SUBCHRONIC EXPOSURE TO METHYLMERCURY IN MALE WISTAR RATS: EFFECTS ON NEUROBEHAVIORAL PERFORMANCE

TÜNDE VEZÉR AND ANDRÁS PAPP

Department of Public Health, University of Szeged, Hungary

ABSTRACT: Humans are exposed to mercury by industrial emission, dental fillings, use of mercury-based fungicides and bactericides, and via food. Mercury exposure results in altered behavioral, cognitive, coordinative, and emotional functions of humans and animals. Cognitive behavior, open field activity and psychomotor performance was studied in young adult male Wistar rats treated subchronically with methylmercury (II) chloride (0.5 and 2.0 mg/kg b.w.) per os by gavage. Spatial learning of the animals, investigated in a maze, was altered, demonstrated by lower performance in short-term, long-term and reference memory compared to the controls. Depending on the dose and the length of treatment, the number of animals making mistakes and the number of errors per animal was increasing. In the open field test, the treated rats showed decreased locomotor activity and diminished spontaneous exploratory activity. The changes seen in the methylmercury-treated rats were similar to those found in exposed humans which stresses the importance of monitoring and control of direct and indirect environmental exposure.

KEY WORDS: Methylmercury, spatial learning, locomotor activity, sensorimotor performance, rat

INTRODUCTION

Neurotoxic metal compounds exist in various chemical forms. In case of mercury, the relevant forms from toxicological viewpoint are the metallic form, also called the elemental form, the divalent inorganic, and the organic forms (ATSDR, 1999). Both organic and inorganic mercury are known to diminish mental performance in humans (especially children) and in young experimental animals by inducing deficits in coordination, emotionality and other behavioral features, and causing neurological disorders (O'Flaherty, 1998). The functional state of the brain is affected by mercury entering the blood stream and passing the blood-brain barrier above a concentration threshold (Nielsen, 1992). Mercury is deposited in the cortex and in subcortical nuclei, causing morphological and functional deficit of transmitter receptors, synapses and neurons (Friberg and Mottet, 1989; Warfringe et al., 1992;

Corresponding author: Tünde Vezér
Department of Public Health
University of Szeged
Dóm tér 10
H-6720 Szeged, Hungary
Phone: +36-62-545-119
Fax: +36-62-545-120
E-mail: vezer@puhe.szote.u-szeged.hu

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Möller-Masden, 1994). Functional neurotoxic consequences of mercury exposure are present at various organizational levels of the central nervous activity. In our earlier studies, mercuric chloride, an inorganic form, was subchronically given and alterations in the spontaneous (Dési et al., 1996; Nagymajtényi et al., 2000) and stimulus-evoked (Schulz et al., 1997; Papp et al., 2000) cortical activity were observed.

The hippocampus, an important neurobiological substrate of learning and memory processes in mammals, is one of the sites where mercury is acting following exposure (Yuan and Atchison, 1995). In rats, the spatial memory, depending on information provided by spatial cues in the environment, remains highly accurate for as long as 8 hours and seems to be very resistant to disruption by a wide variety of environmental influences (Beatty and Shavalia, 1982) making it suitable for experimental work. In the present work, male Wistar rats were treated for 10 weeks with low-level methylmercury chloride (MEM) by gavage. Our aim was to test how these doses of the metal compound affected various processes of spatial maze-learning, short-term and long-term memory (acquisition, working and reference memory etc.), locomotor activity, and sensorimotor performance. It was also investigated what heavy metal-specific functional neurotoxicological changes are seen in the central nervous system generally, and in brain areas having a special role in learning (hippocampus CA1 region).

