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# Behavioral and neurotoxic effects seen during and after subchronic exposure of rats to organic mercury

Tünde Vezér[a], András Papp[a,*], Anita Kurunczi[b], Árpád Párducz[b], Miklós Náray[c], László Nagymajtényi[a]

[a] *Department of Public Health, University of Szeged, H-6720 Szeged, Dóm tér 10, Hungary*
[b] *Biological Research Center of the Hungarian Academy of Sciences, Szeged, Hungary*
[c] *National Center for Public Health, Budapest, Hungary*

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**Abstract**

Young adult male Wistar rats (24/group) were treated for 5 weeks with methyl mercury(II)chloride (corresponding to 0.5 and 2.0 mgHg°/kg b.w., control: distilled water) by gavage, followed by a 19 weeks post-treatment period. Spontaneous motility, psychomotor performance and sensorimotor gating was repeatedly tested, electrophysiological recordings done, in the rats throughout the whole experiment.

Decreased horizontal open field activity, reduced number of "noise positive" startle responses, as well as increase of startle response onset latency and peak time, and decrease of peak amplitude, was seen in the treated animals. Most changes disappeared in the post-treatment period.

In the spontaneous cortical and hippocampal activity, altered distribution of the frequency bands was seen after 5 weeks of treatment but not at the end of the post-treatment period. Hippocampal population spikes in the treated animals were depressed and showed less potentiation, which effect was still present 19 weeks after finishing the treatment. The duration of the sensory cortical evoked potentials was shorter than in the controls. In the treated rats, tyrosine hydroxylase-immunoreactive boutons in the substantia nigra pars reticulata were shrunk; blood and brain Hg levels were significantly higher and decreased only slowly.

Considering the continuous presence of low levels of mercurials in the human environment, effects of this kind may be supposed as the background of some human neurobehavioral abnormalities.


*Keywords:* Methyl mercury; Spontaneous exploratory activity; Psychomotor performance; Electrocorticogram; Evoked activity; Rat

## 1. Introduction

In the exposure of the population to organic mercury compounds, phenyl mercury in dispersion wall paints and methyl mercury (MEM) as contaminant in food of sea (and freshwater) origin are the most important sources. Within the organism, dealkylation can occur (Gallagher and Lee, 1980) so that both organic and inorganic forms of Hg must be taken into account when investigating toxic effects.

MEM was found to affect several of the known transmitter systems. Hippocampal population spikes (generated by glutamatergic transmission) in in vitro slices were reduced by MEM most probably by a postsynaptic mechanism (Yuan and Atchison, 1994). In the extracellular space, glutamate (Glu) level is likely to increase, as suggested by the MEM-induced inhibition of Glu uptake (Aschner et al., 2000) and conversion to glutamine (Kwon and Park, 2003) in astrocytes. Muscarinic ACh receptors in the rat brain, and especially in the hippocampus, increased in number in animals with oral MEM exposure (Coccini et al., 2000), possibly leading to altered memory and psychomotor functions. Extracellular level of dopamine (DA), a transmitter with a crucial role in extrapyramidal motor control and in several elements of behavior, was increased by systemic MEM treatment (Faro et al., 1997), probably due to the action of MEM on the DA transporter (Faro et al., 2002a) and/or, indirectly, to a glutamatergic mechanism (Faro et al., 2002b). GABAergic

* Corresponding author. Tel.: +36 62 545 119; fax: +36 62 545 120.
*E-mail address:* ppp@puhe.szote.u-szeged.hu (A. Papp).

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transmission, involved in sensorimotor regulation and locomotor activity (Koch et al., 2000) is also known to be affected by MEM (Yuan and Atchison, 1994). The effects of MEM on $Ca^{2+}$ homeostasis (Denny and Atchison, 1996) may influence neural functions via the mentioned transmitter systems or by direct action. MEM-dependent block of mitochondrial Ca-ATPase (Freitas et al., 1996) causes increase of cytosolic $Ca^{2+}$ levels, affecting the gating function of ion channels.

The behavioral methods used in the present experiment (see below) are known to be sensitive in detecting changes in the above-mentioned transmitter systems. There have been, in fact, several publications on the effect of mercury exposure on behavioral outcomes in rats (open field: Rossi et al., 1997; T-maze: Zenick, 1974; open field and several maze types: Fredriksson et al., 1996). These, however, provided no correlation with morphological and physiological parameters, and included no follow-up in the after-exposure period. In the present study, a comprehensive analysis of MEM effects on functions of the nervous system was attempted; by oral exposure of rats for 5 weeks with two doses, which proved to be toxicologically relevant in behavioral endpoints in different settings from prenatal (Fredriksson et al., 1996; Rossi et al., 1997) to young adult (Coccini et al., 2000). Observations were continued for a long post-treatment period, and two doses of d-amphetamine (d-AM), a dopaminerigc agonist, were applied to detect possible alterations in the neurotransmitter systems. A spectrum of endpoints was investigated, from mercury levels (in the blood, cortex and hippocampus) and histological changes (tyrosine hydroxylase immunoreactivity in the substantia nigra pars reticulata) to cortical electrophysiological (ECoG, evoked potential) and behavioral (open field activity, acoustic startle response, prepulse inhibition) alterations.

## 2. Methods

### 2.1. Animals and treatment

Male Wistar rats (obtained at the University's Breeding Center, 10 weeks old, 160 ± 10 g b.w.) were treated, 5 days in a week, with methylmercury chloride ($CH_3HgCl$; analytical grade, Aldrich). The animals were housed under conventional conditions (22–24 °C, 12 h light/dark cycle with light starting at 6:00 a.m., up to four rats in one cage). Standard rodent chow and drinking water was given ad libitum.

Three groups of rats were used, consisting of 24 animals each, one receiving 2.0 mg/kg b.w. MEM (high dose group), another receiving 0.5 mg/kg, and a third, control group receiving distilled water. MEM was dissolved in distilled water b.w. to 1 ml/kg administration volume, pH neutralized as necessary, and administered by gavage. All 24 animals in the groups received the same treatment for 5 weeks (1st to 5th treatment week, Table 1). From each group, 12 animals were kept, without treatment, for further 19 weeks (1st to 19th post-treatment week) to see the elimination of MEM; and were used for the behavioral tests before, during and after the treatment period; and for electrophysiological recording at the end of the post-treatment period. From the other 12 rats per group, seven were used for electrophysiological recording in the 1st, and three in the 4th, post-treatment week, and two went for tyrosine hydroxylase immunochemistry at the end of the 5th treatment week. Blood and brain (cortex and hippocampus) samples were taken in the 1st, 4th and 19th post-treatment week.

During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed.

