Gender Differences in the Effects of Haloperidol on Avoidance Conditioning in Mice

M. CARMEN ARENAS†, ANDRÉS PARRA* AND VICENTE M. SIMÓN*1

*Area de Psicobiología, Facultad de Psicología, Universitat de València, 46071 Valencia, Spain
†Area de Psicobiología, Facultad de Psicología, Universidad de Málaga, Málaga, Spain

Received 7 February 1994

ARENAS, M. C., A. PARRA AND V. M. SIMÓN. Gender differences in the effects of haloperidol on avoidance conditioning in mice. PHARMACOL BIOCHEM BEHAV 51(4) 601-609, 1995. — Gender differences in the effects of haloperidol (0.075 mg/kg per day for 5 days) on avoidance conditioning were evaluated. We also studied performance of the subjects free of the drug and the acute effects of haloperidol in animals trained without drug 48 h after the last haloperidol administration. Latencies of escape and avoidance responses, number of nonresponses, escapes, avoidances, crossings during the adaptation period, crossings during intertrial intervals, and total crossings per minute were analyzed. This dosage impaired conditioning of the male animals but did not attain the same effects on females. Haloperidol did not deteriorate performance of the task when it had been learned previously without drug. The results confirm the existence of gender differences in haloperidol effects on avoidance conditioning in mice and suggest that these differences are related to the learning process and not only to the impairment of motor behavior characteristic of neuroleptic drugs.

Haloperidol Gender differences Escape Avoidance Mice

Numerous investigations have shown that haloperidol produces a dose-dependent impairment in active avoidance conditioning (4,5,15,20,23,39,40,46,49,51,53,60-62). These effects of haloperidol on the learning of a conditioned avoidance response have been explored in studies using acute administration (4,5,23,40,46,49,51,60-62) as well as in those in which haloperidol was administered repeatedly (15,20,53).

To find the specific effects of haloperidol on acquisition it is appropriate to study the action of the drug over several training sessions. Blackburn and Phillips (15) found that a dose of 0.15 mg/kg per day completely blocked avoidance responses, whereas a smaller dose of 0.075 mg/kg per day deteriorated acquisition, although a certain amount of avoidance responses were still present.

On the other hand, haloperidol deteriorates, in a dose-dependent manner, the motor activity of experimental animals (3,13,19,21,35,44). This action affects behaviors that require a motor response, thus influencing all studies that use operant behavior (6,10,11) and making a careful differentiation between effects on learning and purely motor effects necessary.

Generally, male rodents are used in this kind of study because the activity levels of these animals are not subject to the cyclic oscillations of their female conspecifics (11). Despite this, some gender differences in the effects of haloperidol have been detected. Using a differential reinforcement of low rate (DRL) program, Van Hest et al. (59) found that male mice were more sensitive to the inhibitory effects of the drug than were females. On the contrary, Dalton et al. (22) found that haloperidol increased the lever pressing used to produce cocaine self-administration more in females than in males.

The prevalence of side-effects in the clinical use of neuroleptics has also shown differences according to gender. tardive dyskinesia is more frequent in postmenopausal females than in a group of males of a similar age (54). Likewise, some clinical studies have found a superior therapeutic action of neuroleptics in women than in men (37).

In a previous work (5) we intended to find out whether these gender differences of haloperidol effects were evident in a unique training session of an active avoidance task. Using a dose (0.25 mg/kg) that clearly deteriorates avoidance re-

1 Requests for reprints should be addressed to Vicente M. Simón, Area de Psicobiología, Facultad de Psicología, Universitat de València, Aptdo. 22109, 46071 Valencia, Spain.
Drugings during the adaptation period (Adapt-Cross), number of measures of conditioning [(response latencies of avoidances (LAT-As), response latencies of escapes (LAT-Es), number of avoidances (As), number of escapes (Es), and number of nonresponses (NRs)); b) activity measures [number of crossings during the adaptation period (Adapt-Cross), number of crossings during intertrial intervals (ITI-Cross), and total crossings (As + Es + Adapt-Cross + ITI-Cross)/minute (T-Cross/minute)].

Acquisition phase. The subjects were randomly assigned to one of four groups (n = 18): saline males, saline females, haloperidol males, and haloperidol females. A dose of 0.075 mg/kg per day of haloperidol was administered for 5 consecutive days. The same volume of saline was injected in control animals. Each subject was tested in the shuttle-box 30 min after injection. The tests were run between the second and the 10th h of the dark phase.

Performance test. Forty-eight hours after the last experimental session of the preceding phase, all subjects were tested to exclude the possibility of state-dependent learning (47). In each group half of the animals continued the same treatment; the other half was shifted to the contrary pharmacologic condition. Thus, we obtained the following groups in each gender (n = 9): saline-saline (S-S), saline-haloperidol (S-H), haloperidol-saline (H-S), and haloperidol–haloperidol (H-H).

