Pergamon

Pharmacology Biochemistry and Behavior, Vol. 51, No. 4, pp. 601–609, 1995

0091-3057/95 $9.50 + .00

0091-3057(94)00382-3

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 autoadministration 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.

sponses (53), we found that males had fewer escape responses and showed more nonresponses than females. Both genders presented a reduced number of avoidances that were practically inhibited by the drug. Subjects did not show gender differences in motor activity, as measured by the number of crossings during the adaptation period and between trials, which suggests that the differences found in escape-avoidance behavior did not reflect a purely motor inhibition.

The present work was designed to further evaluate gender differences in the acquisition and performance of active avoidance in mice, also controlling the effects of the drug on motor behavior. For this purpose the effects of the daily administration (for 5 days) of 0.075 mg/kg haloperidol on the acquisition of a conditioned avoidance response were explored. Forty-eight hours after the last drug administration, performance was evaluated in the drug-free subjects and some of the saline-treated animals were tested under haloperidol. After this lapse of time no residual effects of haloperidol on behavior are usually found (2,44).

METHOD

Subjects

Thirty-six female and 36 male OF1 mice from Iffa Credo (Lyon, France), weighing between 24 and 32 g and 32 and 44 g, respectively, at the start of the experiment, were used as experimental animals. They arrived in the laboratory at 42 days of age and were housed for 12 days, males and females separate, in groups of five or six animals in translucent plastic cages (25 × 25 × 14.5 cm) under a reversed light–dark cycle (lights off at 0700–1900 h) and with controlled room temperature (22 ± 3°C).

Drug

Haloperidol (Syntex Latino, Spain) was diluted with 0.9% saline to obtain a dose of 0.075 mg/kg. Controls received 0.9% saline alone. Injections were administered intraperitoneally (IP) in a volume of 10 ml/kg.

Apparatus

We used a computerized, two-way shuttle-box (Shuttle Scan Model SC-II; Omnitech Electronics, Inc., Columbus, OH) described in detail elsewhere (5) and RMS V.2.02 software (Omnitech Electronics).

Procedure

The test consisted of a) 3 min of adaptation to the apparatus, in which animals could explore the box and move freely; and b) 30 trials of two-way escape-avoidance (intertrial interval, 30 ± 10 s). Each trial consisted of the presentation of a light (6 W) in the compartment occupied by the mouse; after 5 s, the presentation was accompanied by a 0.3-mA foot-shock 10 s in duration. A conditioned avoidance response was defined as crossing to the opposite side during the only light period, an escape as crossing when the shock was on, and a nonresponse as the absence of crossing.

We determined the following behavioral parameters: a) 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, appropiate 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 [$F(1, 27) = 4.39$; $p < 0.045$]. The interaction of Gender × Treatment was statistically significant on day 4 [$F(1, 49) = 6.56$; $p < 0.013$]. The

TABLE 1

TOTAL NUMBER OF AVOIDANCES, ESCAPES, AND NONRESPONSES, AND MEAN LATENCIES OF ESCAPE AND AVOIDANCE RESPONSES ± SD

		Males				Females			
		Saline		Haloperidol		Saline		Haloperidol	
Parameters	Days	*N*	Latencies	*N*	Latencies	*N*	Latencies	*N*	Latencies
Avoidances	1	16	2.77 ± 1.49	14	2.32 ± 1.22	10	1.61 ± 1.60	6	1.33 ± 1.09
	2	53	2.68 ± 1.06	15†	1.98 ± 1.59	25	2.70 ± 1.12	6	3.19 ± 1.98
	3*	112	2.74 ± 1.05	11‡	2.33 ± 1.72	47**	3.29 ± 0.76	17	2.71 ± 1.34
	4*	209	3.08 ± 0.61	34‡	2.31 ± 1.13§	108#	2.90 ± 1.02	20†	3.51 ± 0.5‡‡
	5*	223	2.83 ± 0.55	47‡	2.65 ± 1.14	136	2.95 ± 1.17	42	3.26 ± 1.04
Escapes	1	207	8.49 ± 1.18	87	10.88 ± 1.56¶	157	10.24 ± 1.55‡‡	105	9.96 ± 1.88
	2	293	8.71 ± 1.50	181	9.83 ± 2.00§	258	8.72 ± 1.53	196	9.57 ± 1.44
	3	322	8.25 ± 1.40	166‡	9.41 ± 1.41§	236	8.38 ± 1.40	209	9.44 ± 1.87
	4	270	8.02 ± 1.68	217	9.11 ± 1.09§	214	7.71 ± 1.61	224	8.91 ± 1.44§
	5	242	7.52 ± 1.35	180	9.43 ± 1.51¶	184	8.25 ± 2.09	201	9.68 ± 1.96¶
Nonresponses	1	317		439		373		397	
	2	194		344†		257		308	
	3*	106		360‡		257		284	
	4*	61		289‡		218		266	
	5*	65		313‡		220		267	