### 2.2. Open field, startle response and psychomotor gating tests

The animals' spontaneous motor behavior was first tested; in the 0th week (before commencing MEM administration), then in the 5th treatment week, and the 12th post-treatment week (17th week of the experiment); on the 12 animals per group defined above (Table 1). The rats were brought to the test room for accommodation 30–40 min before testing. Open field (OF) activity was investigated in 10-min sessions between 9.00 a.m. and 2.00 p.m. Horizontal motiliy (run length), and vertical (rearing) as well as local (grooming)

Table 1
Time scheme of the experiment in the pre-treatment week and in the treatment and post-treatment period

| Investigations | Number of investigated animals per group over the whole course (0–24th weeks) of the experiment | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Pre-treatment | Treatment period | | Post-treatment period | | | | | | | | | |
| | 0th | 1st–4th | 5th | 1st | 2nd–3rd | 4th | 5th–11th | 12th | 13th | 14th | 15th | 16–18th | 19th |
| Open field | 12 | – | 12 | – | – | – | – | 12 | *0.5 d-AM 12* | – | *1.5 d-AM 12* | – | – |
| ASR/PPI | | – | | – | – | – | – | | – | – | – | – | – |
| TH-IR | – | – | – | 2 | – | – | – | – | – | – | – | – | – |
| Electrophys. | – | – | – | 7 | – | 3 | – | – | – | – | – | – | 10 |
| Blood Hg level | – | – | – | 3 | – | 3 | – | – | – | – | – | – | 6 |
| Tissue Hg level | – | – | – | | – | | – | – | – | – | – | – | |

Tests and investigations performed are indicated in the column of the corresponding week. There were three groups (high dose, low dose and control) investigated, the number in the cells show the number of animals used in the different tests. Abbreviations: ASR/PPI, acoustic startle response and pre-pulse inhibition; TH-IR, brain tissue sampling for tyrosine hydroxylase immunhistochemistry; electrophys., electrocorticogram (ECoG) and evoked potential (EP); blood Hg level, blood sampling from tail vein for Hg level determination; tissue Hg level, cortex and hippocampus sampled for Hg level determination; d-AM, d-amphetamine.

activity, was detected by arrays of infrared LEDs and sensors (ACTIFRAME, Gerb Electronic, Berlin, Germany) around a 40 cm × 40 cm × 40 cm OF box. Illumination at the floor of the OF was 15–25 lux, with ca. 40 dB white background noise. The animals were placed into the center of the open field box and their activity was recorded for 10 min.

In the 13th and 15th post-treatment week (18th and 20th weeks of the experiment), the same rats tested before were i.p. injected with 0.5 or 1.5 mg/kg b.w. of d-AM, respectively. Fifteen minutes later, their OF activity was recorded again.

Acoustic startle response (ASR) is a fast involuntary contraction of facial and body muscles evoked by sudden and intense acoustic stimuli (Koch, 1999), and prepulse inhibition (PPI) is the normal suppression of the startle response by a preceding stimulus and is used as an operational measure for early sensorimotor gating mechanisms (Braff and Geyer, 1990). ASR and ASR with PPI were tested, following the OF test, on the same day (Table 1), using the ResponderX (Columbus Instruments, Ohio, USA) equipment (plexiglass chamber of 16 cm × 28 cm, 18 cm high, with a piezo force transducer in the bottom).

Parameters of the eliciting (base) acoustic stimulus were 5000 Hz, 110 dB and 200 ms, for the pre-stimulus, 1000 Hz, 73 dB and 500 ms (preceding the eliciting stimulus with 200 ms). One animal at a time was put into the box, and, after 10 min accommodation, 10 stimuli per session were applied with a fixed interval of 15 s, then, after 15-min rest, another 10 with prepulse. In both tests, a whole-body twitch resulting in more than 50 g force to the cage floor was accepted as "noise positive response", of which latency, time to peak and startle amplitude were measured. Stimulation and data acquisition was controlled by a PC.

### 2.3. Electrophysiological investigations

These were done on seven animals per group in the 1st, and three per group in the 4th, post-treatment week (from those 12 not used for behavioral testing); and in the 19th post-treatment week on 10 animals per group of those having undergone the behavioral tests. The animals were anaesthetized with 1000 mg/kg urethane i.p. (Bowman and Rand, 1980), the left hemisphere was exposed by removing the bony skull, and the rat was put aside for at least 30 min for recovery covered with a warm cloth. The recording room was shaded and low-noise.

For recording spontaneous and evoked cortical activity, the animal's head was fixed in a stereotaxic frame and silver electrodes were placed on the primary somatosensory, visual and auditory areas. Electrocorticogram (ECoG) was recorded from the three sensory areas simultaneously for 15 min. From the recordings, the relative spectral power of the frequency bands: delta, 0.5–4 Hz; theta, 4–7 Hz; alpha, 8–13 Hz; beta1, 13–20 Hz; beta2, 20–30 Hz; gamma, 30–50 Hz (Kandel and Schwartz, 1985) was electronically determined. For group to group comparison, "ECoG index": activity ratio of [delta + theta]/[beta1 + beta2] (Dési and Nagymajtényi, 1999) was calculated. Cortical evoked potentials were recorded subsequently via the same electrodes. Somatosensory stimulation was done by a pair of needles inserted into the whiskery skin, using square electric pulses (1 Hz, 3–4 V, 0.05 ms). Visual stimulation was performed by flashes (1 Hz, ca. 60 lux) delivered from a flashbulb via an optical fiber directly into the contralateral eye of the rat. For acoustic stimulation, clicks (1 Hz, ca. 40 dB) from a small earphone were applied into the contralateral ear of the rat. All stimuli were just supramaximal and well above background. Fifty stimuli of each modality per rat were applied and the evoked activity recorded. After averaging, latency and duration of the main waves was measured manually.

For recording hippocampal activity, a steel needle recording electrode (ca. 1 MΩ) was inserted into the left hippocampal CA1 region (stereotaxic coordinates: AP −3, L 2, V 2–3; Paxinos and Watson, 1982). Population spikes (resulting from simultaneous discharge of numerous neurons on a synchronous input; Andersen et al., 1971), were elicited by stimulating the perforant path (AP −6, L 4.5, V 4) by a bipolar steel electrode at 0.3 Hz.

All recording of spontaneous and evoked activity and their off-line analysis was performed by a PC using the NEUROSYS 1.11 software (Experimetria Ltd., UK).

### 2.4. Tyrosine hydroxylase-immunreactivity (TH-IR)

Tyrosine hydroxylase is a key enzyme of catecholamine biosynthesis (Kalsner and Westfall, 1990) making TH activity a suitable indicator of disturbances in e.g. the dopaminergic system. Two animals per group were, at the end of the 5th treatment week, transcardially perfused first with ice-cold saline, then with 4% paraformaldehyde solution for 10 min. The brains were removed and postfixed overnight, cryoprotected by 30% sucrose in phosphate buffer for 16 h, and sectioned on a freezing microtome at 50 μm thickness. The free floating coronal sections of the substantia nigra region were stained using a common immunhistochemical procedure against tyrosine hydroxylase (Rabbit anti-TH, Chemicon, 1:5000). The reaction was visualized with the avidin–biotin–peroxidase complex plus 3,3-diaminobenzidine, and intensified by nickel (ABC-DAB/nickel method).