METHODS

We used male Wistar rats (200–220 g body weight, in groups of 16 at start) housed under controlled conditions of temperature (22 to 24°C) and photoperiod (12-hour light/dark cycle with light starting at 06:00), with free access to drinking water. Three weeks before starting the treatment, the animals were put in polypropylene cages of 28×40×20 cm, 4 rats/cage. The animals were treated with MEM (CH_3HgCl, analytical grade, Aldrich) per os by gavage for 10 weeks. The doses were 0.5 and 2.0 mg/kg b.w., dissolved in 0.01 mol/L $NaHCO_3$ (for pH correction) to 1 ml/kg administration volume. Control animals received distilled water. The memory test used required that during the 10 weeks of treatment the animals had a restricted access to food (1 hour/day) resulting in a small (ca. 20-25%) body weight loss (Beatty and Shavalia, 1982). Body weight was measured and the animals' general state was observed every day. All behavioral tests were performed in a room different from that used for keeping and treating the animals.

Test procedure for spatial working memory. The animals' spatial learning ability was tested in an 8-arm radial maze (Columbus Instruments, Ohio, USA). In the first week of treatment, the animals had 10-minute training sessions twice a day in which the rats, under partial food deprivation, were adapted to find food pellets in the maze arm ends. During acquisition (the next week), with one training per day, the rats learned to visit the farthest points of each arm. This way, the rats were forced to learn a win-shift food search strategy (Beatty and Shavalia, 1982). All animals had a run performance of over 85%. In the spatial short-term working memory test

(weeks 3 and 5) the rats were allowed in the first run to enter four of the arms, and their task in a second run was to enter only arms not entered 2 or 4 hours before. This was the "event-to-be-remembered".

Reference memory was tested on the 4th week, here food reward was put only in the 4 arms preferred by the individual rats.

Long-term retention memory test. Following 2 weeks of rest, memory return was induced on the 8th week by re-acquisition. Then, the 2- and 4-hour spatial working memory was tested again. In all tests with the 8-arm maze, run performance was calculated from the proportion of errors to all responses (entering any arm).

Locomotor activity and the psychomotor performance of the rats were tested on the 5th and 10th weeks of treatment. These tests were performed during the early part of the light cycle (9:00 to 14:00) after the maze-learning test. Before the tests, the animals were allowed to accommodate in the testing room for 30-40 minutes. Motility and spontaneous horizontal, vertical and local exploratory activity was scored during a 10-minute interval spent in a dimly lit open field box (40 × 40 × 40 cm, ACTIFRAME, Gerb Electronic, Berlin, Germany). The movements of the rat were detected by two arrays (3 and 15 cm above floor level) of infrared sensors with 1.1 cm distance between the beams. *Psychomotor performance* – acoustic startle response (ASR) and pre-pulse inhibition – of the rats was measured, after the open field sessions, in a commercially available reflex monitor (Responder X System, Columbus Instruments, Ohio, USA). During testing, each animal was placed into a small plastic cage enclosed within a light- and sound-attenuated chamber. Following a 10-min accommodation, a series of 10 consecutive tones (5 kHz, 110 dB, 200 ms, 15 s interval) as test stimuli were applied. In another series following 15 min rest, inhibiting prepulses (1 kHz, 50 dB, 500 ms) were applied 200 ms before each test stimulus. A whole body motor response with more than 50 g force on the cage floor was accepted as positive response, on which latency, time to peak and peak amplitude were measured.

The functional changes in the central nervous system were studied following the 5th or 10th weeks of the treatment. In a room separate from those used for behavioral tests, the animals were anesthetized with 1000 mg/kg urethane and the left hemisphere was exposed by removing the bony skull. Surface electrodes were placed on the primary somatosensory, visual and auditory cortical focus and a steel needle electrode was inserted into the hippocampal CA1 region. Spontaneous electrical activity was recorded for 5 min by means of the NEUROSYS system (Experimentria, UK). The records were analyzed by a software yielding the relative power distribution among the standard frequency bands (delta to gamma).

All behavioral results were analyzed by ANOVA or Kruskal-Wallis test following a Kolmogorov-Smirnov normality analysis. The functional changes of the central nervous system were assessed by two-factor ANOVA.

RESULTS

The mercury doses applied in the present investigation had no general toxic effect and no significant change in the body weight gain. However, the relative weight of the femur (related to brain weight) showed a dose-dependent decrease, and that of the kidneys, an increase.