*Kruskal-Wallis test showed significant variance at $p < 0.01$.
†$p < 0.05$; ‡$p < 0.01$ (Mann-Whitney *U* test); §$p < 0.05$; ¶$p < 0.01$ (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.

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 × 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 [$F(1, 53) = 10.35, p < 0.002$], and differences due to pharmacologic treatment in all sessions in the case of the males [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 ($p < 0.01$). This effect 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 [$F(1, 35) = 5.28, p < 0.03$], day 4 [$F(1, 35) = 9.95, p < 0.003$], and day 5 [$F(1, 35) = 5.49, p < 0.03$].

Gender × Treatment interaction was significant on day 3 [$F(1, 71) = 4.28, p < 0.05$] and day 4 [$F(1, 71) = 7.04, p < 0.01$]. 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 2

TOTAL NUMBER OF CROSSINGS DURING THE ADAPTATION (ADAPT-CROSS) AND DURING ITIs (ITI-CROSS) AND MEAN TOTAL CROSSINGS PER MINUTE (T-CROSS/MIN) ± SD

		All Animals		Males		Females		
Parameters	Days	Saline	Haloperidol	Saline	Haloperidol	Saline	Haloperidol	All Males vs. All Females
Adapt-Cross	1	196	156	100	74	96	82	NS
	2	72	89	40	71	32	18	$p < 0.03$
	3	155	92	84	60	71	32	NS
	4	255	158	146	76	109	82	NS
	5	320	174†	175	86†	145	88	NS
ITI-Cross	1	227	205	121	112	106	93	NS
	2	259	179	155	115	104	64	$p < 0.05$
	3	515	213†	369	118	146	95	NS
	4*	702	287†	515	169†	187**	118	$p < 0.01$
	5*	858	370‡	560	228‡	298#	142	$p < 0.04$
T-Cross/min	1	0.97 ± 0.67	0.69 ± 0.75	1.07 ± 0.71	0.66 ± 0.62	0.87 ± 0.63	0.72 ± 0.89	NS
± SD	2	1.24 ± 0.9	0.83 ± 0.74§	1.39 ± 0.77	0.91 ± 0.76	1.09 ± 1.00	0.76 ± 0.73	NS
	3	1.81 ± 1.39	0.9 ± 0.68¶	2.31 ± 1.56	0.86 ± 0.61¶	1.3 ± 1.01††	0.93 ± 0.76	$p < 0.05$
	4	2.38 ± 1.63	1.2 ± 0.81¶	3.14 ± 1.7	1.22 ± 0.79¶	1.62 ± 1.15‡‡	1.17 ± 0.85	$p < 0.006$
	5	2.66 ± 1.66	1.29 ± 0.88¶	3.28 ± 1.58	1.33 ± 0.87¶	2.05 ± 1.55††	1.25 ± 0.91	$p < 0.04$

*Kruskal-Wallis test showed significant variance at $p < 0.01$.
†$p < 0.05$; ‡$p < 0.01$ (Mann-Whitney U test); §$p < 0.05$; ¶$p < 0.01$ (ANOVA), compared with their saline controls.
#$p < 0.05$; **$p < 0.01$ (Mann-Whitney U test); ††$p < 0.05$; ‡‡$p < 0.01$ (ANOVA), compared with saline treated males.

Performance Test

Table 3 presents the results of the performance test. Kruskal-Wallis analysis showed significant variance among groups in the number of avoidances ($p < 0.0003$), nonresponses ($p < 0.04$), and ITI-crossings ($p < 0.03$).

