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A pilot study with simultaneous recording of changes in motility and cortical electrical activity of rats during four weeks of oral manganese exposure

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Manganese as an environmental neurotoxicant can cause oral exposure. Six rats were equipped with a connector ''crown'', allowing repeated recording of electrocorticogram (ECoG) with simultaneous recording of motor activity in an open field box. Weekly one 30-min recording session was held, and after two control sessions, four of the six rats had 2.5 mg/ml manganese chloride in their drinking water. The treated rats showed higher motility during the exposure period than the untreated ones; and substantially decreased total ECoG power without marked change the spectrum. The changes of both motility and ECoG were correlated to the individual brain Mn levels, and the activity decrease during a session was correlated to the total ECoG power. These effects can be likened to early adult manganism and to symptoms of children exposed to Mn via drinking water. Repeated simultaneous recording of open field motility and spontaneous cortical activity seems suitable to detect early electrophysiological and behavioral effects of an oral neurotoxic exposure.

Keywords: manganese; electrocorticogram; open field; chronic recording; rat

Introduction

Manganese is one of the known neurotoxic heavy metals which is able to cause human exposure by inhalation but also by ingestion. Nervous system effects of high-Mn drinking water have been described in several parts of the world (Australia: Kilburn 1987; Greece: Kondakis et al. 1989; Japan: Kawamura et al. 1941). In the affected Greek population, motor symptoms and hair Mn levels were strongly correlated in persons over 50 years of age.

Motor disorders are typical consequences of chronic Mn overexposure. The resulting human disease is often (first of all when of occupational origin) called manganism, and progresses in three stages (Saric et al. 1977; Calne et al. 1994). The first stage is marked by non-specific symptoms like apathy, anorexia, asthenia, headache, hypersomnia, spasms, arthralgia, weakness of the legs, and irritability. In the second stage, motor (dysarthria and difficulty in walking) and psychic disturbances dominate. The third stage represents a Parkinson-like syndrome with its associated symptoms. Such disorder was described also following non-inhalational

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exposure (as from high-Mn drinking water: Kondakis et al. 1989; or from overdosing a health supplement: Ohtake et al. 2005). Mn-related electrophysiological disorders include epileptic activity, observed in exposed children (Komaki et al. 1999; Hernandez et al. 2003); as well as disturbances of the electroencephalogram (EEG) and evoked potentials following occupational Mn exposure (Sjögren et al. 1996; Sinczuk-Walczak et al. 2001; Halatek et al. 2005).

Chronic Mn exposure and its effects on the nervous system have been modeled in animals repeatedly (general evaluation: Gwiazda et al. 2007). In earlier works of our Department, electrophysiological (spontaneous and evoked cortical activity) and behavioral (open field, acoustic startle response, etc.) effects of subacute oral Mn exposure were investigated by Vezér et al. (2005) – with the shortcoming, however, that cortical electrical activity was recorded in anesthesia and hence could not be directly put in parallel with the behavioral effects.

In this work, rats with implanted chronic cortical electrodes (Papp 2009; Takács and Papp 2010) were exposed to Mn via the drinking water and the resulting changes of spontaneous cortical electrical activity (electrocorticogram, ECoG) and motor behavior were recorded repeatedly, in awake state.

Methods

Preparation and Mn exposure of the rats

Male Wistar rats of 10–11 weeks of age, with ca. 350 g body weight were obtained from the breeding centre of University of Szeged, and were prepared for repeated ECoG recording by means of chronically implanted electrodes.

Four holes of ca. 0.6 mm diameter were drilled in the skull in isoflurane anesthesia (2–3vol% in O_2, open system) down to the epidural space over the right and left frontal and parietal lobe (Figure 1). Two holes received the fine steel screws which served as electrodes and fixed the "crown" used for electrical connection. In the other two holes, silver wire electrodes were placed. The screws and the silver wires were connected to the crown base and the base was secured to the skull with dental acrylic. The skin was sutured and the rats were allowed to recover for 10–14 days before the first recording. Before and after surgery, sufficient analgesic and antibiotic treatment was given (30 mg/kg b.w. amoxicillin plus 0.2 mg/kg. b.w. meloxicam, injected subcutaneously 2 h before the intervention and repeatedly after it as necessary). The animals had to be kept one-by-one in separate cages but needed no extra care. Altogether six such animals were prepared and used.

