ILL EFFECTS OF INORGANIC METAL POLLUTANTS

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ABSTRACT:

The presence of heavy metals in the ecosystem presents a major problem in environmental and occupational medicine. Repeated exposure of humans by Hg and Mn compounds and the resulting pathological changes have been described.

In the present experiments, male Wistar rats were treated for 10 weeks by gavage with low-doses $HgCl_2$ (0.5 and 2.0 mg/kg) and $MnCl_2 \cdot 4H_2O$ (14.8 and 59.4 mg/kg). It was tested how these doses of the two metals affected various elements of spatial learning and short- and long-term memory, spontaneous exploratory locomotion, and sensorimotor performance with psychomotor gating. Metal-specific functional neurotoxic effects in the CNS in general and in centers with special role in learning (hippocampus) were also looked for.

Both metals caused a dose-dependent significant decrease in the memory performance and in the local locomotor activity. In the sensorimotor (startle) reaction, the number of responses dose-dependently and significantly (high dose vs. control) decreased. In the Hg^{2+}-treated animals, spontaneous cortical activity was shifted to higher frequencies. The effect on the evoked cortical activity was below significance.

The Hg and Mn doses applied altered in the higher nervous functions of the treated adult rats. In cases of human exposure, similar effects can be expected.

KEYWORDS:

mercury, manganese, neurotoxicity, 8-arm radial maze; spontaneous locomotor activity; acoustic startle response

1. INTRODUCTION

Manganese is toxic in high but an essential micronutrient in low dose. Occupational Mn exposure comes from ore and metal dusts [11] while Mn burden of the population originates from the environment (due to Mn-containing waste, methylcyclopentadienyl manganese tricarbonyl used as anti-knock petrol additive, and organo-Mn agricultural fungicides). The brain is among the primary target organs in chronic Mn exposure [20]. A "Parkinson-like syndrome" develops with functional [22] and structural [26] damages of the dopaminergic systems. The release of other transmitters (Glu and GABA) is also reduced by Mn^{2+} in moderate doses [24]. In Mn-exposed humans, impairment of several memory and motor function parameters was observed [14]. In rats, increase or decrease of the spontaneous motor activity was seen depending on the dose and duration of Mn treatment [2].

The important forms of mercury from neurotoxicological viewpoint are the metallic form, and the divalent inorganic and organic forms. Humans are exposed to mercury by the industry, from dental fillings, by the use of mercury-based fungicides and bactericides, and via food. Inorganic mercury is known to diminish mental performance in humans (especially children) and in young experimental animals by inducing deficits in coordination, emotionality and other behavioural features, and causing neurological disorders [17]. Mercury induces pathomorphological changes, affecting the higher order functions of the central nervous system. By stimulating serotonin receptors, Hg causes increased motivation, aggressiveness and impulsive behaviour [5], and by stimulating striatal dopamine release, decreases the intraneuronal dopamine degradation [7]. Several authors described the decrease of spontaneous locomotion [10,21] and startle response [5]. In animal experiments, Hg^{2+} was found to inhibit presynaptic Ach release and postsynaptic muscarinic receptor activation [4], and to damage motor axons [18]. The irreversible inhibition of transmitter release is supposed to depend on generation of disulfide bridges [13]. Effects of Hg^{2+} on GABA receptors [15] have likewise been described. Hg^{2+} and Mn^{2+} block Ca^{2+} channels [3], and Hg^{2+} also affects Na^{+}/K^{+} ATP-ase [23,25]. In occupational exposure to inorganic mercury, alterations of the spontaneous cortical activity [19] and delayed waves in the brainstem auditory evoked potential [6] were found.

The aim of the present work was to investigate the effects of subacute (10 weeks) oral exposure of rats to inorganic Mn^{2+} and Hg^{2+} with behavioral and electrophysiological methods.

2. METHODS

Male Wistar rats (220-250 g body weight at start) were used in both treatments. The animals were treated with 14.8 and 59.4 mg/kg b.w. Mn^{2+}, or 0.5 and 2.0 mg/kg b.w. $HgCl_2$ (low and high dose, respectively) per os by gavage for 10 weeks (5 days a week). Control animals received distilled water. The animals were 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. 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 mild (ca. 20-25%) body weight loss [1]. 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, between 08:00 and 14:00.

The animals' spatial learning ability was tested in an 8-arm radial maze. In the first week of treatments, the rats were adapted to find food pellets in the maze arm ends. During acquisition (2^{nd} week), the rats learned to visit the farthest points of each arm. All animals had a run performance of over 85%. In the spatial short-term working memory test (3^{rd} and 5^{th} week) 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 ago (the “event-to-be-remembered”). Reference memory was tested on the 4^{th} 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 observed on the 8^{th} week. Then, the 2- and 4-hour spatial working memory was tested again (9^{th} and 10^{th} week). In all tests with the 8-arm maze, run performance was calculated from the proportion of errors (entering a false arm) to all responses (entering any arm).