During the short-term and long-term retention tests of the maze learning (2^{nd}–10^{th} weeks of treatment) the MEM-treated animals showed, compared to controls, a significant and dose-dependent decrease in the average memory performance (*Fig. 1*). During acquisition (7^{th}–12^{th} days of the treatment) MEM-treated rats had a dose-dependently lower performance (high and low doses vs. control: $p < 0.001$). During the short-term (2 and 4 hours) working memory tests (3^{rd} and 5^{th} weeks of treatment), the error frequency of the treated rats was significantly and dose-dependently higher than in the controls (high dose vs. control $p < 0.001$; low dose vs. control $p < 0.01$ on the 3^{rd} and $p < 0.001$ on the 5^{th} week). The reference memory of the animals' spatial learning showed in both treated groups a significant ($0.001 < p < 0.01$) performance deficit. After a 2-week rest period, the control and low dose group both showed a memory return to the level on the 2^{nd} week of treatment but the level reached by the high dose animals was 5 to 6 % below that. In the long-term retention test (from the 43^{rd} day on) both MEM-treated groups showed a further significant memory deficit vs. control ($p < 0.001$). Comparison of the long-term memory retention within the same MEM-treated group indicated an increase of errors by 3 to 4% from the 9^{th} to the 10^{th} week.

Open field tests revealed decreased locomotor activity in the treated animals both on the 5^{th} and 10^{th} weeks of MEM administration. The diminished spontaneous exploratory activity of the animals was mainly due to decreased vertical and horizontal exploration. *Fig. 2* shows three different elements of locomotor activity: vertical exploration (rearing), local motility (grooming) and horizontal ambulation (running); on the 10^{th} week, in the 1^{st}, 5^{th} and 10^{th} min of the 10-min open field session. Habituation in the exploratory activity (over the 10-min session) was increased in both treated groups vs. control.

Startle reactions of the animals were recorded on the 5^{th} and 10^{th} weeks of treatment. The number of positive ASR responses displayed a decreasing trend in the treated groups on the 5^{th} week. On the 10^{th} week, the ASR score of the low dose MEM-treated animals was slightly reduced. In the high dose animals the score was similar to that of the controls (*Fig. 3*). Onset latency and peak time were also dose-dependently decreased. The average amplitude of ASR was reduced on the 10^{th}, but not the 5^{th}, week. The number of noise-positive responses after pre-pulse inhibition was insignificantly increased in the treated animals vs. controls on the 10^{th} week (not shown).