Weekly one recording session of 30 min duration was held with each animal. After the first two weeks as control period, four of the six rats had 2.5 mg/ml manganese chloride ($MnCl_2 \cdot 4H_2O$ analytical grade, Reanal, Hungary) in their drinking water while the remaining two had normal tap water and served as parallel controls. To prevent precipitation, 0.125 mg/ml citric acid was also added to the Mn solution. The addition of citric acid had apparently no aversive taste because the rats' measured consumption was not less than that of rats in other experiments drinking plain tap water (the natural Mn level of the local tap water was very low, 0.03 μg/ml). The intended length of the Mn exposure period was four weeks. One treated rat lost the crown after three weeks Mn exposure and was left out from further evaluation.

The procedures applied were approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged. During the whole

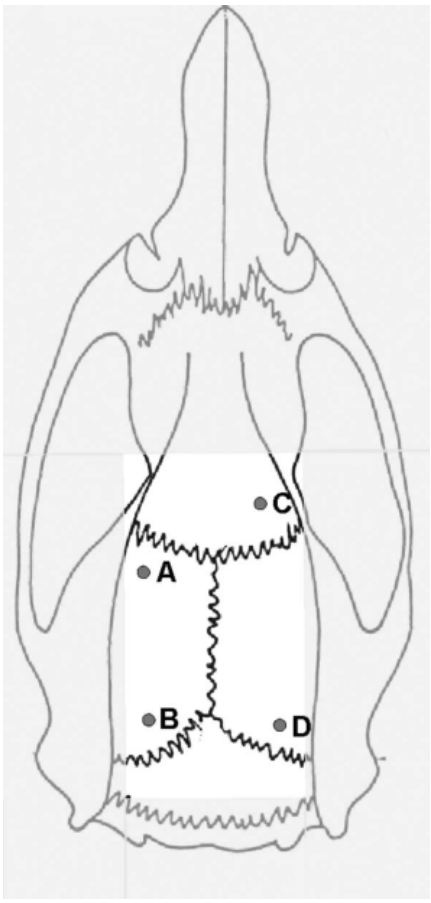

Figure 1. Position of the holes drilled into the rat's skull. In the recording, channel 1 was bipolar with points A and B, and channel 2, with C and D.

procedure, the principles (based on the EU-conform Hungarian law) of the Committee were strictly followed.

Recording

A combined system (provided by Experimetria Ltd, Hungary) was used for parallel recording of motility and cortical activity. The rat was in an open field (OF) box, detecting and analyzing its movements; while cortical electrical activity was recorded via a cable attached to the crown. The OF box was of 48 cm × 48 cm × 40 cm size, equipped with an array of infrared light gates at floor level. The rat's movements were detected by the light beam interruptions.

The cortical electrical activity was taken up by a preamplifier mounted on the end of the flexible lead-off cable, attached to the socket of the crown. The two electrodes above the left and right hemisphere, respectively, gave one bipolar lead-off each. Signals from the left hemisphere (channel 1) were always used for analysis unless they were strongly distorted for technical reasons. The amplified signals were fed via swivel contact in the main amplifier. Overall amplification was $10^4\times$ with low- and high-pass filters set to 1.6 and 75 Hz. The ECoG signals were visualized on the PC monitor in real time and stored on the HDD.

Manganese level determination

For Mn level determination, the whole brain of all five rats who completed the exposure period was removed and dried at 80°C to constant weight, and was then digested in 5 ml 65% HNO_3 at 90°C for 90 min. Mn determination was done by inductively coupled plasma mass spectrometry.

Analysis

From the beam interruptions in the OF box, counts, time and run length of the basic activity forms (ambulation, local activity, and immobility) were computed by the software of the OF box (Conducta 1.3, Experimetria, Hungary) as follows: More than 40 mm shift in the location of interrupted beams at the floor level during a time unit of 1 s was interpreted as horizontal activity; less shift, as local activity; and no shift at all, as immobility. These were calculated on 10 min periods or the total 30 min. Rearing was deliberately omitted because of the false signals produced by the crown and cable.

