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The Circling Training Rat Model as a Behavioral Teratology Test

JUAN L. BRUSÉS, [1] PATRICIA M. BERNINSONE, SILVIA I. OJEA AND JULIO M. AZCURRA[1]

Laboratorio de Biologia Celular, Depto. de Ciencias Biológicas
Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires
1428 Buenos Aires, Argentina

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BRUSÉS, J. L., P. M. BERNINSONE, S. I. OJEA AND J. M. AZCURRA. *The circling training rat model as a behavioral teratology test.* PHARMACOL BIOCHEM BEHAV **38**(4) 739–745, 1991.—The properties of circling training (CT) for detecting behavioral teratologic drug-induced effects was evaluated by prenatal administration of two behavioral teratogenic drugs: vitamin A (80,000 IU/kg/day) and haloperidol (2.5 mg/kg/day). The circling training was started at 30 days of age and performed for 8 days in an automated apparatus. Statistically significant differences between drug-treated and control animals regarding the measured response (turns per minute) were found. Two components may affect the response measured by the CT: associative learning and motor performance. The incidence of these components was discriminated with behavioral and mathematical approaches. In the experimental conditions used the most affected parameter was motor performance. The results indicate that CT can be used as an instrumental conditioning test where the quantifiable endpoint is the on-going motor performance. Further applications of the CT for neurochemical evaluation of drug induced effects are also discussed.

Behavioral teratology Operant conditioning Circling training Caudate nuclei Vitamin A Haloperidol

THE value of behavioral tests as indicators of neurotoxicity due to drugs and chemicals has recently been ratified since they are sensitive and display the altered final output of the brain through a specific behavior (17). On this trend, the design of tests with a defined quantitative behavioral endpoint and which can be automated, may be useful for behavioral teratology studies.

On the other hand, the availability of biological models in which a defined animal behavior is related to measurable biochemical changes in a specific brain area, would be of value for neurochemical studies of drug-induced permanent effects. The need of such models for studies on functional teratology has been recently discussed (14).

On this basis, we focused on the circling trained rat model, in which the circling behavior has been described to be related to an increase in dopamine and dihydroxyphenylacetic acid concentrations in the caudate contralateral to the turning direction (6,21).

As a first approach in this trend, we explored the possibility of using the circling training of rats as a behavioral teratology test. Pups delivered from dams treated during gestation with two well known behavioral teratogenic drugs (vitamin A 80,000 IU/kg/day and haloperidol 2.5 mg/kg/day) were trained to turn in a circle employing an automated apparatus.

In order to discriminate the factors affecting the behavioral endpoint, a mathematical analysis and a comparison with two tests with different characterized endpoints (negative geotaxis and T-maze) have been applied. Data reported here provide evidence that the circling training of rats can be used as a behavioral teratology test.

METHOD

Animals, Treatments and Biochemical Measurements

Primiparous Sprague-Dawley female rats (local facilities, originally purchased from Holtzman Inst.) were used for breeding. Cages containing three female rats (about 200 g) and one male (about 380 g) each were placed in a temperature-controlled environment (20–22°C) under a 12-h light-dark cycle (lights on 8:00 a.m.) with food and water available ad lib. The date of conception was determined by the presence of a copulating plug in the vagina and was considered day 0 of gestation.

Pregnant rats were randomly allocated to three groups, namely, control (C) group (n = 7), vitamin A (VA) group (n = 7) and haloperidol (HP) group (n = 6). Each rat was kept in an individual cage. The three groups were injected daily, intraperitoneally, at 12:00 a.m. during gestation as follows: controls 200 μl of sterile physiological solution from day 5 to 20, hydrosoluble vitamin A (as palmitate; Abbott Laboratories) 80,000 IU/kg/day (0.27 g/kg/day; using sterile physiological solution as vehicle)

[1]Requests for reprints should be addressed to Juan L. Brusés at his present address: The University of Connecticut, Department of Physiology and Neurobiology, Box U-42, 75 North Eagleville Road, Storrs, CT 06269-3042, or Julio M. Azcurra, Laboratorio de Biologia Celular, Depto. Ciencias Biológicas, Facultad de Ciencias Exactas y Naturales, Universidad de Buenos Aires, 4° piso, Pabellón II, Ciudad Universitaria, 1428 Buenos Aires, Argentina.

from day 11 to 15, and haloperidol (halopidol, Janssen ampule, 5 mg/ml) 2.5 mg/kg/day from day 5 to 20 of gestation. Both drugs were injected in a volume of 1 ml/kg as a rule.