When tested free of drug, the subjects trained on haloperidol made fewer avoidances than those trained off haloperidol (H-S vs. S-S, $p < 0.003$). These differences were observed in males (H-S vs. S-S, $p < 0.02$) but not in females. No statistically significant differences were found between H-S and H-H groups (either in males or females).

In animals pretrained with saline, haloperidol decreased the number of ITI-crossings (S-H vs. S-S, $p < 0.035$). However, the S-H subjects showed a higher number of avoidances and a lower number of nonresponses than the H-H ones ($p < 0.001$ and $p < 0.005$, respectively). These differences were found in males (S-H vs. S-S, ITI-crossings: $p < 0.02$; S-H vs. H-H, avoidances: $p < 0.001$, and nonresponses: $p < 0.01$), but not in females.

The ANOVA analysis performed with data of the latencies of avoidances, latencies of escapes, and total crossings per minute showed that the factor Treatment was significant for the three parameters [$F(3, 47) = 2.85, p < 0.047$; $F(3, 59) = 3.15, p < 0.032$; $F(3, 64) = 6.77, p < 0.0005$, respectively]. Pairwise comparisons showed that the H-H group had shorter avoidance latencies than the S-H group ($p < 0.05$), and longer escape latencies ($p < 0.05$) and fewer total crossings per minute ($p < 0.01$) than the S-S group. Also, the H-S group presented fewer total crossings per minute than the S-S group ($p < 0.01$). In two measures, the simple main effects showed that Treatment was significant in males [LAT-Es: $F(3, 59) = 3.77, p < 0.015$; T-crossings per minute: $F(3, 64) = 5.41, p < 0.002$] but not in females [NS]. The factor Gender and the interaction Gender × Treatment were not significant for any of the parameters.

In summary, with respect to gender differences in the effects of haloperidol, the drug clearly increased the number of nonresponses in males, and did not produce significant changes in this variable in females (Fig. 1). Avoidance responses in the treated males decreased significantly from the 2nd training day, whereas in females this decrease only reached significance on the 4th day of treatment (Fig. 2). Likewise, haloperidol treatment increased the latencies of escape responses, an increase that was significant on all last training days for the males but only on the last 2 days for the females. In general, haloperidol deteriorated behavior more in males than females.

Such differences became more evident in the group of animals tested free of drug in the performance test. Males trained (in the acquisition phase) under the influence of haloperidol and tested later without drug (H-S) had fewer avoidance responses than their saline controls (S-S). Furthermore, their performance did not differ from that of the animals that received haloperidol before the performance test (H-H). On the contrary, the performance of females was not significantly affected by the haloperidol treatment (S-S = H-S = H-H), although the tendency observed, especially in avoidances and nonresponses, was the same as in males: an impairment of the acquisition of avoidance responses (Fig. 3).

DISCUSSION

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-

TABLE 3

TOTAL NUMBER OF AVOIDANCES, ESCAPES, NONRESPONSES, CROSSINGS DURING THE ADAPTATION (ADAPT CROSS), AND CROSSINGS DURING ITIs (ITI + CROSS); MEAN LATENCIES OF AVOIDANCES RESPONSE (LAT-As) AND ESCAPE RESPONSES (LAT-Es) ± SD, AND MEAN TOTAL CROSSINGS PER MINUTE (T-CROSS/MIN) ± SD

	Males				Females			
Parameters	S-S	H-S	H-H	S-H	S-S	H-S	H-H	S-H
Avoidances*	123	32#	13	71¶	75	27	22	59
LAT-As ± SD	2.71 ± 0.43	2.55 ± 1.31	1.72 ± 1.38	2.97 ± 0.66	2.65 ± 0.40	2.54 ± 1.25	2.34 ± 1.01	3.13 ± 0.47
Escapes	92	114	93	147	114	138	113	143
LAT-Es ± SD‡	7.11 ± 1.26	8.73 ± 2.31	9.49 ± 1.09	7.99 ± 1.44	7.97 ± 1.92	8.71 ± 1.33	8.68 ± 1.48	8.40 ± 1.35
Nonresponses†	55	124	164	52§	81	105	105	68
Adapt-Cross	86	42	63	71	88	65	64	74
ITI-Cross†	343	89	99	175#	201	99	47	153
T-Cross/min ± SD‡	3.6 ± 2.24	1.45 ± 1.06	1.28 ± 0.58	2.43 ± 1.00	2.61 ± 1.89	1.66 ± 1.15	1.41 ± 1	2.22 ± 1.09

*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.
S-S: Saline–saline; H-S: haloperidol–saline; H-H: haloperidol–haloperidol; and S-H: saline–haloperidol.