Off-line analysis of the ECoG records (using a purpose-developed software by Experimetria) provided the power spectrum between adjustable limits with 0.5 Hz resolution. The complete spectrum between 1 Hz and 50 Hz was generated, and the total power (the sum of the power in the 0.5 Hz wide bins) was calculated. The analysis results provided by the record from the left hemisphere were used mostly (see above). To see the trend of change, the power spectra were calculated for the same periods (10 min and 30 min) which were used for OF activity analysis.

Due to the small data pool, a simple statistical evaluation was done. Corresponding data of the treated and the control rats were taken as one data set each and compared by means of two-sample *t*-test. Significance was accepted at $p < 0.05$. Linear regression between different data was calculated, and its significance tested, by the ''linear fit'' function of MS Excel.

Results

During the six weeks of recording, the weight gain of the Mn-treated rats was 98.8 ± 36.2 g, and of the controls, 90.0 ± 35.4 g; indicating the lack of gross general toxicity. Their actual daily water consumption was 56.0 ± 11.6 ml in the exposed and 52.4 ± 8.3 ml in the control, practically not different from each other and also not much different from that seen in previous experiments with oral Mn exposure (Máté et al. 2009).

The tissue Mn levels at the end of the four-week exposure period, resulting from the consumed water, are given in Table 1.

During the weeks of Mn treatment, significant difference developed between the treated and control rats' motility. The general trend of less and less time spent in ambulation, and more and more in immobility, was similar. However, as shown in Figure 2, the change of both relative ambulation time and immobility time – calculated from 30-min data on the basis of the mean of the two pre-treatment weeks – was dissimilar, that is, the Mn-treated animals' motility decreased less strongly than that of the controls. The difference (between all data of the control vs. the treated rats in the four treatment weeks) was significant ($p < 0.05$). This difference could also be demonstrated as correlation between brain Mn levels and

the relative immobility time in the sixth week (again, calculated on the basis of the pre-treatment period), showing that in the treated rats, who had higher brain Mn levels, the time of immobility increased much less than in the controls (Figure 3).

The time trend of the total ECoG power was strongly dissimilar: it did not change much in the controls but decreased after starting the oral Mn exposure in the treated rats (Figure 4). The difference, calculated as described for the data in Figure 2, was significant ($p < 0.01$). The ECoG power spectra underwent, at the same time, minimal alteration in the treated rats, that is, the power decrease was approximately even in the studied frequency range. Between ECoG total power of the sixth week and the brain Mn levels, a similar correlation was found as with the rate of immobility (Figure 3).

Table 1. Tissue Mn levels in the control and treated rats' tissue samples.

	Mn level (μg/kg)	
	Treated	Control
Blood	281.35 $\pm$ 63.62*	181.46 $\pm$ 60.17
Brain	1931.88 $\pm$ 156.93***	984.71 $\pm$ 132.56
Liver	8022.97 $\pm$ 609.25*	7132.24 $\pm$ 471.74

Note: *, ***$p < 0.05$, 0.001 vs. control. Mean $\pm$ SD, $n = 3$ (treated) or 2 (control).