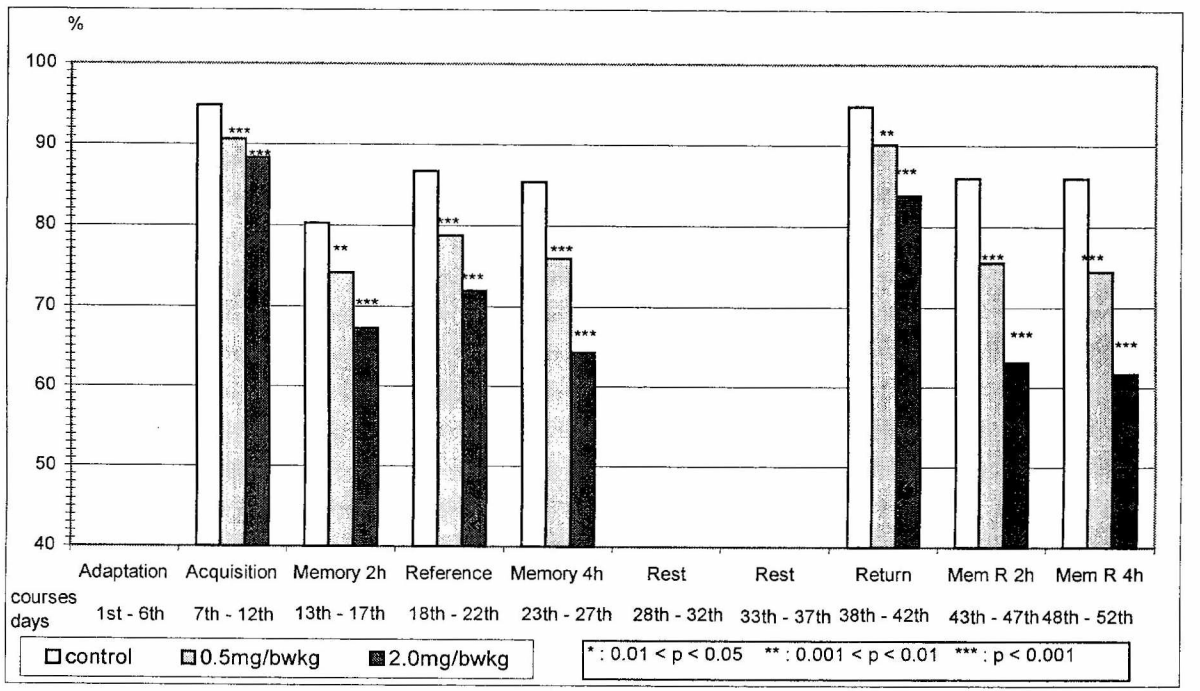

Fig.1. Memory performance alterations in male Wistar rats treated with organic mercury. Ordinate: performance % (correct response/all responses). Abscissa: phases of the memory test.

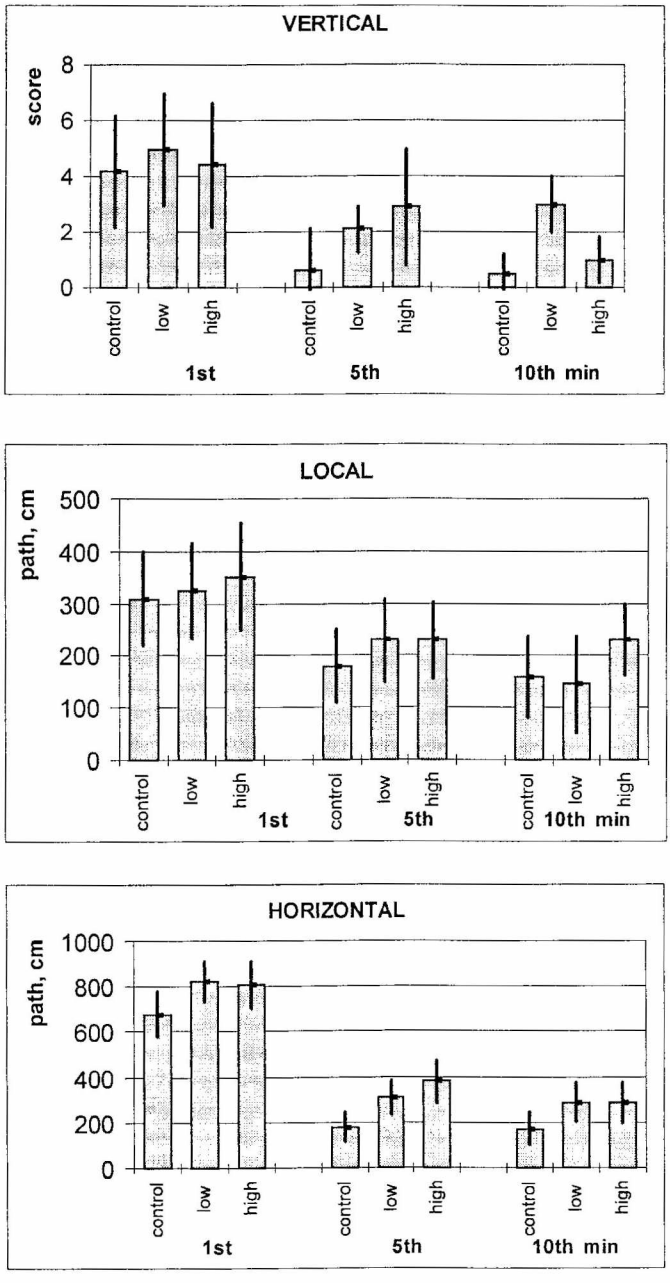

*Fig. 2. Effects of MEM on rearing activity, motility and local activity of rats in the 10^{th} week of treatment, in the 1^{st}, 5^{th} and 10^{th} min of the 10-min session. Ordinate: number of events (VERTICAL) or distance run (LOCAL, HORIZONTAL). Abscissa: time of record and dose groups. Error bar: SEM; n=16; * $p<0.05$.*