The VA dose was chosen according to previous reports where it was shown to be subteratogenic but induced functional deficits (16). The calculation of HP dose was done on the basis of body weight, taking into account that man is generally more vulnerable than experimental animals by a factor of about ten to toxic drugs effect (10). The dose used in this work was ten-fold higher than a medium one used in therapy.

The date of birth was considered day 0 of age and the total number of pups per dam, including the dead ones, was recorded. All alive pups were sexed and the viable litters (more than six pups) with more than ten pups were reduced to ten.

Body weights were recorded weekly, measuring total weight of the litter during the nursing period, the mean pup weight was calculated by dividing the litter weight by the number of pups. The litters were weaned at day 21, and rats were marked. Six rats of the same sex, two of each treated group (different litters), were assigned to each cage.

The offspring from the control group were assigned, at the moment of birth, to two different subgroups: one to be used in the learning tasks, and the other to be kept as a nondeprived control group. Both subgroups were kept with the mother until weaning.

Animals were kept in the housing room (under the environmental conditions described above) until they were sacrificed. The tests were performed in a room, specially constructed for behavioral testing with sound-insulated walls and a baseline noise, placed close to the housing room.

After 80 days of age, 6 males of each group were sacrificed by decapitation, brains were rapidly removed and cerebral cortex, caudate nucleus, hippocampus and cerebellum were dissected. Then each area was homogenized in a glass/teflon homogenizer in 40 vol. of saline and assayed for DNA by the method of Burton (2). Proteins were determined by the method of Lowry et al. (11).

Surface Righting and Negative Geotaxis

All the pups were tested by the appearance of the surface righting reflex and by the negative geotaxis test during the nursing period. Those tests were performed as previously described (18). Briefly, for surface righting reflex, each pup was placed in a supine position and the time required for placing all four feet in contact with the surface was recorded. Rats were observed daily from day 3 of age and were given two trials per day. The rats were tested in an all-or-none evaluation procedure, until all pups of a litter could right themselves in less than 2 s on both trials on a given day.

For the negative geotaxis test, the pups were timed for completing a 180° turn when placed in a head-down position on a 25° inclined plywood surface. Pups were given one trial per day on days 6 to 12 and allowed a maximum time of 60 s per trial. The mean of the litter in each day was scored.

T-Maze

At day 30 of age, rats were placed in a T-maze with a drinking bottle place at the end of the arms. The stem of the maze measured 14×60 cm (20 cm of the exit box included), and the arms 14×35 cm. The maze was 17 cm high. Only one arm had the reward in the drinking place (10% sucrose/water solution) and it was randomly assigned to each rat. There were four painted lines on the floor: at the end of the exit box, at the end of the stem and at the beginning of each arm. Each rat (water-deprived for 22 h) was placed for 15 s in the exit box before opening the door. One error was computed when the animal ran into the wrong arm or crossed in the opposite direction one of the other lines. Once the animal had arrived to the drinking place it was allowed to drink for five s, after that the animal was taken out and put again in the exit box. The subjects were given 2 blocks of 10 trials per day, the learning criterion was 90% correct arm choice in 20 trials. After reaching this criterion the correct arm was reversed and the same learning criterion was used. After the 2 blocks of trials the animals received water ad lib for two h in an individual cage. Testing was ended when the subject reached the learning criterion eight times, the original learning (OL) and seven succesive reversals (R1 to R7).

Circling Training (CT)

The model described by Yamamoto was used (21), in which animals deprived of water for 24 h are trained to turn in a circle for a reward (10% sucrose/water solution). The left-hand or right-hand turning direction was randomly assigned to each rat. Turns per min (calculated as total turns in a session divided by the required time) and total errors in each session were recorded.