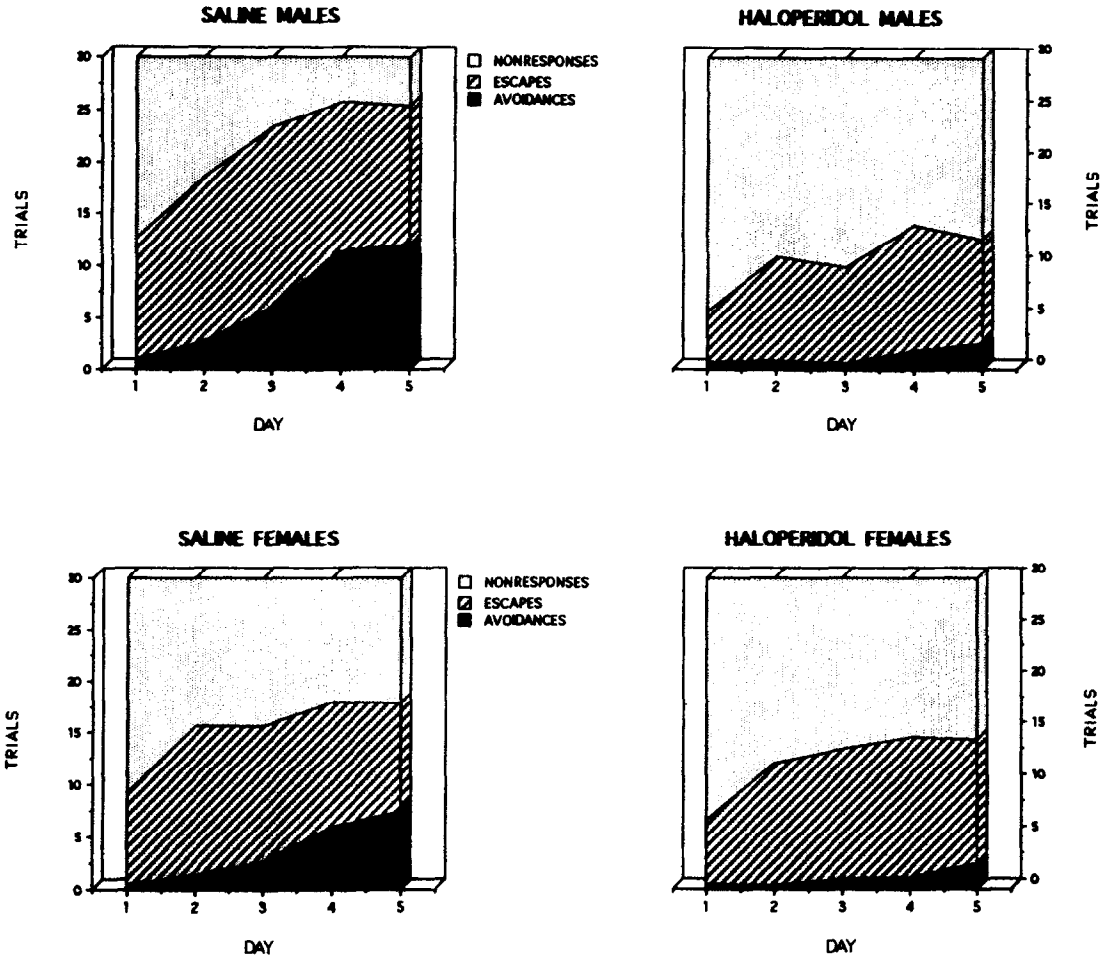

FIG. 1. Response distributions in nonresponses, escapes, and avoidances during the acquisition phase of the avoidance conditioning in a two-way shuttle-box.