Locomotor activity was tested on the 5th and 10th weeks of treatments. Spontaneous horizontal, vertical and local exploratory activity was scored automatically by means of a PC during a 10-minute session in a dimly lit open field box (40x40x40 cm) equipped with two arrays (3 and 15 cm above floor level) of infrared movement detectors with 1.1 cm distance between the beams.

Acoustic startle response (ASR) and prepulse inhibition (PPI) of the rats was measured on the 5th and 10th weeks, after the open field sessions, using a commercially available acoustic reflex monitor. The animals were one by one put in the test box. After 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, the test stimuli were by 200 ms preceded by inhibiting prepulses (1 kHz, 73 dB, 500 ms). A whole body twitch resulting in more than 50 g force to the cage floor was accepted as positive response.

Electrophysiological investigation. After finishing all behavioural tests (i.e. after 5 and 10 weeks of Hg- and Mn-administration), the animals were anesthetized with 1000 mg/kg urethane ip. and the left hemisphere was exposed by removing the bony skull. Following a recovery of 30 minutes minimum, 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 (electrocorticogram, ECoG) was recorded for 5 min, and subsequently analysed for the relative power distribution among the standard frequency bands (delta to gamma). Cortical evoked potentials were recorded subsequently via the same electrodes. (Somatosensory stimulation - square electrical pulses {1 Hz, 3-4 V, 0.2 msec} to the whiskers, visual stimulation - flashes {1 Hz, 60 lux} to the contralateral eye, acoustic stimulation - clicks {1 Hz, 40 dB}to the contralateral ear.) Fifty stimuli per modality per rat were applied. After averaging, latency and duration of the main waves was measured manually. All recording of spontaneous and evoked activity and off-line analysis was performed by a PC using the NEUROSYS 1.11 software (Experimetria Ltd., U.K.).

All data were analyzed by ANOVA or Kruskal-Wallis- and Mann-Whitney U-test following a Kolmogorov-Smirnov normality analysis.

3. RESULTS

The manganese and mercury doses applied in the present investigation had no general toxic effect.

During all phases of the maze learning test, both $MnCl_2$ treated groups showed, compared to control animals, a decrease in the average memory performance (*Fig.1*). Acquisition (7th-12th days of the treatment) was dose-dependently impaired in $MnCl_2$ treated rats (high dose vs. control: $0.001<p<0.01$; low: $0.01<p<0.05$). The reference memory of the animals' spatial learning (4th week) showed in both treated groups a significant (high dose vs. control: $p<0.001$; low dose vs. control: $0.001<p<0.01$; and high dose vs. low dose: $0.01<p<0.05$) performance deficit. During the short-term (4 hours) working memory test (5th week), the error frequency of the treated rats was significantly and dose-dependently higher that in the controls (high dose vs. control: $p<0.001$; low dose vs. control: $0.01<p<0.05$; high vs. low dose: $0.01<p<0.05$). After 2 weeks rest period, the control and low dose group both showed a memory return to the level on the 2nd week of treatment but the level reached by the high dose animals was about 20 % below that. In the long-term retention test (from the 43rd day on) both $MnCl_2$ treated groups showed a further

significant memory deficit vs. control ($p<0.001$) and the high vs. low difference was also significant ($0.001<p<0.001$). Comparison of the long-term (9^{th} and 10^{th} week) and short-term (3^{rd} and 5^{th} week) memory retention showed that the error level of both treated groups remained nearly unchanged but the difference vs. control group increased in both long-term memory tests.