The training was performed in an apparatus in which turns detection, reinforcer delivery and time counting were automatically executed (design details will be provided on request). It was constructed with two circular steel (1.8 mm thick) 21 cm high drums (24 cm and 40 cm inner and outer diameter respectively) mounted on a wood base covered with antislipping rubber. Four infrared transmitters were located in the inner drum and four detectors in the outer drum. Each pair was separated by 90°. The detectors were connected to a memory chip, so that the first one must have been activated to permit the recorder of the activation of the second one on the memory chip and so on. The sequence in which the detectors must be activated in order to assign different directions of training (left or right hand) can be chosen by the operator. The apparatus was connected to an electronic timer and counter. The liquid dispenser was made with an electromagnetic valve connected to a bottle containing the reward. The bottle was located higher than the end of the tube where the reward was delivered, in order to permit the circulation of the solution when the valve was opened. When the third receptor was activated, the circuit sent an electric pulse to the electromagnetic valve which opened it for enough time to deliver a 50 μl drop on the floor. The fourth receptor (which was placed where the drop was delivered) advanced the turn counter and reset the memory.

Two lines painted on the floor divided the circle in two semicircles, one error was scored when the animal crossed one line in the opposite way to the assigned turn direction. The first training session (S0) consisted in rewarding each rat by successive approximation to a full turn in the prescribed direction during 30 min: the first ten min a quarter turn, the next ten min a half turn and the last ten min a full turn in the appropriate direction were rewarded. Then training was conducted daily for seven days according to a continuous reinforcement schedule. Each rat was required to perform 100 complete turns in each of the first three sessions (S1–S3), and 150 complete turns on each of four subsequent days (S4–S7). If this condition was not fulfilled within 30 min, the session was terminated (not frequent). The only water available during the training period was that provided during the training session. To analyze the data, the following comparisons were done: all sessions S1 and S7 (group main effect) and each individual session (simple main effects).

Statistical Analysis

Results with no repeated measures were analysed by a one-way ANOVA test. Those which had repeated measures were

TABLE 1

PRENATAL TREATMENT AND POSTNATAL OBSERVATION OF THE OFFSPRING

Treatment	Period Gestational (days)	No. of Mothers	Length of Gestation (days)	No. of Pups Born Per Litter	Ratio Males/ Females	Total Offspring Mortality Day 0	Day 21
Control Saline	5–20	7	21.6 ± 0.5	10.0 ± 3.1	1.0 ± 0.3	3	3
Vitamin A 80,000 UI/kg	11–14	7	21.6 ± 1.9	10.1 ± 2.3	1.1 ± 0.6	4	1
Haloperidol 2.5 mg/kg	5–20	6	21.8 ± 0.8	10.5 ± 2.9	1.1 ± 0.7	0	3

Data are expressed as the mean value per litter ± SD. No statistically significant differences were observed.

analysed by the ANOVA test using the split-plot repeated measures design, which is a factorial design with block-treatment as a between group factor (9). When the statistical evaluation was significant using the F distribution, the analysis was followed by a posteriori comparisons by the Tukey's test (9).

RESULTS

Litter Data and Physical Development

No significant differences were found between control and drug-treated groups on the following parameters: duration of gestation, number of pups born per litter, ratio male/female of the litters, offspring mortality at birth and at weaning, and body weight (Table 1, Fig. 1). At 80 days of age, 6 males per group were randomly selected for assessment of μg of DNA per mg of protein in four different brain areas (Table 2). No significant differences in this ratio between control and treated animals were found [according to one-way ANOVA, cerebral cortex, $F(2,15) = 0.22$, $p>0.1$; caudate nucleus, $F(2,15) = 0.44$, $p>0.1$; hippocampus, $F(2,15) = 0.22$, $p>0.1$; and cerebellum, $F(2,15) = 0.02$, $p>0.1$].

Surface Righting

All pups from each experimental group were evaluated daily for the appearance of surface righting reflex beginning at day 3, until all pups in the litter reached the criterion. Age (mean ± SD) to reach the criterion was: 10.0 ± 1.0, 12.7 ± 1.0 and 15.5 ± 2.6 for C (n = 65), VA (n = 66) and HP (n = 63) groups, respectively. Development of this reflex was delayed for both drug-treated groups [according to one-way ANOVA, $F(2,17) = 18.52$, $p<0.005$, and a posteriori Tukey's test: VA, $p<0.05$ and HP, $p<0.01$, vs. controls].