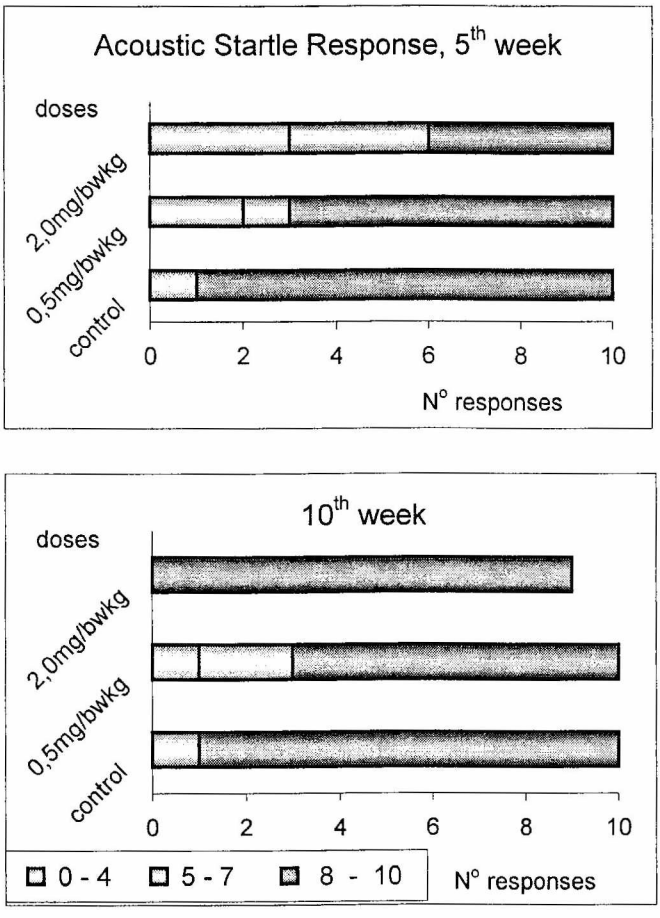

Fig.3. Acoustic Startle Response results in the 5th and 10th week. The bars show how many of the rats (n=10) gave how many times a positive reaction during the session of 10 acoustic stimuli.

The functional changes in the central nervous system were studied at the end of the 5th or 10th weeks, in 1 or 2 days following the open field and ASR tests. The general trend seen in the spontaneous cortical and hippocampal activity was decreased activity in the lower and increased activity in the higher frequencies. The effect was the most characteristic and significant ($p < 0.05$) on the visual cortex (*Fig. 4*). The other cortical areas showed similar but statistically not significant trend. In the hippocampal activity, the alteration was similar but more marked.