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

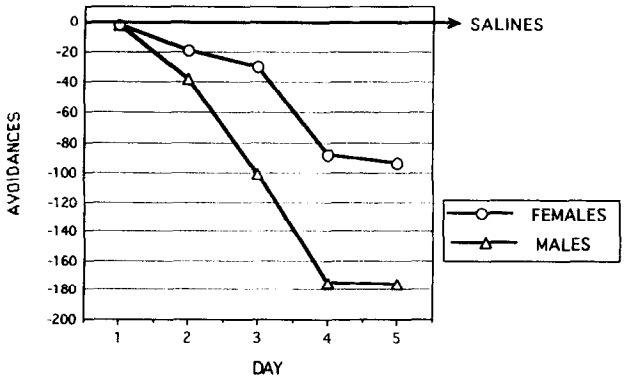

FIG. 2. Decrease in the number of avoidances in haloperidol-treated subjects during the acquisition phase.

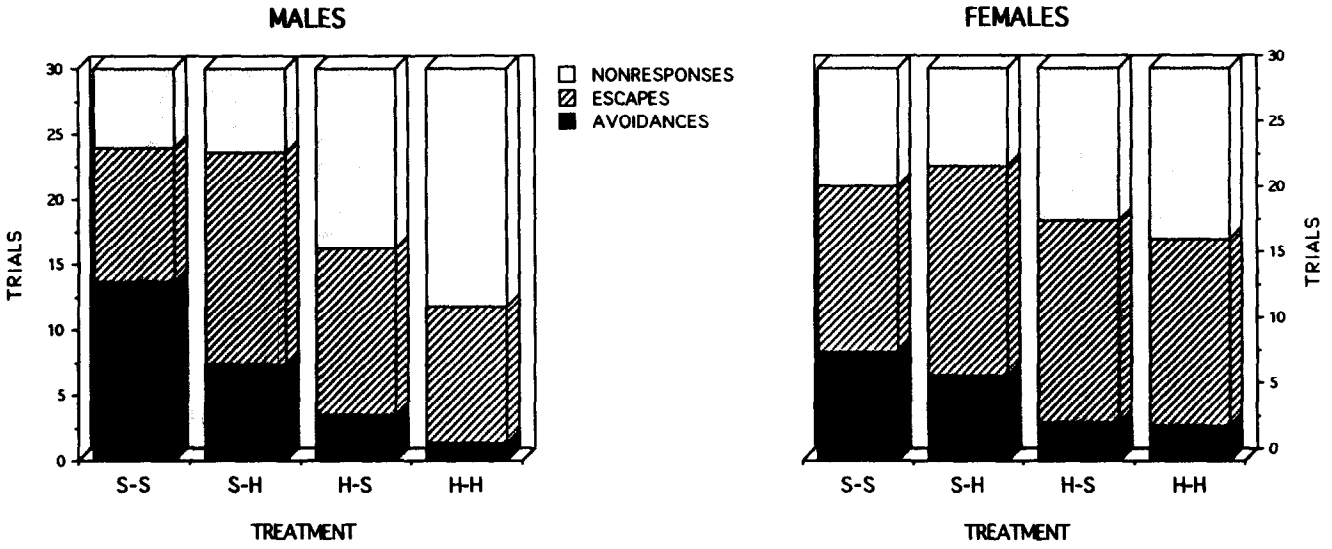

FIG. 3. Response distributions in nonresponses, escapes, and avoidances during the performance test of the avoidance conditioning in a two-way shuttle-box. S-S: Saline-saline; S-H: saline-haloperidol; H-S: haloperidol-saline; and H-H: haloperidol-haloperidol.

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 appropiate 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 chloridic 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 transmision (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.