Statistical Analysis

Nonparametric Kruskal-Wallis tests were used to assess the variance of the behavioral measures (escapes, avoidances, nonresponses, crossings during the adaptation period, and crossings during ITIs) over different groups. Subsequently, appropriate paired comparisons were carried out using Mann-Whitney U tests to contrast the behavior among different treatment groups. Latencies of avoidance responses, latencies of escape responses, and total crossings were subjected to analysis of variance (ANOVA) for each day, with Gender and Treatment as the main factors, supplemented by Newman-Keuls pairwise comparisons and tests of simple main effects.

RESULTS

Acquisition Phase

Tables 1 and 2 show the results of this phase. Table 1 presents measures of avoidance conditioning. With the Kruskal-Wallis test, significant differences among groups were found in the number of avoidances and nonresponses in the last 3 days (p < 0.01). For both genders, haloperidol-treated subjects had fewer avoidances (day 2, p < 0.027; days 3–5, p < 0.0001), fewer escapes (day 1, p < 0.023, and day 3, p < 0.032), and more nonresponses (day 1, p < 0.021, day 3, p < 0.021, day 4, p < 0.0006, and day 5, p < 0.0007) than their saline controls.

When both genders were compared (independently of the treatment condition), males showed more avoidances than females on days 3–5 (p < 0.05). Taking into account gender and pharmacologic treatment, control males showed higher number of avoidances than control females on day 3 (p < 0.004) and day 4 (p < 0.02). Haloperidol-treated males showed fewer avoidances than control males in four of the five training days (day 2, p < 0.03; days 3–5, p < 0.0001); fewer escapes on day 3 (p < 0.001), and more nonresponses on day 2 (p < 0.03) and days 3–5 (p < 0.0001). However, treated females had fewer avoidances than controls only on day 4 (p < 0.01).

With respect to the latencies of the avoidance responses, males (irrespective of pharmacologic treatment) presented longer latencies than females on day 1 [F1, 27] = 4.39; p < 0.045]. The interaction of Gender × Treatment was statistically significant on day 4 [F1(49) = 6.56; p < 0.013].
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The post-hoc analysis for this interaction showed that haloperidol-treated males had shorter latencies than their female counterparts \( (p < 0.05) \). The simple main effects revealed that also on day 4, haloperidol-treated males had shorter latencies than control males \( F(1, 49) = 4.99; p < 0.03 \), and that the treatment of females was not statistically significant \( F(1, 49) = 2.17; p > 0.05 \).

Haloperidol increased the latencies of the escape responses on all days \( [day 1: F(1, 53) = 6.56, p < 0.013; day 2: F(1, 61) = 5.74, p < 0.02; day 3: F(1, 61) = 8.66, p < 0.005; day 4: F(1, 61) = 9.50, p < 0.003; and day 5: F(1, 63) = 15.48, p < 0.0002] \). The interaction of Gender \( \times \) Treatment was statistically significant on day 1 \( F(1, 53) = 10.63, p < 0.002 \), and the post-hoc analysis showed that control males had shorter latencies than their female counterparts \( (p < 0.05) \) or haloperidol-treated males \( (p < 0.01) \). The simple main effects revealed gender differences for control animals on day 1 \( F(1, 53) = 17.34, p < 0.0001; day 2: F(1, 61) = 4.18, p < 0.045; day 3: F(1, 61) = 5.15, p < 0.027; day 4: F(1, 61) = 4.68, p < 0.035; and day 5: F(1, 63) = 10.50, p < 0.002], and only on the last 2 days in the case of the females \( day 4: F(1, 61) = 4.83, p < 0.032; and day 5: F(1, 63) = 5.49, p < 0.022 \).

Table 2 presents activity measures. Kruskal Wallis test showed significant variance on days 4 \( (p < 0.007) \) and 5 \( (p < 0.003) \) in the number of crossings during ITIs. Irrespective of pharmacologic treatment, males had a higher number of crossings during the adaptation period on day 2 \( (p < 0.03) \) and a higher number of crossings than females during ITIs on days 2, 4, and 5 \( (days 2 and 5, p < 0.05; day 4, p < 0.01) \).

Saline-treated males crossed more than saline-treated females on days 4 \( (p < 0.01) \) and 5 \( (p < 0.04) \) during ITIs. No gender differences were observed in saline-treated animals during the adaptation period.

