced with food or electrical brain stimulation repeated administration of pimozide leads to a gradual decline in responding. This, together with other findings, has been interpreted as showing that dopamine antagonist drugs produce a motivational deficit, positive reinforcers no longer being reinforcing (anhedonia). The present results with haloperidol suggest that a similar effect is seen when responding is motivated by negative reinforcement. It is not apparent that this effect can be encompassed by the anhedonia theory.

In the present experiment the effects of daily administration of a dose of chlordiazepoxide differed from the effects of repeated injection of either clozapine or haloperidol. The effects of chlordiazepoxide to disrupt avoidance responding showed neither tolerance nor an augmentation. The failure to observe any tolerance was somewhat surprising as previous studies have shown that tolerance to the muscle relaxant or sedative effects of benzodiazepines can develop very rapidly [12,19]. However, the rate of tolerance development depends on a number of factors, including drug dose [13]. It is evident that more than four daily administrations of 30 mg/kg of chlordiazepoxide are necessary for tolerance to develop to the disruption of avoidance responding.

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