The size of TH-IR boutons in the substantia nigra pars reticulata was measured by means of a digital system (SONY 950-P CCD camera, Flashpoint 128 frame grabber and Image Pro Plus 4.0 image analysis software) using a 40× objective (at this high magnification different optical planes of the same section could be examined). Boutons, being in focus and recognized and measured by the program as single objects, were selected for determination of cross-sectional areas. In each experimental group 450–500 boutons were analyzed.

### 2.5. Tissue mercury level determination

Blood samples were collected in the 1st, 4th and 19th week of the post-treatment period from three, three and six

animals of each group, respectively (Table 1). The tail vein was punctured and blood was taken to heparinized vacutainers of 7.0 ml capacity (Becton-Dickinson, BD367735). All blood samples were stored at 4 °C until analyzed.

When the electrophysiological recording was finished (1st, 4th and 19th post-treatment week), the animals were sacrificed with an overdose of Nembutal. The thorax was opened and the animals were transcardially perfused with 500 ml saline to remove blood from the organs. The brain was removed whole, without the meninges, and was, under a stereomicroscope, halved and dissected to isolate the hippocampus. The whole hippocampus and 0.3 g of the cortex was taken as sample, kept at −18 °C until analyzed.

Total mercury was determined at the accredited laboratory of the National Center for Public Health, Budapest. The samples were digested in 2 ml concentrated nitric acid, with 0.5 ml hydrogen peroxide added for cortex and hippocampus. Total mercury content was measured by cold vapor atomic absorption photometry.

### 2.6. Statistics

The number of animals giving "noise positive response" in the ASR and PPI tests was evaluated with the $Chi^2$ test. The distribution of other data was checked for normality by the Kolmogorov–Smirnov test. Depending on the distribution, the statistical analyses were carried out by two-way ANOVA or Kruskal-Wallis one-way ANOVA for the open field results in the post-treatment period (with MEM dose and d-AM administration as independent variables). One-way ANOVA was used to evaluate the results of ASR/PPI, Hg level determination, electrophysiological recordings, TH histochemistry, and OF (in the pre-treatment and treatment periods). The confidence level was always set to $p < 0.05$. Post hoc analysis of group differences was performed by Scheffe's test; group comparisons following the Kruskal-Wallis test were performed by the Mann–Withney *U*-test (also here, $p < 0.05$). For the statistical analysis, Statistica for Windows 4.0 software package was used.

## 3. Results

### 3.1. Blood and tissue mercury levels

The levels of total mercury (μg/g) in the blood and brain (cortex and hippocampus) samples of the MEM-treated and control animals were measured three times during the post-treatment period (Table 2). Blood level in the low and high dose group was in the 1st post-treatment week about 40 and 160 times higher, respectively, than in the controls. Later during the post-treatment period, Hg levels decreased a lot but the difference between the high dose and control group remained significant even in the 19th post-treatment week. In the cortex samples, the metal level difference between treated and control animals, and the rate of elimination, was comparable to what was seen in the blood. In the hippocampus, Hg concentrations were less (but still significantly) elevated versus control and the elimination was faster.

Table 2
Total mercury levels (μg/g, mean ± S.D.) in blood, cortex and hippocampus samples at various times during the post-treatment period (*n*, number of animals/group)

| Hg° (μg/g) in: | Weeks of the post-treatment period | | | | | |
|---|---|---|---|---|---|---|
| | 1st ($n = 3$) | | 4th ($n = 3$) | | 19th ($n = 6$) | |
| | Mean ± S.D. | Significance | Mean ± S.D. | Significance | Mean ± S.D. | Significance |
| Blood | | | | | | |
| Control | 0.2633 ± 0.035 | $F(2,6) = 706.8$, $p < 0.001$ | 0.2000 ± 0.085 | $F(2,6) = 91.5$, $p < 0.001$ | 0.0135 ± 0.007 | $F(2,15) = 29.5$, $p < 0.001$ |
| 0.5 mg/kg | 10.8233 ± 1.954*** | | 1.5900 ± 0.036* | | 0.1222 ± 0.031 | |
| 2.0 mg/kg | 42.1467 ± 1.490***,### | | 4.5667 ± 0.693***,### | | 0.4197 ± 0.159***,### | |
| Hippocampus | | | | | | |
| Control | 0.1783 ± 0.083 | $F(2,6) = 42.5$, $p < 0.001$ | 0.1300 ± 0.105 | $F(2,6) = 8.2$, $p < 0.05$ | 0.1446 ± 0.128 | $F(2,15) = 0.26$, $p > 0.05$ |
| 0.5 mg/kg | 0.6200 ± 0.072*** | | 0.2533 ± 0.015 | | 0.1015 ± 0.117 | |
| 2.0 mg/kg | 0.6633 ± 0.057*** | | 0.4467 ± 0.129* | | 0.1135 ± 0.065 | |
| Cortex | | | | | | |
| Control | 0.0250 ± 0.005 | $F(2,6) = 38.9$, $p < 0.001$ | 0.0247 ± 0.005 | $F(2,6) = 103.8$, $p < 0.001$ | 0.0214 ± 0.012 | $F(2,15) = 2.78$, $p > 0.05$ |
| 0.5 mg/kg | 2.1233 ± 0.637*** | | 0.2800 ± 0.027*** | | 0.0155 ± 0.009 | |
| 2.0 mg/kg | 2.9133 ± 0.329*** | | 0.4767 ± 0.061***,## | | 0.0331 ± 0.019 | |

* $p < 0.05$ treated vs. control group.
*** $p < 0.001$ treated vs. control group.
## $p < 0.01$ high vs. low dose.
### $p < 0.001$ high vs. low dose.