Negative Geotaxis

Motor coordination was assessed by the negative geotaxis test from day 6 to 12 in all the pups (C n = 65, VA n = 66 and HP n = 63) (data not shown). The mean time each group required to complete a 180° turn in each trial was recorded and the mean value of each litter was computed. Although both treated groups performed worse than control groups, statistical analysis revealed that only VA group was significantly different with respect to control group [according to ANOVA test, $F(2,17) = 4.76$, $p<0.05$, and a posteriori Tukey's test: VA, $p<0.05$ and HP, $p>0.05$].

Instrumental Conditioning

At 30 days of age, three groups of males born from C- (n = 7), VA- (n = 8) and HP- (n = 7) treated dams and two groups of females born from C- (n = 7) and VA- (n = 8) treated dams were tested in the T-maze; and three groups of males (C n = 7, VA n = 9 and HP n = 9) and two groups of females (C n = 6 and VA n = 6) were tested by the circling training.

T-Maze

Days required and errors made to achieve the criterion in original learning and seven successive reversals were scored, and analyzed by an ANOVA test using a split-plot design (9). When performance was analyzed considering gender as a between group factor, no statistically significant differences were found with the

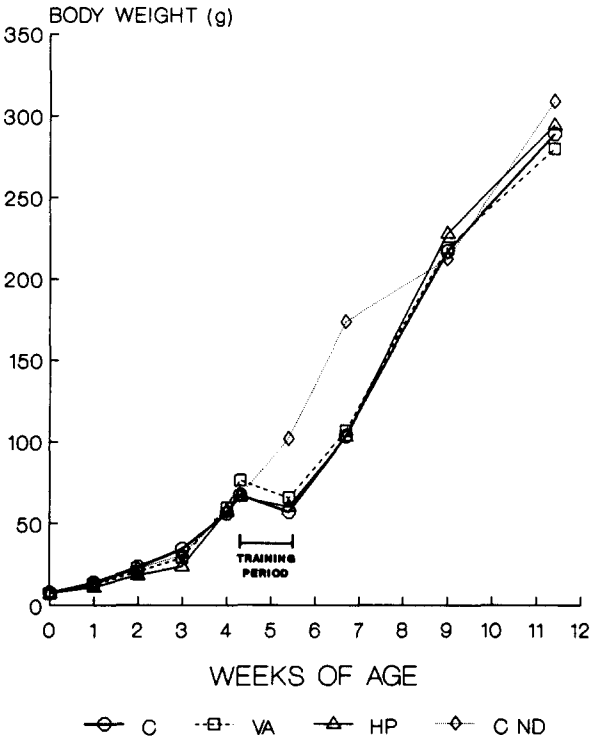

FIG. 1. Mean body weight of pups used for testing the CT during the study. Control and drug-treated groups were compared with a nondeprived control group (C ND).

TABLE 2

μg DNA/mg PROTEINS*

		Cortex	Caudate Nucleus	Hippocampus	Cerebellum
C	(6)	6.25 ± 1.50	6.93 ± 1.27	7.22 ± 1.47	18.82 ± 5.13
VA	(6)	6.21 ± 2.62	7.87 ± 2.10	6.81 ± 2.55	19.10 ± 5.27
HP	(6)	6.89 ± 1.76	7.43 ± 1.76	6.37 ± 2.47	19.29 ± 2.79

*Each value represents the mean ± SD of the group in the different areas. Number of animals assayed are indicated between parentheses.

See the Results section for statistical evaluation.

overall test and with the interaction term in any case [days: $F(4,32)=0.51$, $p>0.1$; interaction: $F(28,224)=1.19$, $p>0.01$; errors: $F(4,32)=0.66$, $p>0.1$; interaction: $F(24,228)=1.26$, $p>0.1$]. Pharmacological treatments did not induce evident changes in male and female performance and no statistically significant differences between males and females were detected.