REFERENCES

1. Acquas, E.; Carboni, E.; Leone, P.; DiChiara, G. SCH 23390 blocks drug-conditioned place-preference and place-aversion: Anhedonia (lack of reward) or apathy (lack of motivation) after dopamine-receptor blockade? Psychopharmacology 99:151-155; 1989.
2. Aguilar, M. A.; Rodríguez-Arias, M.; Miñarro, J.; Simón, V. M. Time course of the effects of haloperidol on agonistic behaviour in male mice. Psicothema 6:191-196; 1994.
3. Ahlenius, S.; Hillegaart, V. Involement of extrapyramidal motor mechanisms in the suppression of locomotor activity by antipsychotic drugs: A comparison between the effects produced by pre and postsynaptic inhibition of dopaminergic neurotransmission. Pharmacol. Biochem. Behav. 24:1409-1415; 1986.
4. Anisman, H.; Zacharko, R. M. Stimulus change influences escape performance: Deficits induced by uncontrollable stress and by haloperidol. Pharmacol. Biochem. Behav. 17:263-269; 1982.
5. Arenas, M. C.; Parra, A.; Simón, V. M. Gender differences in escape-avoidance behavior of mice after haloperidol administration. Pharmacol. Biochem. Behav. 44:233-236; 1993.
6. Asin, K. E.; Fibiger, H. C. Force requirements in lever-pressing and responding after haloperidol. Pharmacol. Biochem. Behav. 20:323-326; 1984.
7. Asin, K. E.; Wirtshafter, D. Evidence for dopamine involvement in reinforcement obtained using a latent extinction paradigm. Pharmacol. Biochem. Behav. 36:417-420; 1990.
8. Bauer, R. Ontogeny of two way avoidance behavior in male and female rats. Dev. Psychobiol. 11:103-116; 1978.
9. Beatty, W. W. Gonadal hormones and sex differences in nonreproductive behaviors in rodents: Organizational and activational influences. Horm. Behav. 12:112-163; 1979.
10. Beninger, R. J. The role of dopamine in locomotor activity and learning. Brain Res. Rev. 6:173-196;1983.
11. Beninger, R. J. Dissociating the effects of altered dopaminergic function on performance and learning. Brain Res. Bull. 23:365-371; 1989.
12. Beninger, R. J.; Mason, S. T.; Phillips, A. G.; Fibiger, H. C. The use of conditioned suppression to evaluate the nature of neuroleptic-induced avoidance deficits. J. Pharmacol. Exp. Ther. 213: 623-627; 1980.
13. Bernardi, M. M.; De Souza, H.; Palermo-Neto, J. Effects of single and long-term haloperidol administration on open field behavior of rats. Psychopharmacology 73:171-175; 1981.
14. Beyer, C.; Pilgrim, C.; Reisert, I. Dopamine content and metabolism in mesencephalic and diencefalic cell cultures: Sex differences and effects of sex steroids. J. Neurosci. 11:1325-1333; 1991.
15. Blackburn, J. R.; Phillips, A. G. Blockade of acquisition of one-way conditioned avoidance responding by haloperidol and metoclopramide but not by thioridazine or clozapine: Implications for screening new antipsychotic drugs. Psychopharmacology 98: 453-459; 1989.
16. Blackburn, J. R.; Phillips, A. G. Enhancement of freezing behaviour by metoclopramide: Implications for neuroleptic-induced avoidance deficits. Pharmacol. Biochem. Behav. 35:685-691; 1990.
17. Blackburn, J. R.; Phillips, A. G. Role of prior experience in blocking the disruptive effects of neuroleptic drugs on active avoidance by rats. Psychobiology 18:35-42; 1990.
18. Bowers, M. B. Jr.; Meltzer, H. J.; Menninger, G. R. Correlation of plasma and CSF prolactin with CSD GABA during neuroleptic treatment. Life Sci. 31:59-62; 1982.
19. Bunsey, M. D.; Sanberg, P. R. The topography of the locomotor effects of haloperidol and domperidone. Behav. Brain Res. 19: 147-152; 1986.
20. Carey, R. J.; Kenney, S. A delayed onset of haloperidol effects on learned escape and avoidance behavior. Pharmacol. Biochem. Behav. 28:203-208; 1987.
21. Carey, R. J.; Kenney, S. Operant conditioning and haloperidol-induced hypokinetic effects. Neuropsychobiology 18:199-204; 1987.
22. Dalton, J. C. H.; Vickers, G. J.; Roberts, D. C. S. Increased self-administration of cocaine following haloperidol: Sex-dependent effects of the antiestrogen tamoxifen. Pharmacol. Biochem. Behav. 25:497-501; 1986.
23. Davidson, A. B.; Weidley, E. Differential effects of neuroleptic and other psychotropic agents on acquisition of avoidance in rats. Life Sci. 18:1279-1284; 1976.
24. Davis, K. L.; Kahn, R. S.; Ko, G.; Davidson, M. Dopamine in schizophrenia: A review and reconceptualization. Am. J. Psychiatry 148:474-1486; 1991.
25. Doherty, M.; Gratton, A. Behavioral evidence of depolarization block of mesencephalic dopamine neurons by acute haloperidol in partially 6-hydroxydopamine-lesioned rats. Behav. Neurosci. 105:579-587; 1991.
26. Ellenbroek, B. A.; Artz, M. T.; Cools, A. R. The involvement of dopamine D_1 and D_2 receptors in the effects of the classical neuroleptic haloperidol and the atypical neuroleptic clozapine. Eur. J. Pharmacol. 196:103-108; 1991.
27. Ettenberg, A. Dopamine, neuroleptics and reinforced behavior. Neurosci. Biobehav. Rev. 13:105-111; 1989.
28. Ettenberg, A.; Camp, C. H. Haloperidol induces a partial reinforcement extinction effect in rats: Implications for a dopamine involvement in food reward. Pharmacol. Biochem. Behav. 25: 813-821; 1986.
29. Ettenberg, A.; Camp, C. H. A partial reinforcement extinction effect in water-reinforced rats intermittently treated with haloperidol. Pharmacol. Biochem. Behav. 25:1231-1235; 1986.
30. Ettenberg, A.; Duvauchelle, C. L. Haloperidol blocks the conditioned place preferences induced by rewarding brain stimulation. Behav. Neurosci. 102:687-691; 1988.
31. Fowler, S. C.; Ford, K. E.; Gramling, S. E.; Nail, G. L. Acute and subchronic effects of neuroleptics on quantitative measures