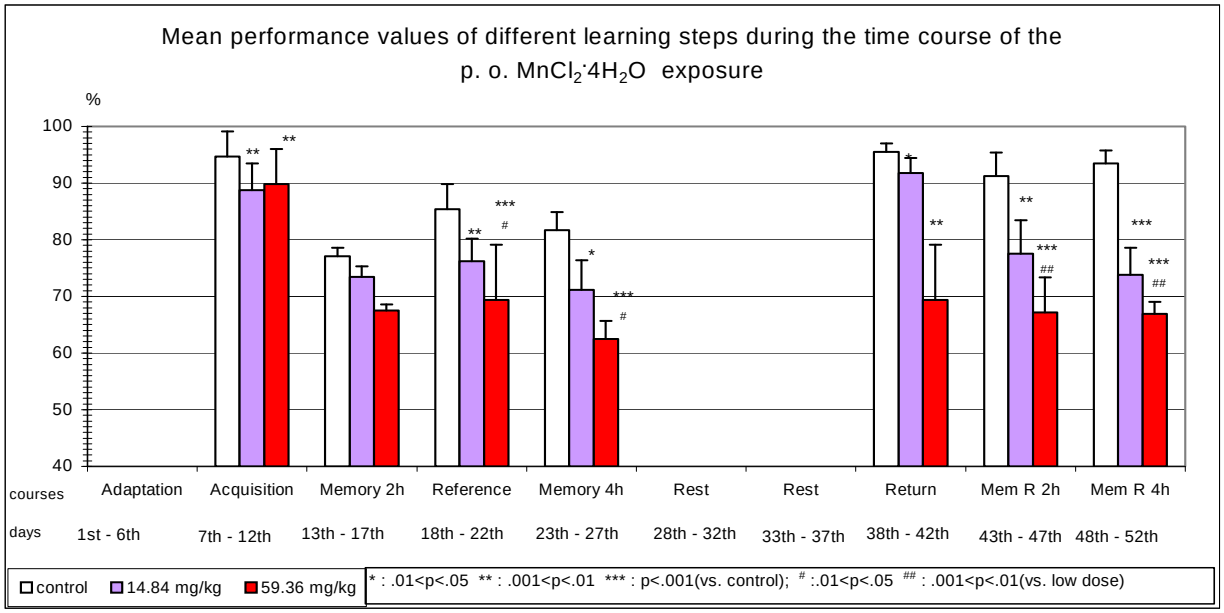

Fig.1. Memory alteration of male Wistar rats treated with $MnCl_2$ p.o. by gavage.

During acquisition and short-term maze learning (2^{nd}- 5^{th} week of treatment), the $HgCl_2$ treated and control animals showed dissimilar trends in the averaged memory performance (*Fig.2*). On the 13^{th} to 17^{th} and 23^{rd} to 27^{th} day of behavioural investigation (short-term retention tests), a significant (high dose vs. control: $p<0.001$, low dose vs. control: $0.01<p<0.05$) memory deficit developed in the groups treated with $HgCl_2$. In the long term retention test (43^{rd} to 57^{th} day of treatment) the 2 and 4 hours memory performance of the treated groups decreased by further ca. 10 %. The reference memory of the animals (4^{th} week of treatment) showed also a significant dose-dependent alteration (high dose vs. control: $p<0.01$, low dose vs. control: $p<0.001$).

Open field tests revealed a decreased locomotor activity in the treated animals on the 5^{th} and 10^{th} weeks of $MnCl_2$ administration (*Fig.3*). The diminished spontaneous exploratory activity of the animals was mainly due to decreased vertical and horizontal activity, and was significant (both doses vs. control: $p<0.01$). On the 10^{th} week, local motor activity was significantly reduced in both treated groups ($0.001<p<0.01$ vs. control).