Circling Training

Turns per minute and errors made in each training session were scored in the CT. The results were statistically evaluated by the ANOVA test using a split-plot design (9). Males and females from all treated groups were analyzed with gender as a between group factor and no statistical differences were detected ($p>0.05$) showing no interaction between sex and drug treatment. To evaluate the incidence of the pharmacological treatments, males and females were separately analyzed. Table 3 shows the value (mean ± SD) of turns per min performed by males and females in each individual session and the results of statistical comparisons of group main effect and simple main effects. Statistically significant differences in turns per min were found for both sexes in group main effect ($p<0.01$). When drug-treated male groups were pairwise compared against control group, a statistically significant difference for VA and HP was found (Tukey's test, $p<0.01$).

Errors made by control and drug-treated groups did not show any statistically significant difference [group main effect males: $F(2,22)=0.57$, $p>0.1$ and females: $F(1,10)=2.00$, $p>0.1$, according to ANOVA test, simple main effect $p>0.05$ in all sessions]. Total errors counted during the CT testing were (mean ± SD): males 92.1 ± 41.0, 93.2 ± 48.0 and 113.1 ± 44.0 for C, VA and HP groups, respectively; and according to one-way ANOVA, $F(2,22)=0.60$, $p>0.1$; females 71.8 ± 27.3 and 91.4 ± 34.3 for C and VA groups respectively, $F(1,10)=2.00$, $p>0.1$. Since maintenance of a rate of turning over six turns per min during 20 min has been postulated as critical to establish measurable biochemical differences in the caudate contra- vs. ipsilateral to the turning direction (6,21), we observed the time course of turning behavior for control animals after the last session (Fig. 3) (see the Discussion section). Animals were individually weighed after weaning until 80 days of age (Fig. 1). The evolution of the weights of the males in the three trained groups was compared to a non-deprived control group. Body weights decreased about 15% during the training period. At the end of the training period animal

TABLE 3

CIRCLING TRAINING

Session Age	1 30	2 31	3 32	4 33	5 34	6 35	7 36
				Males*			
C (n=7)	1.1 ± 0.4	5.2 ± 1.2	8.4 ± 2.4	9.0 ± 2.9	10.7 ± 4.1	12.3 ± 3.2	13.5 ± 2.9
VA (n=9)	0.4 ± 0.3	1.8 ± 1.2[b]	4.5 ± 2.0[b]	6.4 ± 1.8[a]	6.7 ± 2.0[b]	9.2 ± 2.8[b]	9.1 ± 2.2[b]
HP (n=9)	0.9 ± 0.7	2.9 ± 1.7	5.7 ± 2.2[a]	6.5 ± 1.7[a]	7.9 ± 1.1[a]	9.4 ± 2.1[b]	9.2 ± 2.2[b]
				Females†			
C (n=6)	2.9 ± 1.7	5.8 ± 1.8	9.1 ± 0.8	8.8 ± 0.9	10.4 ± 1.1	10.3 ± 1.5	9.5 ± 1.3
VA (n=6)	3.0 ± 0.7	6.0 ± 1.8	6.7 ± 1.0[b]	6.2 ± 0.7[b]	7.8 ± 0.3[b]	8.2 ± 0.8[a]	8.7 ± 1.1

Values are in turns per min (mean ± SD). Statistical analysis was done by ANOVA test using a split-plot design.

*Group main effect, $F(2,22)=9.51$, $p<0.01$. Pairwise comparison of differences among means compared to control group by a posteriori Tukey's ratio were: VA, $p<0.01$ and HP, $p<0.01$.

†Group main effect, $F(1,10)=11.74$, $p<0.01$.

Simple main effect was analyzed by ANOVA test and differences among means were tested by pairwise comparison with respect to control group by a posteriori Tukey's ratio.

[a]$p<0.05$, [b]$p<0.01$.