of discriminative motor control in rats. Psychopharmacology 84: 368-373; 1984.
32. Fowler, S. C.; LaCerra, M. M.; Ettenberg, A. Effects of haloperidol on the biophysical characteristics of operant responding: Implications for motor and reinforcement processes. Pharmacol. Biochem. Behav. 25:791-796; 1986.
33. Fowler, S. C.; Liao, R. M.; Skjoldager, P. A new rodent model for neuroleptic-induced pseudo-parkinsonism: Low doses of haloperidol increase forelimb tremor in the rat. Behav. Neurosci. 104:449-456; 1990.
34. Fowler, S. C.; Mortell, C. Low doses of haloperidol interfere with rat tongue extensions during licking: A quantitative analysis. Behav. Neurosci. 106:386-395; 1992.
35. Fujiwara, H. Comparative studies of sulpiride and classical neuroleptics on induction of catalepsy, locomotor activity, and brain dopamine metabolism in mice. Pharmacol. Biochem. Behav. 41: 301-308; 1992.
36. Gustafsson, J. A.; Mode, A.; Norstedt, G.; Skett, P. Sex steroid induced changes in hepatic enzymes. Ann. Rev. Physiol. 45:51-60;1983.
37. Halbreich, U.; Asnis, G.; Goldstein, S.; Nathan, R. S.; Zander, K.; Herne, J. V. Sex differences in response to psychopharmacological interventions in humans. Psychopharmacol. Bull. 20:526-530; 1984.
38. Harrer, S.; Schmidt, W. J. Oestrogen modulates dopamine-controlled behaviours in the male ferret. Eur. J. Pharmacol. 128: 129-132; 1986.
39. Hayashi, T.; Tadokoro, S.; Hashimoto, H.; Nakashima, M. Enhancement of avoidance-suppressing effect after repeated administration of haloperidol and serum haloperidol in rats. Pharmacol. Biochem. Behav. 17:131-136; 1982.
40. Hillegaart, V.; Ahlenius, S.; Magnusson, O.; Fowler, C. J. Repeated testing of rats markedly enhances the duration of effects induced by haloperidol on treadmill locomotion, catalepsy, and a conditioned avoidance response. Pharmacol. Biochem. Behav. 27:159-164; 1987.
41. Horvitz, J. C.; Ettenberg, A. Haloperidol blocks the response-reinstating effects of food reward: A methodology for separating neuroleptic effects on reinforcement and motor processes. Pharmacol. Biochem. Behav. 31:861-865; 1989.
42. Hruska, R. E.; Silbergeld, E. K. Increased dopamine receptor sensitivity after estrogen treatment using the rat rotation model. Science 208:1466-1468; 1980.
43. Levesque, D.; Di Paolo, T. Dopamine receptor reappearance after irreversible receptor blockade: Effect of chronic estradiol treatment of ovariectomized rats. Mol. Pharmacol. 39:659-665; 1991.
44. Lynch, M. R. Behavioral evidence for dopamine receptor subsentivity following chronic haloperidol. Neuropsychobiology 24: 102-108; 1990.
45. McDermott, J. L. Effects of estrogen upon dopamine release from the corpus striatum of young and aged female rats. Brain Res. 606:118-125; 1993.
46. Niemegeers, C. J. E.; Verbruggen, F. J.; Janssen, P. A. J. The influence of various neuroleptic drugs on shock avoidance responding in rats. I. Nondiscriminated Sidman avoidance procedure. Psychopharmacologia (Berlin) 16:161-174; 1969.
47. Overton, D. A. State dependent learning and drug discriminations. In: L. L. Iversen et al., eds. Drugs, neurotransmitters and behavior. New York: Plenum Press; 1984.