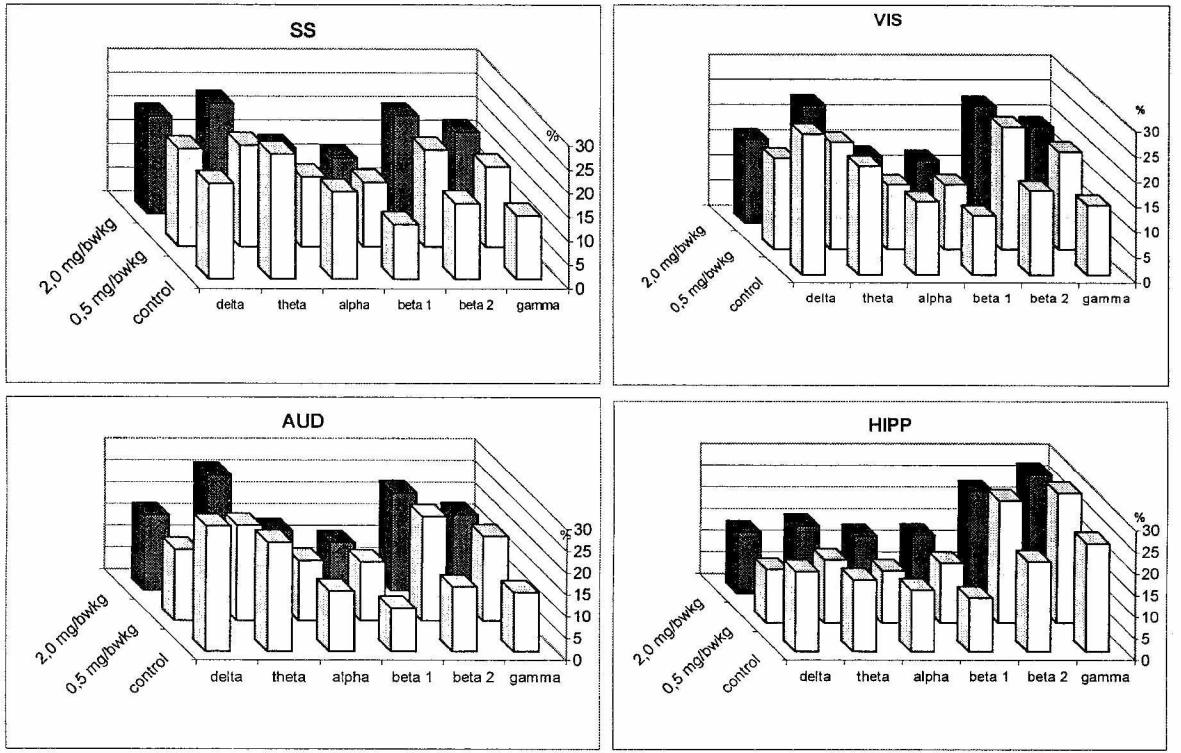

Fig.4. Frequency spectrum of the spontaneous activity recorded from the somatosensory (SS), visual (VIS) and auditory (AUD) cortical areas and the hippocampal CA1 region (HIPP) following 10-week MEM treatment. Ordinate: relative power (%). Abscissa: standard frequency bands.

DISCUSSION

The results showed that MEM in subchronic exposure caused a dose-dependent effect on memory which evolved gradually over the period of treatment. The negative effect of mercurials on mental performance in experimental animals and human subjects has been extensively studied (Fredriksson et al., 1996; Rossi et al., 1997; Grandjean et al., 1998), and some mechanistic explanation has been proposed.

Being neurotoxic, Hg affects synaptic plasticity, an essential element of learning processes (Alkon et al., 1991). The decreased locomotor activity and the impaired short- and long-term spatial memory performance, both observed in our experiments, point to the involvement of the hippocampus in the neurotoxic damage by Hg (Petit, 1990; Yuan and Atchison, 1995).

Mercury is known to affect several transmitter systems, including those involved in memory functions. The changes of vertical and horizontal motor activity are supposed to be due to alterations in the serotoninergic and dopaminergic transmission, respectively (Faro et al., 2000; Dirks et al., 2001). In rats, acute methylmercury administration increased the release of dopamine. The mesolimbic and nigrostrial dopaminergic systems in rats are involved in the spontaneous motor activity (Fink and Smith, 1980). These, too, are affected by Hg (Slotkin and Bartolome, 1987; Rossi et al., 1997).

Hippocampal muscarinic receptors are extremely sensitive to mercury, (Coccini et al., 2000) which explains its strong memory effect and supports our finding that hippocampal spontaneous electrical activity was more affected than cortical activity. The effect of Hg on the cholinergic system (Cagiano et al., 1990) may explain the diminished reactions of the treated animals in the ASR test and memory processes (Fibiger, 1991). The reduction of prepulse inhibition, as it was found in our experiments, can be due to the effect of Hg on GABAergic synapses (Narahashi et al., 1994; Yuan and Atchison, 1995).

The above results seem to be in line with effects described in the literature and may possibly contribute to early recognition of mercury-induced alterations in exposed animals and humans.

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