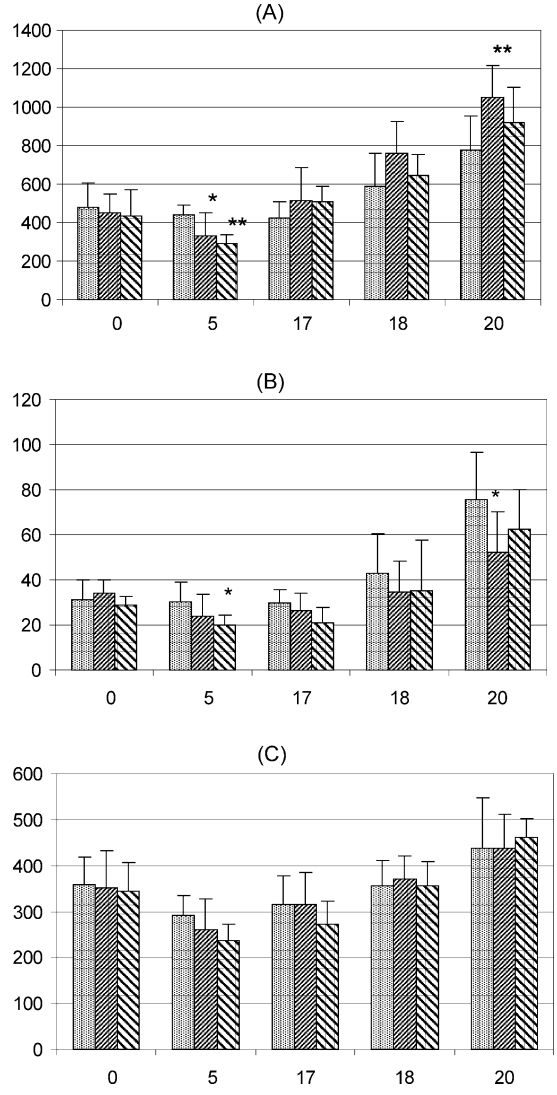


Fig. 1. Open field performance ((A) horizontal activity [running]; (B) vertical activity [rearing]; (C) local activity [mostly grooming]) in the pre-treatment week, and in the treatment and post-treatment period, in control and MEM-treated rats. Mean ± S.D., $n = 12$/group. Abscissa: number of experimental weeks: 0 (pre-treatment week), 5 (5th treatment week), 17, 18, 20 (12th, 13th, and 15th post-treatment weeks); see Table 1 for the course of experiment. Ordinate: (A) length run, cm; (B) counts, rearing; (C) counts, local activity (note different scales). * $p < 0.05$, ** $p < 0.01$ treated vs. control group. Bar pattern: control ( ); low dose (0.5 mg/kg) ( ) ; high dose (2.0 mg/kg) ( ).

### 3.2. Open field activity

The effect of MEM was seen on each of the elements of OF behavior studied.

The OF performance of the treated and control groups was compared separately in the 0th and 5th treatment weeks, and in the 12th, 13th and 15th post-treatment weeks (17th, 18th and 20th week of the experiment; Fig. 1). As seen in Fig. 1A (left two columns), a clear-cut decrease of horizontal activity developed during the 5 weeks of MEM treatment. In the 5th week, the difference of treated versus control rats was significant ($F_{2,24} = 8.53$, $p < 0.01$; high dose versus control, $p < 0.01$, low dose versus control, $p < 0.05$).

The effect on the vertical activity was similar (Fig. 1B). The decrease in rearing activity in the MEM-treated animals was significant in the 5th treatment week ($F_{2,24} = 3.47$; $p < 0.05$; high dose versus control, $p < 0.05$). The decreasing trend of the local motor activity (Fig. 1C) was not significant.

The OF sessions repeated in the 12th post-treatment week (17th week of the experiment) showed that the effect on the horizontal activity disappeared fully (Fig. 1A, third versus second column). On the vertical activity (Fig. 1B), the differences between the values of the treated and control groups indicated that some effect of MEM may have remained although significance was no more present.

These records were used as controls versus those taken in the 13th and 15th post-treatment week (18th and 20th weeks of the experiment), when the effect of d-AM on the open field activity of the control and MEM-treated rats was studied (Fig. 1A–C, columns for 17th experimental weeks and later). In the 13th post-treatment week, 0.5 mg/kg d-AM was given to the rats i.p., which caused no significant alteration in the rearing and horizontal activity of the MEM treated rats, compared either to the untreated control group or to the data of the same group before d-AM administration.

In the 15th post-treatment week, 1.5 mg/kg d-AM was applied. In the low dose (but not the high dose) group, this caused significant increase (versus control) in the horizontal ($F_{2,24} = 5.67$, $p < 0.01$; low dose versus control, $p < 0.01$) and significant decrease in the vertical ($F_{2,24} = 3.54$, $p < 0.05$, low dose versus control, $p < 0.05$) activity.

In the horizontal activity, comparison of the data of the 12th, 13th and 15th post-treatment week revealed a significant effect of the MEM dose ($F_{2,72} = 9.74$, $p < 0.001$) and of d-AM administration ($F_{2,72} = 57.12$, $p < 0.01$).

In the vertical activity, comparison of data from the same period as above also showed significant MEM dose dependence ($F_{2,72} = 3.25$, $p < 0.05$) and d-AM administration dependence ($F_{2,72} = 35.05$, $p < 0.001$).

### 3.3. Acoustic startle response and psychomotor gating

The number of ASR reactions (see Section 2.2) is given in Fig. 2. By the end of the 5th treatment week, MEM significantly ($Chi^2 = 30.73$, $p < 0.001$) decreased the animals' reactivity on the eliciting stimulus (Fig. 2A) but seemed to turn the inhibiting effect of the prepulse into facilitation (Fig. 2B; $Chi^2 = 20.85$, $p < 0.001$). In the 7th post-treatment week, the effects were largely reversed but without a clear dose dependence (Fig. 2C; $Chi^2 = 7.40$, $p < 0.10$). The anomalous effect of the prepulse, however, remained, but only in the low dose group (Fig. 2D; $Chi^2 = 15.58$, $p < 0.01$).

The measured parameters of the ASR motor reaction of the control and MEM-treated animals are summarized in Table 3. In the 5th week of MEM treatment (Table 3A), the onset latency of the ASR increased in a dose-dependent way

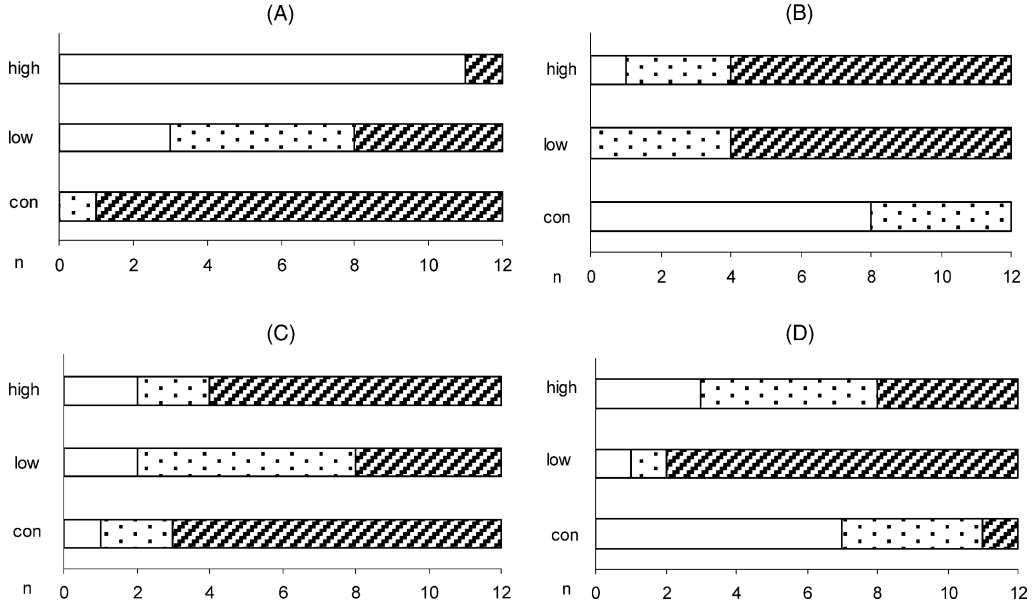


Fig. 2. Number of noise-positive startle responses given on 110 dB acoustic stimulus without (A and C) and with (B and D) prepulse inhibition, in the 5th treatment week (top) and in the 7th post-treatment week (bottom); in control, low dose (0.5 mg/kg) and high dose (2.0 mg/kg) MEM treated rats. The bars show the distribution of rats ($n = 12$/group) according to the number of responses (0–4, (□) ; 5–7, (▨) ; 8–10, (▨)) given on 10 consecutive stimuli.