48. Palermo-Neto, J.; Dorce, V. A. C. Influences of estrogen and/or progesterone on some dopamine related behavior in rats. Gen. Pharmacol. 21:83-87; 1990.
49. Petty, F.; Mott, J.; Sherman, A. D. Potential locus and mechanism of blockade of conditioned avoidance responding by neuroleptics. Neuropharmacology 23:73-78; 1984.
50. Reisert, I.; Pilgrim, C. Sexual differentiation of monoaminergic neurons—genetic or epigenetic? Trends Neurosci. 14:468-473; 1991.
51. Rodríguez, R. Effect of various psychotropic drugs on the performance of avoidance and escape behaviors in rats. Pharmacol. Biochem. Behav. 43:1155-1159; 1992.
52. Salamone, J. D. The actions of neuroleptic drugs on appetitive instrumental behaviors. In: L. L. Iversen et al., eds. Handbook of psychopharmacology, vol. 19. New York: Plenum; 1987.
53. Sanger, D. J. The effects of clozapine on shuttle-box avoidance responding in rats: Comparisons with haloperidol and chlordiazepoxide. Pharmacol. Biochem. Behav. 23:231-236; 1985.
54. Smith, J. M.; Oswald, W. T.; Kucharski, L. T.; Waterman, L. J. Tardive dyskinesia: Age and sex differences in hospitalized schizophrenics. Psychopharmacology 58:207-211; 1978.
55. Spyraki, C.; Fibiger, H. C.; Phillips, A. G. Attenuation by haloperidol of place preference conditioning using food reinforcement. Psychopharmacology 77:379-382; 1982.
56. Tonnaer, J. A. D. M.; Leinders, T.; van Delft, A. M. L. Ovariectomy and subchronic estradiol-17β administration decrease dopamine D_1 and D_2 receptors in rat striatum. Psychoneuroendocrinology 14:469-476; 1989.
57. Vadasz, C.; Laszlovszky, I.; DeSimone, P. A.; Fleischer, A. Genetic aspects of dopamine receptor binding in the mouse and rat brain: An overview. J. Neurochem. 59:793-808; 1992.
58. van Haaren, F.; van Hest, A.; Heinsbrock, R. P. W. Behavioral differences between male and female rats: Effects of gonadal hormones on learning and memory. Neurosci. Biobehav. Rev. 14: 23-33; 1990.
59. van Hest, A.; van Haaren, F.; van de Poll, N. E. Haloperidol, but not apomorphine, differentially affects low response rates of male and female Wistar rats. Pharmacol. Biochem. Behav. 29: 529-532; 1988.
60. Wadenberg, M. L.; Ahlenius, S. Effects of raclopride and haloperidol on spontaneous motor activity and on conditioned avoidance behavior in rats. Drug Res. 41:692-695; 1991.
61. Weiner, I.; Feldon, J.; Katz, Y. Facilitation of the expression but not the acquisition of latent inhibition by haloperidol in rats. Pharmacol. Biochem. Behav. 26:241-246; 1987.
62. White, I. M.; Ciancone, M. T.; Haracz, J. L.; Rebec, G. V. A lever-release version of the conditioned avoidance response paradigm: Effects of haloperidol, clozapine, sulpiride, and BMY-14802. Pharmacol. Biochem. Behav. 41:29-35; 1991.
63. Wise, R. A. Neuroleptics and operant behaviour, the anhedonia hypothesis. Behav. Brain. Sci. 5:39-87; 1982.
64. Yonkers, K. A.; Kando, J. C.; Cole, J. O.; Blumenthal, S. Gender differences in pharmacokinetics and pharmacodynamics of psychotropic medication. Am. J. Psychiatry 149:587-595; 1992.