The number of crossings made by haloperidol-treated animals (irrespective of sex) during the adaptation period was significantly smaller than that of their controls on the last training day \( (p < 0.02) \), and during ITIs, on the last 3 days \( (days 3 and 4, p < 0.05; day 5, p < 0.01) \). Haloperidol-treated males had fewer crossings during the adaptation period than saline-treated males on day 5 \( (p < 0.04) \), and during ITIs, on days 4 \( (p < 0.02) \) and 5 \( (p < 0.01) \), which was not observed in females.

The total number of crossings per minute in untreated animals was higher than in the treated ones in all sessions except the first \( [day 2: F(1, 71) = 4.41, p < 0.04; day 3: F(1, 71) = 12.7, p < 0.01; day 4: F(1, 71) = 18.27, p < 0.001; day 5: F(1, 71) = 20.99, p < 0.001] \). Concerning gender (irrespective of treatment), male mice showed a higher number of total crossings per minute than females on days 3-5 \( [day 3: F(1, 71) = 4.03, p < 0.05; day 4: F(1, 71) = 8.16, p < 0.006; and day 5: F(1, 71) = 4.65, p < 0.04] \). Saline-treated males showed more total crossings per minute than saline-treated females on day 3 \( F(1, 35) = 5.59, p < 0.03 \), and day 4 \( F(1, 35) = 5.49, p < 0.03 \).

Gender \( \times \) Treatment interaction was significant on day 3 \( F(1, 71) = 4.28, p < 0.05 \) and day 4 \( F(1, 71) = 7.74, p < 0.01 \). Haloperidol-treated males showed a lower number of total crossings per minute than saline-treated ones \( [day 3: F(1, 35) = 12.31, p < 0.001; day 4: F(1, 35) = 18.83, p < 0.0001; and day 5: F(1, 35) = 20.97, p < 0.0001] \). No statistically significant effects were observed for females.

### Table 1

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*Kruskal-Wallis test showed significant variance at \( p < 0.01 \).

†p < 0.05; ‡p < 0.01 (Mann-Whitney U test); §p < 0.05; **p < 0.01 (Mann-Whitney U test); ††p < 0.05 (ANOVA), compared with their saline controls.

§p < 0.05; **p < 0.01 (Mann-Whitney U test); †p < 0.05 (ANOVA), compared with their saline treated males.

‡‡p < 0.05 (ANOVA) compared with haloperidol treated males.
The effects of 0.075 mg/kg per day of haloperidol on an escape-avoidance task were studied in male and female mice trained over 5 consecutive days and tested 48 h after the last session of training. Concerning its effects on the learning task, the results are a further example of the well-known inhibitory effect of haloperidol on conditioned responses, diminishing the number of avoidance and escape responses and increasing the latencies of escape responses and the number of nonresponses (4, 5, 15, 40, 53, 60, 61). The decrease found in spontane-
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<td>2.54 ± 1.31</td>
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<td>114</td>
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<td>Nonresponses†</td>
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*Kruskal-Wallis test showed significant variance at p < 0.01.
†Kruskal-Wallis test showed significant variance at p < 0.05.
‡ANOVA showed significant variance in Treatment for males [LAT Es: F(3, 59) = 3.77, p < 0.015; T-Cross/min: F(3, 64) = 5.41, p < 0.01].
§p < 0.05; #p < 0.01 (Mann-Whitney U test), compared with H-H.
#p < 0.05 (Mann-Whitney U test), compared with S-S.
ous motor activity also confirms previous findings that are well documented in the literature (19,21,35,44).

Before discussing gender differences in the effects of haloperidol we must comment on those found in the control animals. Although it has been reported for rats that females learn an avoidance task more quickly than do males (58), we found that untreated male mice had more avoidance responses and shorter escape latencies than their female counterparts. However, such gender differences were only found in rats younger than 90 days of age and when the experiment was carried out during the light phase of the day cycle (8,9). Because our results were obtained in the dark phase of the light cycle they cannot be considered completely contradictory. It is also interesting to note that in other indicators of learning, such as number of escapes, avoidance latencies, or nonresponses, no differences between males and females were found in the control animals. Nevertheless, we observed some gender differences in the motor activity of these subjects, as males had more crossings than females in some sessions. These gender differences in motility were only evident in the control subjects but not in the animals treated with haloperidol, because in the latter the activity of the males was lowered by the drug to the same level as that of the females, which was not affected.

The results concerning gender differences in the effects of haloperidol on the learning of the escape-avoidance task confirm previous findings of our laboratory (5) obtained with

FIG. 1. Response distributions in nonresponses, escapes, and avoidances during the acquisition phase of the avoidance conditioning in a two-way shuttle-box.

FIG. 2. Decrease in the number of avoidances in haloperidol treated subjects during the acquisition phase.
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an identical procedure but applied only to one experimental session. These results are also in accord with those of van Hest et al. (59), who, using a DRL 15 s schedule in an operant chamber, reported that male rats were more sensitive to the inhibitory effects of the drug than were female animals.