($F_{2,27} = 10.46$, $p < 0.001$; high dose versus control, $p < 0.001$; and high versus low dose, $p < 0.01$). MEM in 2.0 mg/kg dose caused also a significant rise of the ASR peak time ($F_{2,27} = 9.53$, $p < 0.001$; high dose versus control, $p < 0.05$; and versus low dose, $p < 0.001$). The startle response peak amplitude decreased in the both treated groups, but for the high dose this was significant ($F_{2,27} = 4.29$, $p < 0.05$; high dose versus control, $p < 0.05$).

Table 3
Parameters (mean ± S.D.) of the acoustic startle response (ASR) without and with prepulse inhibition (PPI); in the 5th week of MEM treatment (A) and in the 7th post-treatment week (B)

| (A) Startle response in the 5th treatment week | Control | MEM | |
|---|---|---|---|
| | | 0.5 mg/kg | 2.0 mg/kg |
| ASR | | | |
| Onset latency (ms) | 20.27 ± 2.12 ($n = 12$) | 21.21 ± 1.82 ($n = 10$) | 24.4 ± 2.11$^{***,\#\#}$ ($n = 8$) |
| Peak time (ms) | 27.89 ± 2.24 ($n = 12$) | 26.71 ± 2.19 ($n = 10$) | 30.92 ± 1.60$^{*,\#\#\#}$ ($n = 8$) |
| Peak amplitude (g) | 257.08 ± 127.60 ($n = 12$) | 177.43 ± 84.21 ($n = 10$) | 129.83 ± 52.32$^{*}$ ($n = 8$) |
| PPI | | | |
| Onset latency (ms) | 23.22 ± 2.59 ($n = 9$) | 18.05 ± 0.76$^{***}$ ($n = 10$) | 21.39 ± 1.15$^{\#\#\#}$ ($n = 10$) |
| Peak time (ms) | 28.44 ± 2.49 ($n = 9$) | 24.91 ± 1.08$^{***}$ ($n = 10$) | 26.59 ± 1.04 ($n = 10$) |
| Peak amplitude (g) | 116.85 ± 45.43 ($n = 9$) | 239.94 ± 139.50$^{*}$ ($n = 10$) | 147.74 ± 38.02 ($n = 10$) |
| (B) Startle response in the 7th post-treatment week | | | |
| ASR | | | |
| Onset latency (ms) | 18.42 ± 1.89 ($n = 11$) | 18.91 ± 1.72 ($n = 10$) | 19.30 ± 1.70 ($n = 9$) |
| Peak time (ms) | 26.34 ± 2.14 ($n = 11$) | 27.071 ± 0.92 ($n = 10$) | 28.32 ± 1.23$^{*}$ ($n = 9$) |
| Peak amplitude (g) | 341.97 ± 167.50 ($n = 11$) | 362.56 ± 138.50 ($n = 10$) | 425.99 ± 196.40 ($n = 9$) |
| PPI | | | |
| Onset latency (ms) | 21.43 ± 1.78 ($n = 11$) | 19.61 ± 1.74 ($n = 10$) | 20.42 ± 2.07 ($n = 8$) |
| Peak time (ms) | 27.66 ± 1.49 ($n = 11$) | 26.14 ± 1.23$^{*}$ ($n = 10$) | 27.53 ± 1.13 ($n = 8$) |
| Peak amplitude (g) | 185.06 ± 74.25 ($n = 11$) | 233.78 ± 125.90 ($n = 10$) | 246.56 ± 138.60 ($n = 8$) |

Onset latency, peak time and peak amplitude were determined from the time course of the force exerted by the startled animal on the piezo force transducer in the cage bottom (n, number of investigated animals in the group).

$^{*}$ $p < 0.05$ vs. control.
$^{***}$ $p < 0.001$ vs. control.
$^{\#\#}$ $p < 0.01$ high vs. low dose.
$^{\#\#\#}$ $p < 0.001$ high vs. low dose.

With prepulse, the response peak amplitude in the low dose group was significantly higher ($F_{2,26} = 5.04$, $p < 0.05$; low dose versus control, $p < 0.05$). It was also greater than without prepulse in the same group. There was also a significant decrease in peak time ($F_{2,26} = 11.03$, $p < 0.001$) and onset latency ($F_{2,26} = 24.14$, $p < 0.001$) of the responses obtained with prepulse in the low dose group (in the high dose group, the changes showed the same trend without being significant).

In the 7th post-treatment week (Table 3B), ASR amplitude and onset latency was no more significantly different between the treated and control animals. Peak time, however, was still significantly longer in the high dose group ($F_{2,27} = 5.05$, $p < 0.05$; high dose versus control, $p < 0.05$). By this time, no significant differences remained in the latency and amplitude of the ASR with PPI in the treated groups versus control, only the peak time of the low dose group was significantly reduced ($F_{2,26} = 4.19$, $p < 0.05$; low dose versus control, $p < 0.05$).

### 3.4. Effects on electrophysiological parameters

The effect of MEM on the ECoG was the most clear-cut (although below significance) in the visual cortex. The power spectrum in the treated rats showed a decrease in the delta, and increase in the beta2 and gamma, bands at the end of the treatment period (1st post-treatment week). In the 19th post-treatment week, these changes were no more present (Fig. 3A and B). In the basal activity of the hippocampal CA1 region (Fig. 3D and E), non-significant decrease was seen in the delta, theta and alpha, and non-significant increase in the gamma bands, which effects also disappeared by the end of the post-treatment period. The ECoG index (see Section