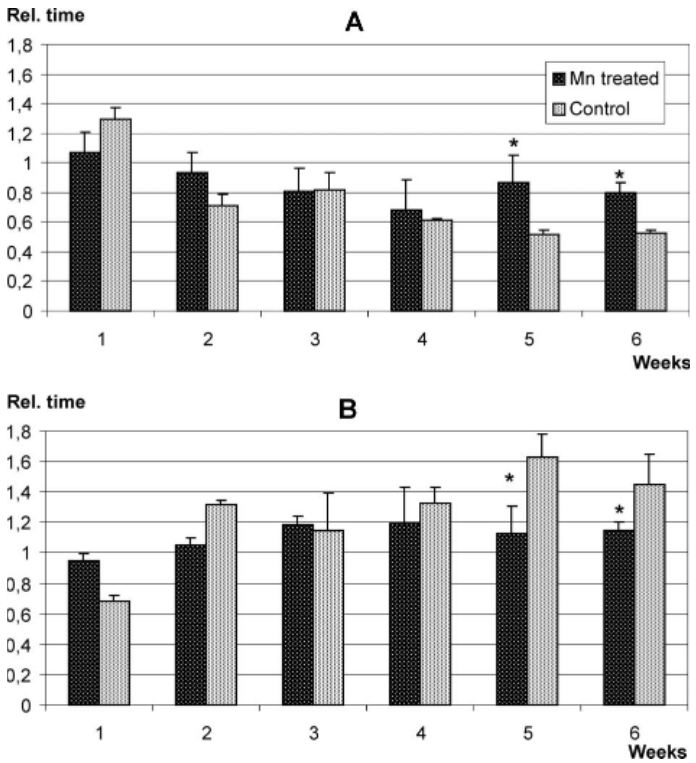

Figure 2. Time course of the time spent in ambulation (A) and immobility (B) by the control and treated rats. Note: The bars (mean + SD, $n = 3$ and 2) represent the relative change of activity compared to the average of the two pre-treatment weeks as basis. *$p < 0.05$ treated vs. control.

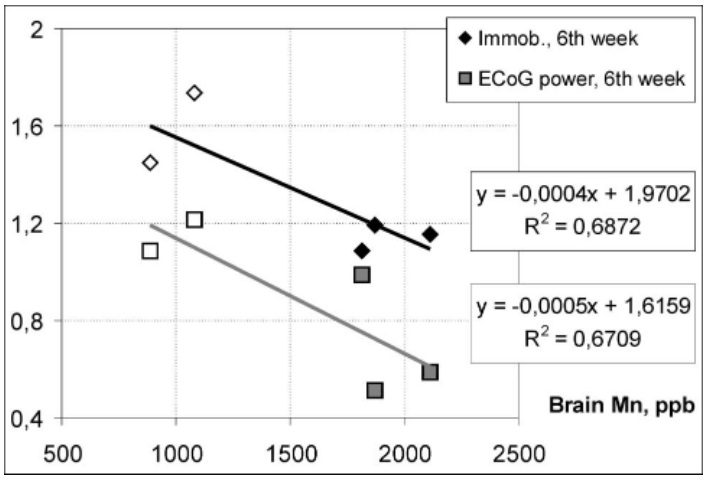

Figure 3. Correlation diagram of the rats' immobility time and ECoG total power (see insert) in the sixth week of experiment (related to the control period, see text) against the Mn levels measured in the brain samples. Note: Solid symbol points, Mn-treated rats; light points, control rats.

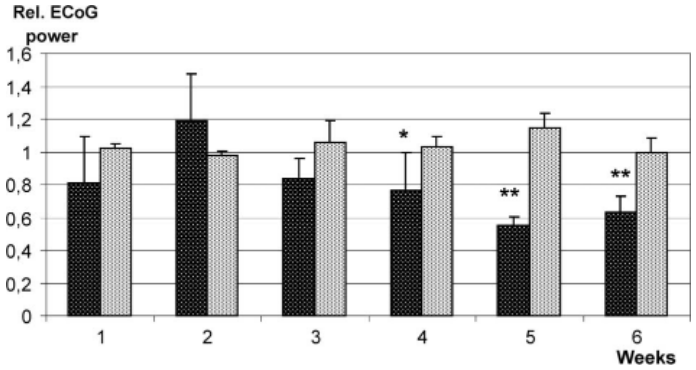

Figure 4. Time course of the ECoG total power of the control and treated rats. Note: The bars (mean + SD, $n = 3$ and 2) represent the relative change of activity compared to the average of the two pre-treatment weeks as basis. *, **$p < 0.05$, 0.01 treated vs. control.

Simultaneous recording allowed us to analyze the relationship between ECoG and motor behavior. Figures 2 and 4 suggested that ECoG total power and the degree of motility were dissimilarly correlated in treated and control rats but the correlation between these parameters turned out as rather weak ($R^2 \leq 0.2$). Another indicator, the shift from ambulation towards immobility during the 30-min OF session, was more sensitive. The ratio of the times spent in immobility during the first and the last 10-min period of the OF session was obtained for the two pre-treatment weeks and the four weeks of oral Mn exposure. When these values were plotted against the ECoG total power (the relative values presented in Figure 4) a considerable correlation was obtained for the treated rats but not for the controls (Figure 5).