With respect to the performance test, our results show that previous training without haloperidol in the acquisition phase prevents part of the deteriorating effects of the acutely administered drug, thus corroborating the viewpoint of Beninger et al. (10) and the results of Sanger (53) and Blackburn and Phillips (17). The group that received haloperidol in the performance test, after having been trained free of drug (S-H), was not statistically different from the group always receiving saline (S-S). We conclude, therefore, that the acute administration of 0.075 mg/kg of haloperidol does not deteriorate the performance of a previously learned task. Our results also exclude the possibility of state-dependent learning, because the animals tested free of drug behaved in a manner similar to that during the training period under haloperidol. This situation was considered by Overton (47) to be an impairment of memorization.

In search of an explanation for the disruptive effects of neuroleptics on conditioned behaviors, several authors have underlined their deleterious effects on motor activity (31–34). They showed that low doses of haloperidol (0.04–0.16 mg/kg) disrupt the process of initiating the response in a way reminiscent of some Parkinsonian symptoms in human beings (33). Nevertheless, a number of studies furnish evidence in favor of mechanisms of action of haloperidol different from an impairment of motor behavior (7,22,28–30,41,55,61). The motor explanation of the effects of haloperidol cannot explain why the animals that were trained under haloperidol but tested free of drug still showed the same impairment of conditioned behavior. In a previously mentioned study (5), haloperidol increased the number of nonresponses in males and not in females, but a similar gender difference was not found in the number of crossings during the adaptation periods or during the ITIs. This absence of gender differences in motility measures seems to stress the specificity of the differences found in the learning task. Likewise, in the present work gender differences in avoidance conditioning appeared earlier in training than similar differences found in motor activity, also suggesting different mechanisms of action for both effects of the drug.

Some authors have proposed that dopaminergic antagonists could impair the establishment of an association between the conditioned stimulus and the appropriate response, which has been called the theory of the dissociative effects (10,12). The fact that haloperidol does not deteriorate the response in animals trained without the drug, as is the case in our experiment, also supports this theory.

The anhedonia hypothesis has been the theoretical approach generating most of the studies attempting to elucidate the mechanism of action of neuroleptics in reinforced behavior (27,52,63). It presumes that neuroleptics interfere with conditioned responses by decreasing the reinforcing power of the stimuli. In the case of aversively motivated behavior it seems more appropriate to speak of an apathy hypothesis in the sense of Acquas et al. (11), where the neuroleptic treatment would produce a lack of motivation to avoid shock.

Another possible explanation of the effects of haloperidol on avoidance behavior may include the possibility of freezing, by which rodents become immobile in the presence of a dangerous stimulus (16). Although this behavior has been observed in our experiments, no exact measurement has been made and we are not presently in the position to draw conclusions about its possible explanatory power.

Numerous explanations for the gender differences found in the action of neuroleptics have been put forward. In the first place we must consider possible sexual disparities in the pharmacokinetics of these and other psychotropic drugs. Higher plasmatic levels of neuroleptics have been found in men than in women, despite administration of equivalent doses (18). This fact could be explained by differences in absorption due to disparities in the gastric secretion of chloride acid in the two sexes, to a prolonged gastrointestinal transit of drugs dur-
ing the luteinic phase of the menstrual cycle (64), or to gender differences in hepatic catabolism (36).

Other studies carried out with tissue cultures have shown that dopaminergic mesencephalic neurons were more numerous in cultures prepared from female mesencephalon than those from male mesencephalon, and female cultures contained more endogenous DA than did males cultures. These differences seem to be under genetic control and are independent of gonadal hormones (14, 50). It is well known that dopamine mesencephalic cells are target cells for haloperidol (24-26, 57), which could be the basis for some of the behavioral differences described. On the other hand, estrogens seem to modulate central dopaminergic transmission (38, 42, 43, 48, 56, 64), although the mechanism by which such modulatory action takes place is not known.

In conclusion, our results confirm the existence of gender differences in the effects of haloperidol on the learning of an avoidance task in mice, and suggest that these differences are related to the learning process and not only to the impairment of motor behavior characteristic of neuroleptic drugs. The dose of 0.075 mg/kg per day used in this experiment was sufficient to impair conditioning of the male animals but was clearly too small to attain the same effects in the females, although one may suppose that higher doses would also produce deteriorating effects in the latter. It has also been demonstrated that this dose is insufficient to deteriorate the performance of the avoidance task, even in males, if the task was previously learned in the absence of haloperidol. It is possible that continued administration of the drug would end by producing the same effect.) These findings could have clear implications for the clinical use of neuroleptics and strengthen the need for more basic as well as clinical research in this field.

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