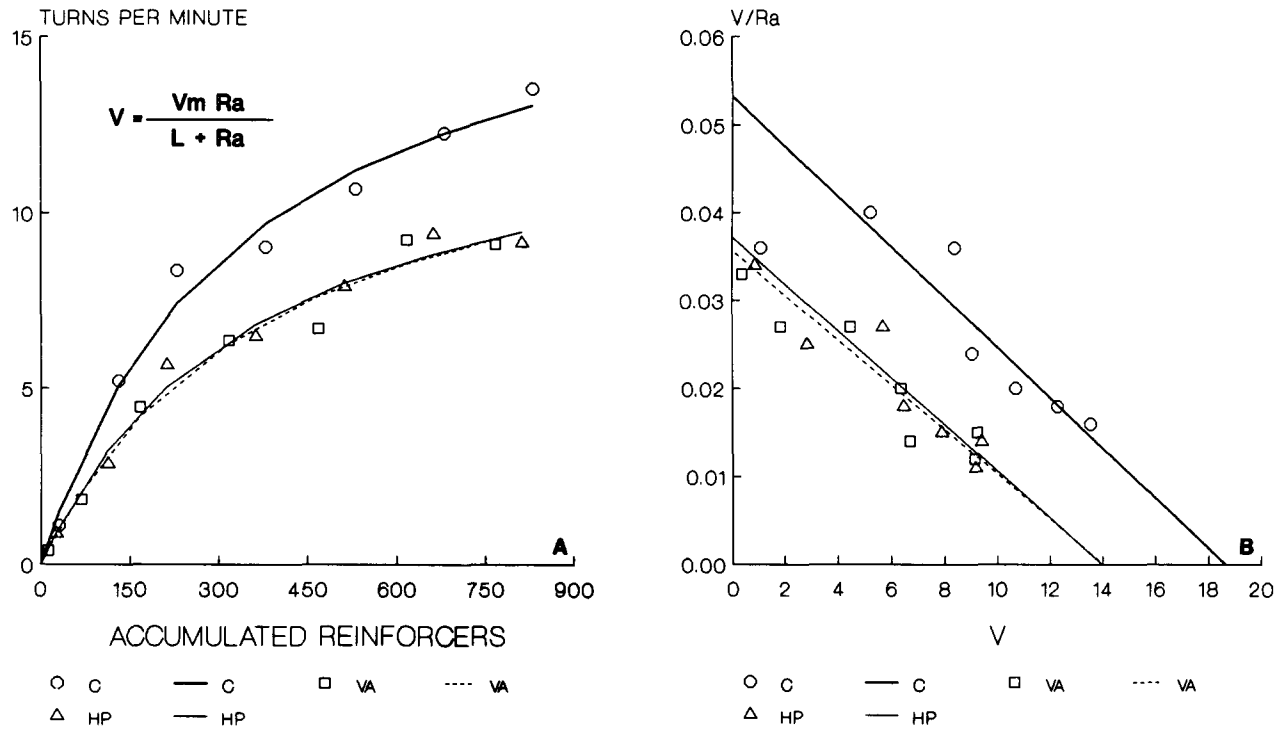

FIG. 2. (A) y-axis is response rate (turns per min) and x-axis is accumulative reinforcers. The line was obtained by fitting the equation shown in the figure with the experimental data (Table 3). V: response rate, Ra: accumulated reinforcers, Vm: asymptotic response rate and L: accumulated reinforcers to reach one-half of the asymptotic response rate. (B) The Scatchard plot of the data shown in A. The slope represents the magnitude of L and the x-axis coordinate of the fitted line at y = 0.0 is equal to Vm.

weights had decreased 45% with respect to nondeprived ones. At 60 days of age the trained animals had recovered the expected weight and no differences were found regarding physical development between trained and nontrained animals after this age. Similar results were found for females.

Mathematical analysis. The circling training can be classified as an instrumentally conditioned behavior whose quantifiable endpoint is the on-going motor performance (15). So that the differences in the animal response (turns per min) observed under pharmacological treatment may be due to specific alterations either of the motor activity or the associative learning process, or both. To discriminate the incidence of these components on the measured response, we followed a mathematical approach based on a derivation of Herrnstein's matching law equation (4). We used the derivation of the mentioned matching law equation described by Heyman and Beer (8). In our case, we used accumulated reinforcers instead of reinforcement frequency.

Figure 2A shows the approximation of data from Table 3 when plotting the rate of responses in turns per min (V) versus the number of reinforcers received up to that moment (accumulated reinforcers) (Ra). The smooth lines were obtained by fitting the equation V = Vm*Ra/(L + Ra) to the experimental data. Fitting values [calculated as (1 − RSE)*100, RSE being the residual standard error described (5) using a weight $1/y^2$] were 82.2%, 90.3% and 91.3% for C, VA and HP groups, respectively.