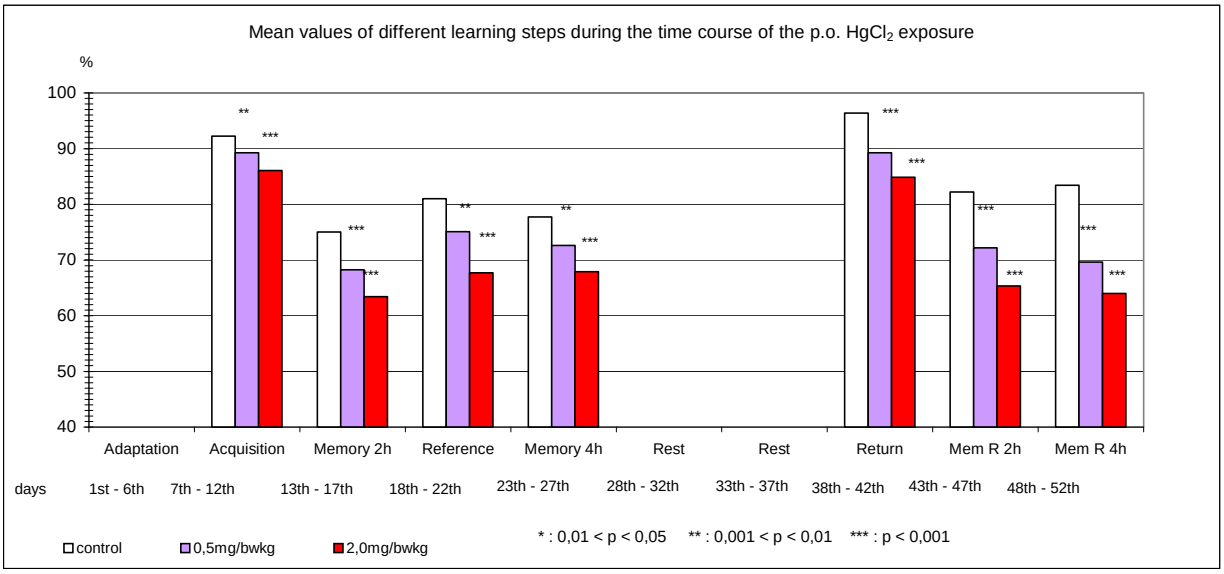

Fig.2. Memory alteration of male Wistar rats treated with $HgCl_2$ p.o. by gavage.

Habituation in the exploratory activity (over the 10 min session) was increased in both treated groups vs. control in the 5th and 10th weeks. Fig. 3 shows the three different elements of locomotor activity - motility, rearing and grooming - on the 10th week. Habituation in the exploratory activity (over the 10 min. session) was increased in both treated groups vs. control.

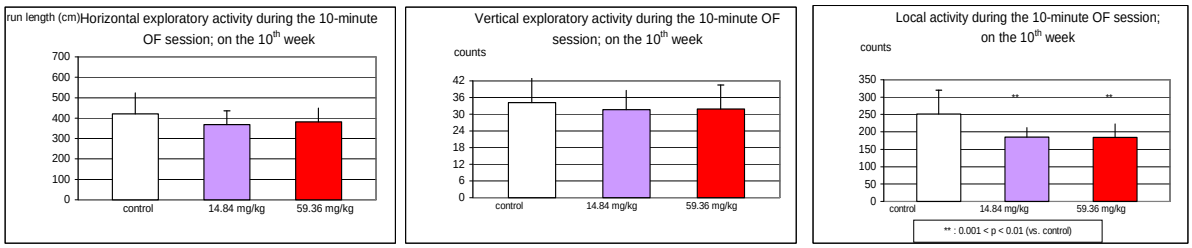

Fig.3. Effects of $MnCl_2$ on horizontalm exploratory activity, rearing activity and local activity of rats over the 10 min open field session in the 10th week of treatment.

Decreased locomotor activity was seen on the 5th and 10th week of hg^{2+} treatment, too. The diminished spontaneous locomotor activity was mainly due to decreased vertical and horizontal activity. *Fig. 4* shows the 1st, 5th and 10th min locomotor activity on the 5th week. Grooming activity/local exploration was reduced by 25 % in the low and 38 % in the high dose group in the 5th week. Continued treatment, however, did not increase this effect. The habituation in the exploratory activity (over the 10 min. session) was decreased in both treated groups vs. control.

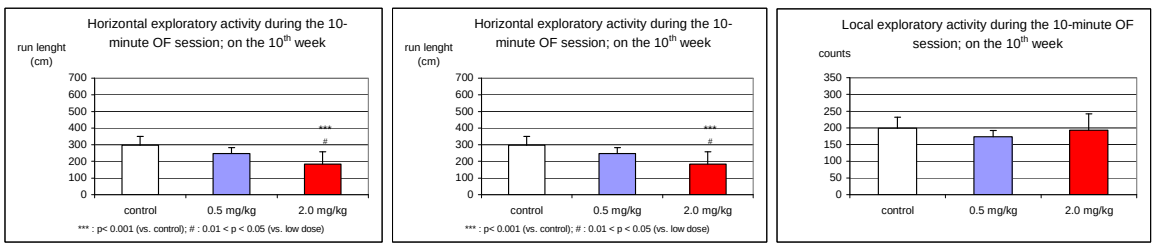

Fig.4. Effects of $HgCl_2$ on spontaneous motility, rearing activity, and grooming activity of rats in the 10th week of treatment.

The number of positive ASR responses decreased significantly ($p<0.01$) in the $MnCl_2$ treated groups by the 10^{th} week (in he 5^{th} week, the difference was not significant). With prepulse (PPI), the number of responses of the treated rats increased, while in the controls, it decreased (significant difference, $p<0.01$ vs. control for both).

