Behavioral Changes of Young Rats Force-Fed Methyl Mercury Chloride[1] (37480)

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Mercury has been recognized as an industrial poison from early times causing death or neurological disturbances. The manufacture of felt hats exposed the workers, especially fur cutters, to serious risk. The tremors that developed in these individuals gave rise to the expression "hatter's shakes" and the mental changes associated with long exposure to mercury coined the common saying "mad as a hatter" (1). However, other incidents of mercury poisoning were recorded as recently as 1971 (2).

Anatomical changes in the brain of humans and animals poisoned with mercury have been well documented (3). However, behavioral changes associated with mercury poisoning have not been extensively studied but descriptions of poisoned individuals indicate that behavioral changes can be expected. Patients suffering from chronic mercury poisoning reportedly exhibit behavioral changes which have been described as embarrassment, timidity, and sudden attacks of anger. Occasionally, a patient has hallucinations and delusion (1).

Controlled experimentation to determine behavioral changes in mercury-poisoned animals is now beginning to appear in the literature (4–8). Spyker *et al.* (4) reported changes in emotionality in offspring of mice exposed to methyl mercury dicyandiamide during pregnancy. Whereas Rosenthal and Sparber (5) reported that chicks poisoned with the same mercury compound during embryonation had retarded detour learning. In view of the paucity of data regarding behavioral changes associated with mercury toxicity, we studied the behavior of rats fed methyl mercury chloride in open-field and T-maze tests.

Materials and Methods. A single dose of methyl mercury chloride was force-fed at 2.0 mg/100 g body weight to 15- and 21-day-old and at 2.5 mg/100 g body weight to 60-day-old male Sprague–Dawley rats (Expts 1, 2, and 3, respectively). Cocoa butter or 1-2-propanediol was used as the carrier of the mercury compound and was force-fed to control rats. Each experiment consisted of 15 treated and 15 control rats. The rats in Expt 1 came from 3 control and 3 treated litters. After force-feeding, they were returned to their mothers and were weaned at 21 days of age. At weaning, these rats as well as those from Expts 2 and 3 were individually kept in suspended wire cages in a laboratory maintained at 22° and 12 hr each of light and darkness.

A nutritionally adequate grain diet and water were available at all times except during learning and testing in the T-maze, when the rat was placed on a 2.5 hr feeding regime commencing at the end of each day of testing.

The sequence of behavioral testing is listed in Table I. The rat was trained to enter the goal box which contained the food reward by having it detect a wire mesh screen on the floor of the T-maze goal where the food cup was located. The screen also extended 12 cm into the alley of the T-maze on the side of the goal. The T-maze was 30.5 cm high, 21.6 cm wide with a 30.5 cm long start box, a base leg 120.7 cm long, and each arm 47.0 cm long. The goal box entrance had a one-way door so that the rat cannot

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retrace. The position of the screen as well as the food was randomly assigned to one arm or goal of the T-maze and the other arm contained food that was covered with a screen to make it unavailable and to control for olfactory cue. Testing in the T-maze consisted of 10 trials/rat/day. The criterion was nine correct responses out of the 10 trials/day. In addition to recording the correct responses made by the rat, latency was also measured from the opening of start box's guillotine door until one of the arms was entered. Extinction trials were identical to learning trials except food reinforcement was removed.

Testing in the open field consisted of recording the frequency of standing upright and circling in the start box for one minute. At the end of the minute, the start box door was opened and the time (latency) when the rat left was recorded. Once the rat entered the open field, standing upright, circling, length of inactivity and number of areas traversed for a period of 5 min were recorded, The events were registered on a recorder with a keyboard operated by the experimenter. Open field consisted of a start box 26.7 × 19.1 × 45.7 cm equipped with a plexiglass guillotine door and a circular field of 76.2 cm diameter surrounded by a sheet metal wall 45.7 cm high. The open field was divided into 7 equal areas consisting of 6 pie-shape areas and a circular area in the middle of the field. The observer watched the behavior of the rat by means of a convex mirror which was positioned over the open field and thus avoided possible introduction of various cues from the movement of the observer.

Five to six days after the last test (Table I) 5 representative rats from each group were lightly etherized and perfused intracardially with buffered 10% formalin to fix the brains *in situ* after which they were removed and stored in buffered formalin. A parasagittal section 0.5 mm lateral to the median line was embedded in paraffin, sectioned and stained with H and E for microscopic examination. All data collected from the behavioral tests were analyzed for statistical differences by analysis of variance with the aid of a computer.

Results and Discussion. Within half an hour after force-feeding, the mercury-fed rats in each experiment were lethargic and ataxic. However, within 2–3 hr, they appeared normal. All rats completed the experiments without again showing any clinical signs.