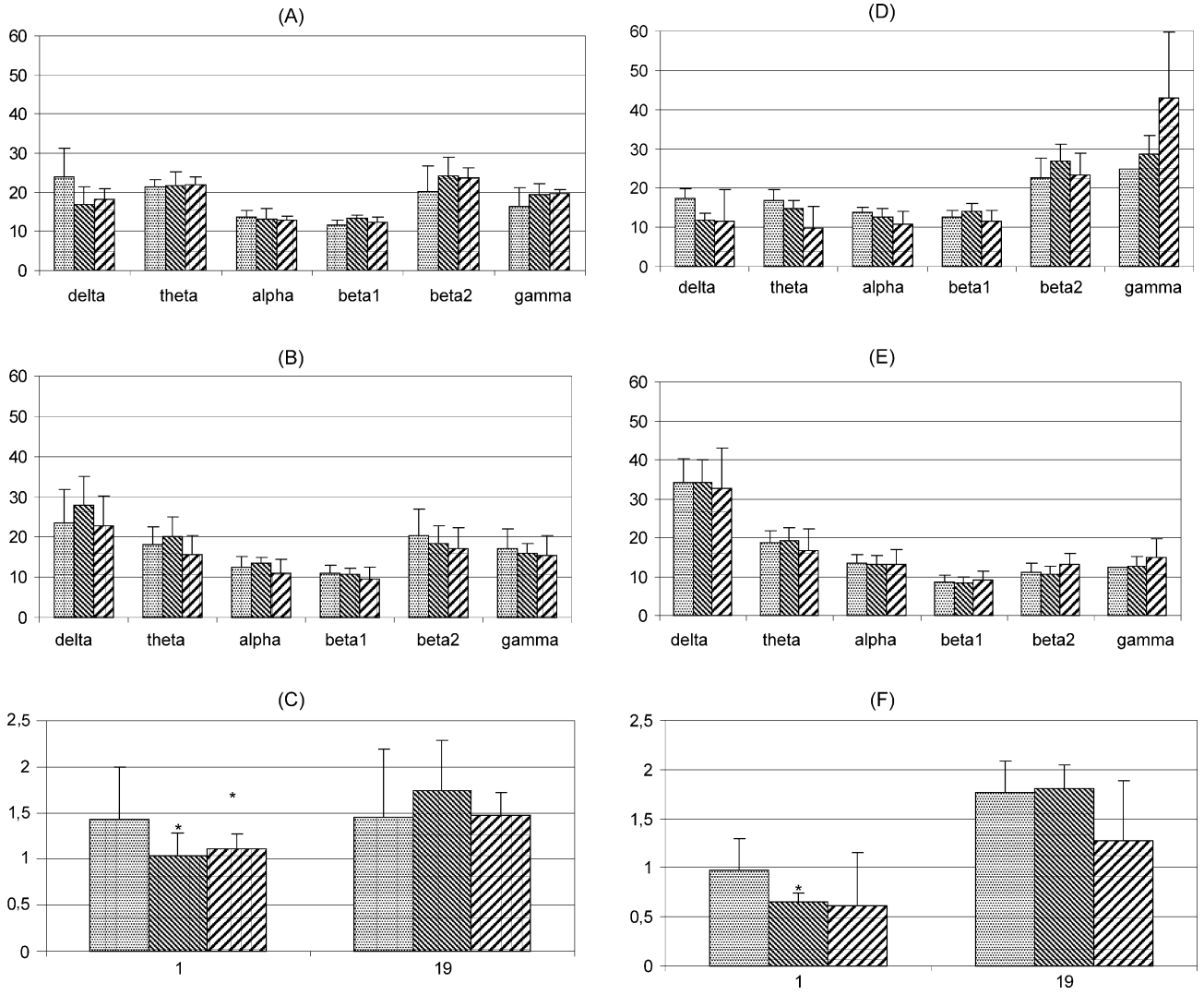


Fig. 3. Power spectrum of the spontaneous activity in the visual cortical area, in the 1st (A), and 19th (B) post-treatment week. Mean ± S.D., $n = 10$ per group. Abscissa: standard frequency bands. Ordinate: relative power by band (in percentage of the total activity). (C) ECoG index values (see Section 2.3) of the visual cortex activity in the 1st and 19th post-treatment weeks. Abscissa: weeks of the post-treatment period. Ordinate: index value. (D and E) Power spectrum (as in A and B) of the hippocampal CA1 activity in the 1st (D) and 19th (E) post-treatment week. (F) Index values, as in (C), of the hippocampal activity. Bar pattern as in Fig. 1. * $p < 0.05$ treated vs. control group.

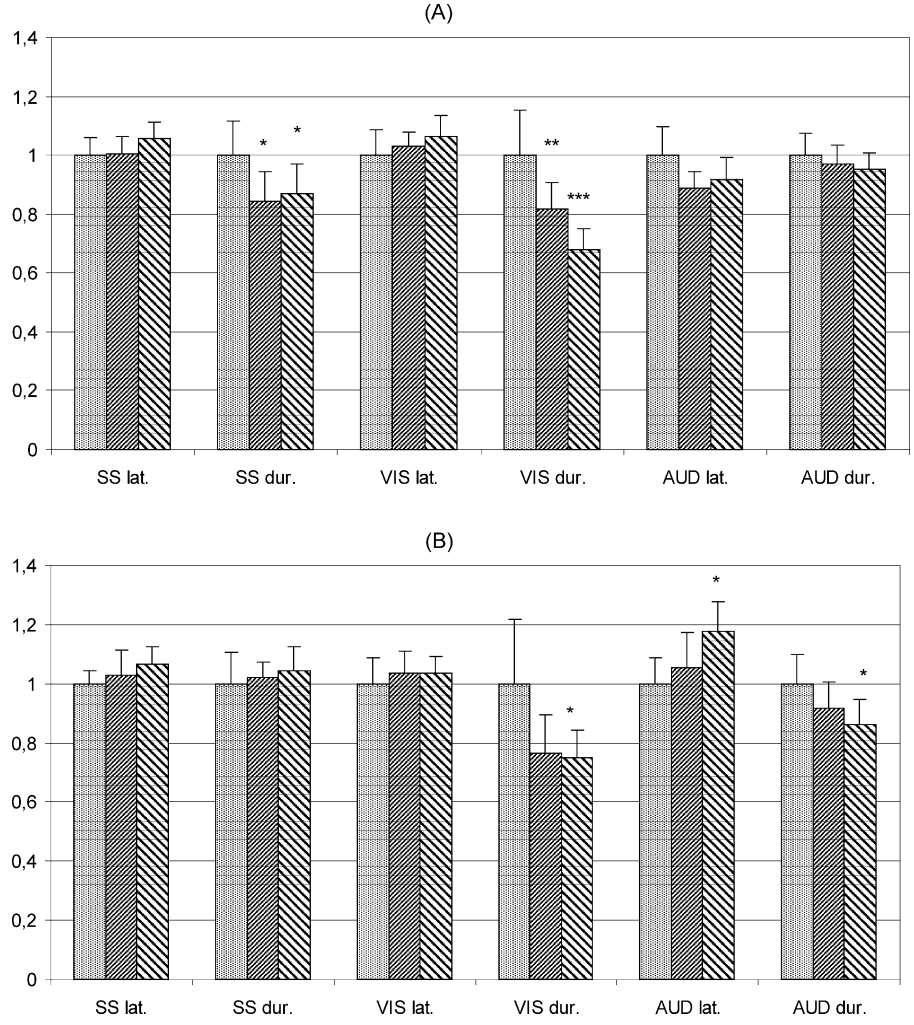


Fig. 4. Relative changes (treated/control) of the latency and duration of cortical evoked potentials in the 1st (A) and 19th (B) post-treatment week. Mean ± S.D., $n = 10$ per group. Abscissa: parameters (SS, somatosensory; VIS, visual; AUD, auditory; lat., latency; dur., duration). Ordinate: relative change, [treated group average]/[control group average], error bars calculated with the same ratio. Bar pattern as in Fig. 1. * $p < 0.05$ treated vs. control group.