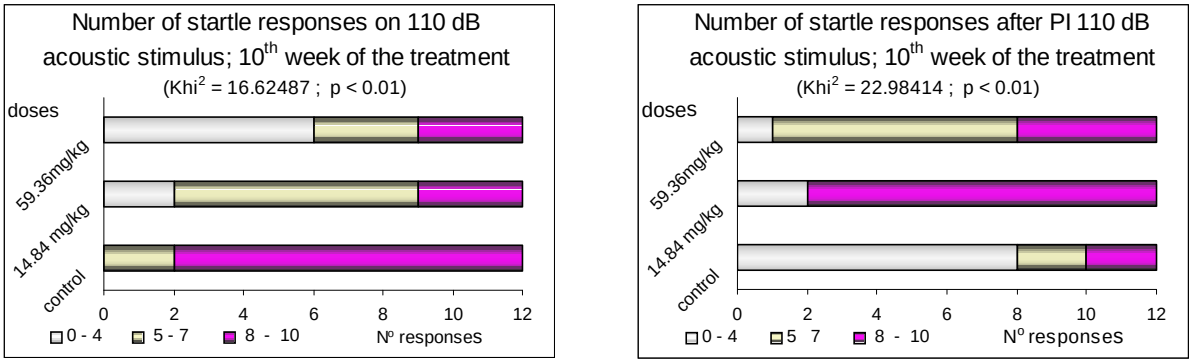

Fig.5. Psychomotor (ASR) and sensorimotor gating (PPI) performance on the 10^{th} week of the $MnCl_2$-treatment

In the high dose $HgCl_2$ group, the number of positive responses was significantly decreased both in the 5^{th} (high dose vs. control $p<0.01$ - not shown) and 10^{th} week ($p<0.05$; *Fig.6*). In the low dose group, the number of startle responses was significantly less than in the control in the 5^{th}, but not in the 10^{th}, week. The number of responses with PPI was not different in the treated and control groups in the 5^{th} week. In the 10^{th} week, however, the low dose rats gave significantly ($p<0.05$) more responses.

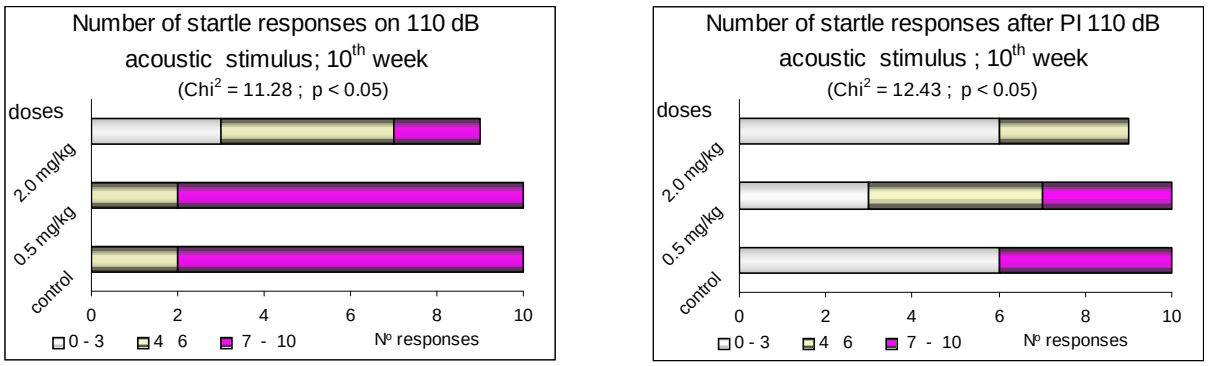

Fig.6. Psychomotor (ASR) and sensorimotor gating (PPI) performance on the 10^{th} week of the $HgCl_2$-treatment

Spontaneous cortical and hippocampal activity was shifted to higher frequencies in the Hg^{2+} treated animals. In the $MnCl_2$ treated ones, the spontaneous activity in the delta and gamma bands decreased, and in the theta and beta1 bands, increased.

4. CONCLUSION

Recall of acquired memory contents was more affected by the high than by the low dose Mn. In the long-term retention, however, the low dose group showed a more severe impairment. Manganese is known to affect several transmitter systems, including those involved in memory functions [12,22]. The effect of Mn on the

cholinergic system [16] can be reflected in altered spatial learning [8]. Spontaneous motor activity in the rat involves both the mesolimbic and nigrostrial dopaminergic system [9], Mn-dependent dysfunction of which is also known.

Hg affects transmitter systems involved in memory functions. The changes of vertical and horizontal motor activity may arise from alterations in the serotoninergic and dopaminergic transmission, respectively [5,7]. Hippocampal muscarinic receptors are extremely sensitive to Hg [4] 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 may explain the diminished reactions of the treated animals in the ASR test and memory processes [8]. The reduction of prepulse inhibition, as it was found in our experiments, can be due to the effect of Hg on GABAergic synapses [15].

The heavy metal doses applied caused alterations in the higher nervous functions of the treated adult rats. In case of human exposure, similar defects can be expected.

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