Figure 2B shows the same data after transformation of the equation V = Vm*Ra/(L + Ra) using a Scatchard plot (13), where y-axis is the ratio of the animal response in turns per min (V) to the accumulated reinforcers (Ra) and the x-axis is the animal response. This transformation provides two different values:

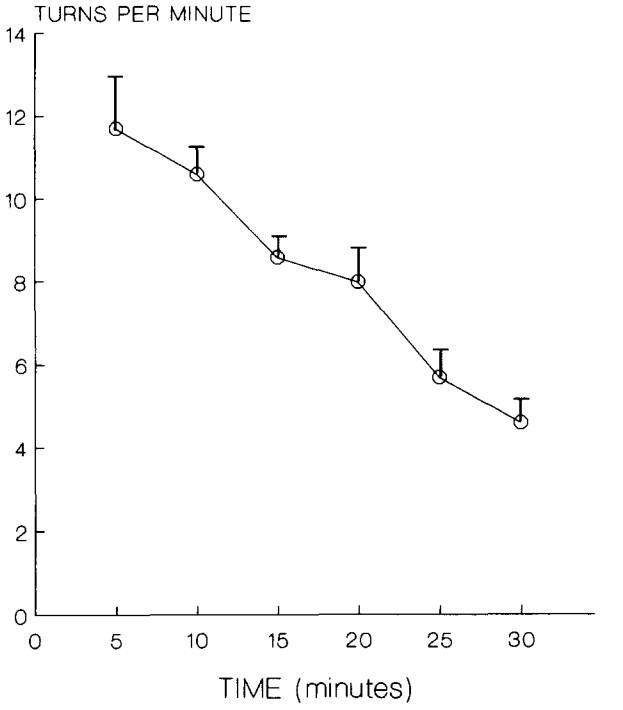

FIG. 3. Time course of circling behavior of control animals (n = 4) after the last training session. Turns counted in five-min intervals. Mean ± SD indicated.

a) the theoretical maximum response of the animal (Vm), and b) the slope, which represents the total number of reinforcers necessary to reach one half of the Vm value (L). L was taken as an indicator of the learning component of the response. Vm and L values and their asymptotic standard error (between brackets) were: Vm—18.4(1.6), 12.2(1.5) and 12.1(1.3) for C, VA and HP groups, respectively; and L—332.3(71.7), 388.8(95.0) and 364.6(80.0) for C, VA and HP groups, respectively.

To assess the detection sensitivity of the CT test we employed the coefficient of detection (CD) used in the Collaborative Behavioral Teratology Study (17), which indicates the percent change required to detect a significant difference using a given α [$CD = (t\alpha/\sqrt{n})(sd/X)*100$]. With this purpose, both CT parameters, Vm and L, from ten experiments performed along three years (1986–1988) were analyzed. As the number of animals tested ranged between 6–7, we used $n=6$ for calculations, and α equal to 0.05 two-tailed or 2.57, $df=5$. The resulting CD were 25.8% and 46.6% for Vm and L respectively, showing that the detection sensitivity of the parameter representing motor performance is greater than the one of learning.

DISCUSSION

It has been reported that prenatal treatment with vitamin A and haloperidol affects the functional neurodevelopment of the offspring (3,19). This was confirmed here by the delayed appearance of the surface righting reflex in both treated groups. However, no differences were detected in the DNA/protein ratio in several brain areas from control and drug-treated animals (Table 2), indicating that such drug treatments did not greatly damage the CNS development as it was previously reported (18). Furthermore, the treatment schedules employed in this study did not affect litter parameters, like offspring mortality and physical development (Table 1, Fig. 1). The evidence that functional neurodevelopment of the animals had been affected and that they did not show noticeable structural alterations of the CNS, allowed us to consider treated groups as positive controls for testing the CT as a behavioral teratology test. The statistically significant difference in the CT response (turns per min) between treated and control animals ($p<0.01$) shows that the CT was able to detect permanent drug effects. Similar results were found with females with respect to VA treatment. When individual sessions were analyzed, the difference between control and drug-treated groups began to be statistically significant after three sessions and increased up to the end of the training for both tested drugs (Table 3).