2.3) proved to be a more sensitive indicator of MEM effects on spontaneous activity, signalizing significant change in the visual area (Fig. 3C; $F_{2,27} = 3.51$, $p < 0.05$; high dose versus control, $p < 0.05$, low dose versus control, $p < 0.05$) and in the hippocampus (Fig. 3F; $F_{2,27} = 3.76$, $p < 0.05$; low dose versus control, $p < 0.05$—the effect of the high dose fit in the trend but was non-significant due to large standard deviation). All changes of the spontaneous activity largely disappeared after the elimination period.

The latency of the cortical sensory evoked potentials (Fig. 4A and B) showed minor, inconsistent changes in the 1st post-treatment week. Significant decrease was seen, however, in the duration of the somatosensory ($F_{2,27} = 4.27$, $p < 0.05$; high dose versus control, $p < 0.05$; low dose versus control, $p < 0.05$) and visual ($F_{2,27} = 13.45$, $p < 0.001$; high dose versus control, $p < 0.001$; low dose versus control, $p < 0.01$) EP. In the somatosensory response, the changes of the duration fully disappeared during elimination. In the visual EP, there was only partial recovery. In the auditory EP, the duration decrease became stronger by the end of the post-treatment period, and a significant increase of latency in the high dose group appeared.

Amplitude and latency of the hippocampal population spikes were weakly influenced by 1st post-treatment week (Fig. 5A). Their potentiation by a train of frequent stimuli (Fig. 3B) was, however, significantly impaired by the high dose MEM ($F_{2,27} = 4.06$, $p < 0.05$; high dose versus control, $p < 0.05$).

### 3.5. Histochemical alterations

In order to find possible structural correlates of changes in the dopaminergic system (reflected in the behavioral alterations) the morphology of TH-immunoreactive neurons was analyzed. In the number of TH-labelled (thus, dopaminergic) cells, no differences were found in the substantia nigra

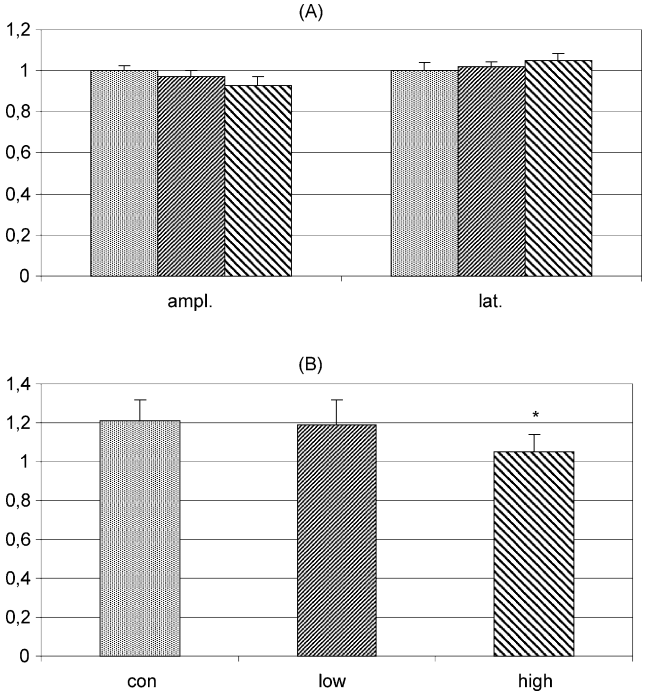


Fig. 5. The effect of MEM on the hippocampal population spike. (A) Relative change (treated/control as in Fig. 4) of amplitude and latency of the hippocampal population spike, in the 1st post-treatment week. Mean ± S.D., $n = 10$ per group. Ordinate as in Fig. 4. (B) Change of amplitude of the population spike, elicited by tetanizing stimulation. Ordinate: group averages (mean ± S.D., $n = 10$ per group) of posttetanic/pretetanic values. Bar pattern as in Fig. 1. * $p < 0.05$ treated vs. control group.

compacta region. In the reticular part, morphometric analysis of the TH-reactive boutons was done and it was found that, following mercury treatment, there was a significant decrease in the bouton size. This could be observed also in animals treated with low dose of MEM. Interestingly, the higher dose did not result in further decrease (Fig. 6).

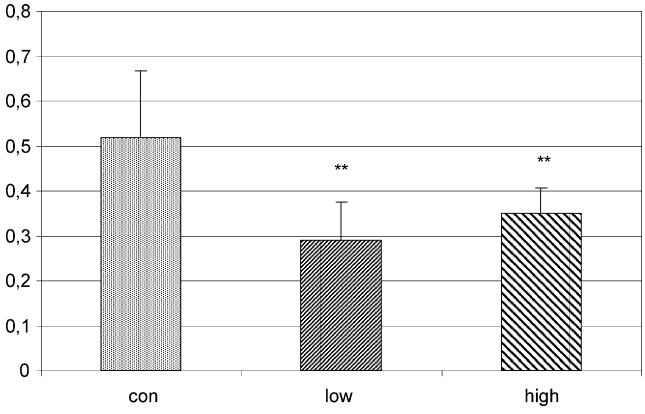


Fig. 6. Size of TH-immunoreactive boutons in the reticular part of the substantia nigra, expressed as the cross sectional area (mean ± S.D.), in brain samples taken at the end of the 5th treatment week. Abscissa: groups. Ordinate: bouton area, $\mu m^2$. Bar pattern as in Fig. 1. ** $p < 0.01$ treated vs. control group.

## 4. Discussion

The measured total concentrations of mercury in blood and brain (Table 2) indicate that the treatment was effective i.e. the introduced amount of MEM was mostly absorbed and followed its known distribution within the body (Rodier and Kates, 1988). Literature data confirm that organic mercury compounds are almost completely (90%) absorbed after ingestion because of their high lipid solubility (Angle and McIntire, 1974). After absorption, MEM easily penetrates the blood-brain barrier and accumulates in the brain. MEM in the CNS has a biological half-life measured in days or weeks (Aschner and Aschner, 1990).

In humans, following fatal acute or chronic MEM poisoning, the amount of mercury absorbed was found 28 and 17%, respectively (Friberg and Mottet, 1989). In male Wistar rats, treated on five consecutive days with 10 mg/kg MEM, the brain total Hg level on the 3rd and 10th days following the last administration was 14 and 18 μg/g (Yasutake et al., 1998).

In our study, the rats' total blood Hg levels were, after treating the rats with lower MEM doses (0.5 and 2.0 mg/kg) for 5 weeks, several hundred times higher than in the controls (Table 2), and a massive deposition of Hg was seen in the cortex and hippocampus. Measurements of total Hg on the 1st and 4th weeks of the post-treatment phase showed different rates of elimination from the blood and the brain. This lasting presence, and effect of MEM, may be due to deposition of Hg in the brain in (partly) inorganic form (Friberg and Mottet, 1989) the CNS half life of which is much longer than that of MEM (Aschner and Aschner, 1990).