Discussion

The treated rats' increased (that is, less strongly decreased) motility can be likened to the early phase of adult human manganism (characterized by behavioral

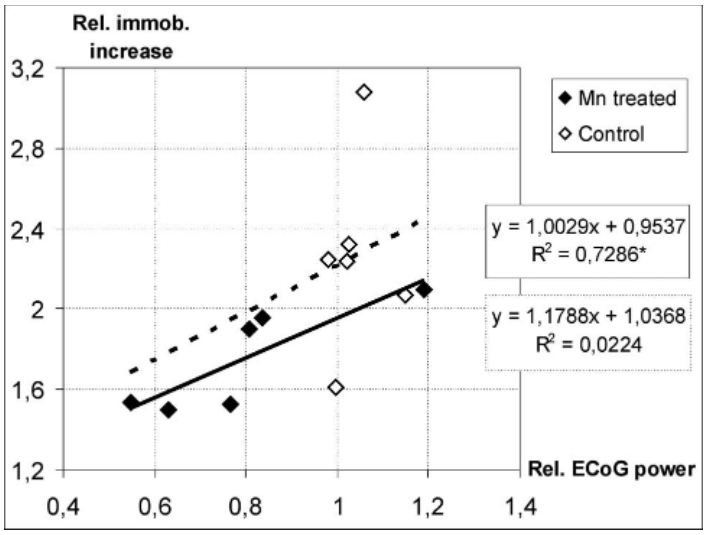

Figure 5. Correlation diagram of the change of immobility during the 30-min open field session (ordinate) against the ECoG total power of the corresponding session (abscissa). Note: Solid trend line and data in solid frame belong to the solid symbol points (Mn-treated rats); and dashed line and data frame to the light points (control rats). *Significant linear fit ($p < 0.05$, F test).

disinhibition) and to symptoms of children exposed to high Mn level via drinking water. In the latter, attention deficit and hyperactivity (Bouchard et al. 2007) or low performance in intelligence tests (Wassermann et al. 2006) was described, and the disturbance of higher order functions was correlated to the bodily Mn load.

Most probably, the damage results from the effects of Mn on the metabolism of transmitters in the CNS, such as glutamate, dopamine or GABA (Fitsanakis et al. 2006). Locomotor activity in rats depends on mesolimbic and mesocortical dopaminergic neuronal transmission (Fink and Smith 1980). It is generally assumed that Mn in the brain diminishes the activity of dopaminergic regulation and, hence, motor activity. There are, however, reports on decreased GABA synthesis in Mn-exposed animals (Chandra et al. 1982), and the disinhibition caused by GABA shortage (observed also during in vivo microdialysis of the rat striatum with high-Mn solution: Takeda et al. 2003) could more than balance out the motility decrease potentially resulting from Mn effects on the dopaminergic systems. Dorman et al. (2000) gave 25 and 50 mg/kg $MnCl_2$ per day by gavage for 21 days to adult male DC rats, and observed no change in motility among rat groups where the brain Mn level difference (treated vs. control) was similar to ours. Cuesta de Di Zio et al. (1995) described, although in mice, a phenomenon in which, in the early phase (two weeks) of parenteral Mn application, the presynaptic inhibitory autoregulation of dopamine release in the striatum was abolished, and the released amount increased, apparently due to an NMDA-receptor mediated glutamatergic effect. In rats treated with an oral Mn dose similar to ours, Calabresi et al. (2001) detected increased efficacy of the presynaptic dopaminergic control of the glutamatergic corticostriatal excitation, and interpreted the effects as a model of the early phase of human manganism (also discussed earlier), which fits well with the preserved motility of the Mn-treated rats (vs. control) in this study.

Our results showed that repeated simultaneous recording of open field motility and spontaneous cortical activity is suitable to detect early electrophysiological and

behavioral effects of oral Mn exposure in rats and, more importantly, to reveal the correlation of these changes to each other and to the Mn load. First of all the decrease of motility within a session seems to be a sensitive indicator which can potentially be developed to a biomarker for experimental studies.

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