TABLE I. Behavioral Testing Sequence (Expts 1, 2 and 3).

Days after treatment	Test
0–2	no testing
2–7	pretraining, T-maze
8–15	training,[a] T-maze
16–20	testing, T-maze
21	no testing
22–26	testing, open field
27	no testing
28–32	retesting, T-maze
33	no testing
34–38	retesting, open field
39–44	no testing
45–47	extinction testing, T-maze
48–53	no testing
54	extinction retesting, T-maze
59–60	sacrificed for histology

[a] Rats in Expt 1 had 7 days of training instead of 8.

Differences in behavioral measurements were either small or statistically insignificant between treated and control rats of Expts 1 and 2. However, in Expt 3, several significant behavioral changes were observed in the treated rats (Tables II, III). The reason for finding more changes in Expt 3 than in 1 and 2 could be due to the higher dose of mercury used in Expt 3 or to the age differences. Statistically significant changes occurred in the following measurements: (a) during T-maze testing treated rats took significantly more days to reach criterion of

TABLE II. Average ± SE Number of Days to Reach Criterion of 9 Out of 10 Correct Responses in T-maze During Test Period.

	Expt no.		
Treatment	1	2	3
Control	4.5 ± 0.4	7.6 ± 0.8	4.8 ± 0.3
Mercury	5.7 ± 0.6	6.6 ± 0.7	6.2[a] ± 0.4

[a] Significantly greater than control ($p < 0.01$).

TABLE III. Average ± SE Voluntary Activities During 5 Min in Open Field of Rats Fed 2.5 mg Methyl Mercury Chloride/100 g Body Weight (Expt 3).

	Open field, test period			Open field, retest period		
Measurements	Control	Hg	*p* values	Control	Hg	*p* values
Areas traversed (no./rat)	72.4 ± 1.8	60.4 ± 3.8	<0.02	74.2 ± 2.1	60.0 ± 0.4	<0.03
Inactivity (sec/rat)	4.7 ± 0.9	27.0 ± 2.7	<0.001	6.6 ± 1.2	18.9 ± 2.3	<0.03
Latency (sec/rat)	33.3 ± 4.2	23.6 ± 3.2	<0.08	37.5 ± 2.2	36.7 ± 2.8	NS[a]
Standing upright (no./rat)	33.4 ± 0.8	28.4 ± 1.5	NS[a]	39.4 ± 1.4	32.5 ± 0.6	<0.06
Circling (no./rat)	3.9 ± 0.4	5.2 ± 0.5	<0.05	5.1 ± 0.5	5.7 ± 0.3	NS[a]

[a] NS denotes no significant differences between control and Hg treated rats.

9 out of 10 correct responses; (b) latency and areas traversed in open field test, treated < control; inactivity and circling, treated > control; (c) during open field retesting, areas traversed and standing upright, treated < control; inactivity, treated > control (Tables II and III).

Histopathological examination of the sections of brains of five representative rats from each group revealed no lesions attributable to the mercury treatment. Whether lesions were produced and had healed by the time the animal was necropsied is not known.

In the T-maze, the rats learned a tactile discrimination and the strength of that learning was tested by the extinction trials. In the open field the rats were examined for emotionality (9). These tests indicated that 60-day-old rats fed a fairly large dose (Expt 3) of mercury had altered emotionality. Despite the difficulty in the interpretation of the behavioral tests and the danger of applying the data to human situations, statistically significant differences found between treated and control rats warrant further research. More importantly, these behavioral changes were detected by relatively simple tests in contrast to the more sophisticated histological examinations that revealed no changes in the brain of the rat. Our data corroborate those reported by Spyker *et al.* (4). They found subtle behavioral changes in progeny of mice treated during pregnancy with mercury but could not find changes in brain weight, protein, choline acetyltransferase and cholinesterase.

Summary. A single dose of methyl mercury chloride was force-fed at 2.0 mg/100 g body weight to 15- and 21-day-old and at 2.5 mg/100 g to 60-day-old male Sprague–Dawley rats. Control rats received the carrier for the mercury compound. T-maze and open-field performances of the rats treated with the 2.0 mg/100 g body weight were not altered when compared to control rats. However, statistically significant differences were found in the T-maze test in number of days to reach criterion between the two groups of 60-day-old rats. Furthermore, statistical differences were found between the two groups in several parameters measured in the open field which indicate alteration in the emotionality of the treated rats. Histological examination of the brain at the end of the behavioral tests revealed no lesions attributable to the mercury treatment. Thus, our data indicated that certain subtle changes due to mercury poisoning can more readily be detected with behavioral tests than by histopathological examination.

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