A number of transmitter systems have been involved in the explanation of MEM neurotoxicity, such as the dopaminergic, serotonergic, GABAergic, cholinergic and glutamatergic ones, each known to interact with cortical and subcortical centers of motor control. In the alterations of the vertical and horizontal motility (dose-dependent significant decrease, Fig. 1), effects of MEM on the serotoninergic and dopaminergic transmission (Faro et al., 1997) can be expressed. In rats, the mesolimbic (running from the ventral tegmental area to cortical structures and responsible for motivation) and the nigrostriatal dopaminergic system, playing (directly or indirectly) an important role in extrapyramidal motor control and sensorimotor integration, were found to be affected by Hg (Rossi et al., 1997). The modifying effect of d-AM on the observed action of MEM also supports the involvement of DA. Passing the blood-brain barrier, d-AM (in the doses applied by us) increases the release of catecholamines, including DA, from the synaptic endings in the nigrostriatal pathway (Clarkson et al., 1988), and in the mesolimbic/mesocortical pathways (Alexander et al., 1990). Our 5-week MEM exposure possibly led to depletion of DA and loss of efficacy of dopaminergic transmission. In such a situation, the sensitivity of the postsynaptic DA receptors could increase which would explain why, in the animals treated with d-AM, spontaneous exploratory activity returned to or beyond control level. The effect of MEM on the DA transporter has a biphasic, increase-decrease

dose dependence (Schweri, 1994) which is a possible explanation of the missing or anomalous dose-dependence of certain effects (e.g. in the open field) in our experiments.

Shrinkage of dopaminergic boutons (indicating DA depletion; Faro et al., 1997) in the substantia nigra pars reticulata (SNR) in the rats after 5 weeks MEM treatment further supports this hypothesis. SNR, connecting the dorsal and ventral striatum with the thalamus, superior colliculus and pontomedullary brainstem, has a strategic role in locomotor activity (stereotypes), and in the sensorimotor regulation of behavior by a tonic nigroreticular GABAergic inhibitory projection to the dorsal and ventral striatal neurons (Koch et al., 2000). The number of TH-immunoreactive cells themselves was, on the contrary, not different in the control and MEM-treated animals, similarly to the findings of Rossi et al. (1997).

In the regulation of locomotor activity and ASR/PPI, the functional connections of the dorsal and ventral hippocampus (the Hg content of which was significantly elevated after 5 weeks MEM administration, Table 2) with the amygdala, nucleus accumbens and prefrontal cortex have also been implicated (Zhang et al., 2002). In the rat, ASR is primarily mediated by a glutamatergic excitatory pathway comprising the cochlear root nucleus and the caudal pontine reticular formation, projecting to facial, cranial and spinal motoneurons (Lee et al., 1996). The dorsal and ventral cochlear nuclei, the ventrolateral tegmental nucleus and the lateral superior olive form late components of the pathway (Koch, 1999). In the ventral tegmental area, DA-containing neurons are thought to play (by connection of D2 and $GABA_A$ receptors) an important role in motivation, emotion, and ASR amplitude (Gifkins et al., 2002). The prepulse inhibition (PPI) is under strong tonic influence of the dopaminergic transmission in the nucleus accumbens (Mansbach et al., 1988). Reversal of the PPI, as was seen in our work, was described in the literature (from lead-treated rats; Commissaris et al., 2000) and was explained by altered dopaminergic modulation in the nucleus accumbens of the GABAergic pathway to the ventral pallidum responsible for decrease in the ASR by PPI (Swerdlow et al., 1990).

Reduced OF activity and decreased number of "noise-positive" ASR responses, together with increased ASR onset latency and peak time in the rats after 5 weeks MEM administration, pointed to the involvement of the serotonergic system. Serotonin receptors (1A and 1B) indeed modulate startle reactivity in rats (Nanry and Tilson, 1989). The serotonin-1B receptor is predominantly located on neurons of various brain regions including motor control centers (basal ganglia) and structures involved in mood control (central grey and hippocampus; Maroteaux et al., 1992), and its inhibition (Boschert et al., 1994) or absence (Dirks et al., 2001) results indirectly in altered modulation of startle reactivity and threshold.

The effect of Hg on the cholinergic system may explain the diminished reactivity of the treated animals in the ASR test (Fibiger, 1991), and the decrement of memory found in a previous experiment (Vezér and Papp, 2002). ACh is one of the transmitters of the projection from the pedunculopontine tegmental nucleus to the caudal pontine reticular nucleus, a part of an inhibitory pathway which mediates the reduction in magnitude of the ASR by PPI. In the MEM-treated animals, higher number of "noise positive" responses under prepulse application (that is, reduced inhibitory modulation of the startle response) and shortened onset latency and peak time was seen (although with no clear dose-dependence). The $Hg^{2+}$ sensitivity of hippocampal muscarinic receptors is extraordinarily high (Coccini et al., 2000) which is in line with the decrease of locomotion and with the stronger alteration of hippocampal versus cortical spontaneous activity (suggested by the changes of the frequency spectra) seen in MEM-treated rats in the present study.

As to the cortical activity, the primary event was likely the shift of the spontaneous activity to higher frequencies, due (at least partly) to the effect of MEM on the ascending cholinergic modulation. Decreased extracellular DA level (see above) may also have contributed to this frequency alteration (Vorobyov et al., 2003). The general depression of cortical evoked responses (longer latency and shorter duration in the treated rats) may be a consequence of increased high-frequency spontaneous activity, a state when intracortical connections predominate over thalamocortical input (Rémond and Lesévre, 1967). Reduced glial glutamate uptake induced by Hg (Aschner et al., 2000) could, in the end, lead to desensitization of the postsynaptic Glu receptors on cortical neurons, contributing to the reduction of evoked cortical responses in a more specific way. Slowed axonal conduction and increased synaptic delay due to ion channel effects of MEM (Sirois and Atchison, 1996) may also have contributed to the altered evoked potentials. Increased spontaneous and decreased evoked cortical activity was observed in our earlier studies with inorganic mercury, too (Schulz et al., 1997). In humans, latency changes of brainstem auditory evoked potential were found to correlate with body burden of both metal and organic mercury (Counter et al., 1998).

## 5. Conclusion

The results of the present study demonstrated the relationship between subtoxic MEM exposure, mercury levels in blood and parts of the brain (cortex and hippocampus), and neurobehavioral alterations. Interestingly, during treatment the animals receiving the higher dose, while during elimination those having received the lower dose showed stronger effects (OF, ASR/PPI). The behavioral alterations can be due to the effect of MEM on neurotransmitter systems. The involvement of the dopaminergic system was indicated by the effect of d-AM, and by the morphological alteration of the boutons in the SNR. However, further systems (serotonergic, GABAergic, glutamatergic and cholinergic) may also be involved.

It was also found that cessation of exposure and decreasing brain levels of mercury were not necessarily paralleled by normalization of the affected functions, suggesting that neurotoxic effects of low dose MEM exposure may be present for a long time even after elimination of the toxicant, and may be detectable by direct recording (like ECoG or ASR/PPI) or by provoking an underlying process of the observable reaction (like in case of d-AM). Although the analogy is not direct, effects similar to those described above may be supposed in the background of human, neurobehavioral abnormalities, considering the continuous presence of low levels of mercurials in the human environment.

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