The circling training may be considered as a variant of the straight alley maze, in which learning was measured as the rate of approach to asymptote and was shown to be independent of motivational factors; notwithstanding, the asymptotic value obtained is affected by motivational manipulations (20). We propose that the CT behavioral response (turns per min) which also approaches an asymptotic value, would result from the interaction of two components: the motor ability and the learning process.

Although motivational factors also contribute to the CT response, we consider that they are not involved in differences in this response as measured in the described experimental conditions because the animals were deprived for the same period of time and they received an equal quantity of reinforcer in each session (see the Method section). However, differential motivational effects of the employed drugs cannot be discarded.

In an effort to distinguish the incidence of both components on the animal response we followed a mathematical approach based on the derivation of Herrnstein's matching law equation (4) described by Heyman and Beer (8). In our case we used accumulated reinforcers (Ra) instead of reinforcement frequency. Thus the derivation describes the relationship between the obtained response (turns per min) and the sum of reinforcers received up to that moment (Ra). A good fit was found with the experimental data using this derivation (Fig. 2A).

Two parameters can be estimated from the equation $V = Vm*Ra/(L + Ra)$: Vm, representing the motor performance and L, which represents the learning process in terms of the quantity of reinforcers required to reach one-half of the maximal velocity (Vm). In order to discriminate more readily these two components affecting the measured response, the same results were plotted in terms of a transformation of this equation (Scatchard transformation) (Fig. 2B). This way of analysing the CT data shows that 1) CT behavioral response can be considered as resulting from two factors (namely, motor performance and learning process) and that they can be quantitatively estimated, and 2) the maximal response the animal can reach (Vm) was the component which was affected most by the pharmacological treatments in our experimental conditions. A derivation of Herrnstein's equation, similar to the one presented here, has been reported using another experimental model (8).

A different approach to elucidate the incidence of the associative learning component in the CT was attempted by analyzing the errors made during the sessions. An equivalent group of animals was tested in an instrumental conditioning test with an associative endpoint, the T-maze. No detectable differences were found by the evaluation of the days required and errors made (see the Results section). In both tests, a spatial discrimination (turn direction choice or arm choice) using a positive reinforcer, is evaluated as an associative learning process. Statistical analysis of this parameter in the CT showed no significant differences between control and drug-treated groups for males and females.

Motor coordination was evaluated by the negative geotaxis test. Pups of dams treated with VA and HP showed an impairment in this test, indicating some disturbance in neuromuscular coordination (see the Results section). A statistically significant difference with respect to controls was found for the VA group.

In this way, control and drug-treated groups performance in the behavioral tests provides further evidence supporting the conclusion obtained by the mathematical analysis, which was able to quantitatively discriminate the incidence of the two components on the measured response. In the particular case of the drugs, doses and period of injection chosen here, the most affected variable was the motor performance. However, the possibility that other drugs and/or treatment schedules modify the learning component or both is not excluded.

In conclusion, the above presented results lead us to propose that the CT can be used as an instrumental conditioning test in which the quantifiable endpoint is the on-going motor performance with certain advantages like its short and strictly defined period of training (8 days) and the possibility of discriminating the learning and motor factors. Besides, it provides objective (automated) measures, and does not require specially trained technical staff.

Searching for new behavioral techniques for detecting the possible interactions of drugs with the developing brain should be combined with the study of the basic neurochemical mechanisms involved. The CT behavior induces a functional variation (dopamine concentration) in a specific brain area, the caudate nuclei (6,21), when a speed over six rpm was maintained. This speed has been reported as critical to detecting a 65% difference in dopamine concentration between caudates ipsi- and contralateral to the turning direction (6,21). Differences in dopamine concentration were not found employing training conditions that did not allow the animal to maintain the mentioned speed for the required time (13). The training conditions here employed maintained this critical speed/time factor (Fig. 3).

The caudate nucleus is a valuable model for the study of the CNS development because the general rules that govern the formation of central neuronal connections are represented in this area (7). Moreover, since the neostriatum performs associative and integrative functions (1), it can be used as a representative brain area and is the target for a wide range of psychotropic drugs. It has defined advantages for neurochemical studies (is anatomically distinct and has well-defined main afferents).

These characteristics and the results presented here support further exploration on the use of the CT and the caudate nuclei as a biological model for combined studies of behavioral and neurochemical drug-induced permanent modifications.

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