NEUROTOXICITY OF MANGANESE ANALYSED BY A NOVEL COMBINED ELECTROPHYSIOLOGICAL-BEHAVIORAL RECORDING SYSTEM

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ABSTRACT

A novel electrophysiological-behavioral recording system, developed by Experimetria Ltd., Hungary in cooperation with our Department, was used to study the effects of manganese, a neurotoxicant frequently causing human nervous system disease in occupational exposure. Male Wistar rats were equipped with a "crown" enabling the recording of electrocorticogram (ECoG) in awake, freely moving state. One 60-min recording session per week was held for 10 weeks, in which the rats' movements in an open field (OF) box and their ECoG were simultaneously recorded. After the 2nd week, treated rats had 7.5 mg/l $MnCl_2$ in the drinking water (control: normal tapwater) and further 8 recordings were made. From the OF records, ambulation distance and the time spent with ambulation, local activity and immobility was obtained. From the ECoG, power spectrum and total power was calculated. In the first weeks of Mn exposure, the rats' motility substantially decreased, and these changes showed little further progression. The decay of motility during one 60-min session was also stronger in exposed rats. The total power of ECoG increased in the first 4 weeks but the spectrum was hardly changed. The total power and spectrum of ECoG and the level of motility were apparently correlated, and the functional alterations showed some dependence on treatment time and/or summed dose. Combined, repeated ECoG and motility recording is suitable to follow-up the development of neurotoxicity induced by Mn, and possibly other environmental neurotoxicants.

INTRODUCTION

The increasing number of xenobiotics with potential risk to human heath urges a need for more sensitive methods in the diagnosis of the alterations caused. To preserve health these effects must be detected as early as possible, because long-term exposure can lead to severe symptoms even in absence of obvious signs. This is especially true to the nervous system where any substance, able to cross the blood-brain barrier, can have a major effect on humans. Various biomarkers are used to detect alterations induced by xenobiotics; these are measurements that indicate the exposure to a chemical, the effect of such exposure, or susceptibility to a (usually toxic) effect of such exposure (Hayes, 2001). For neuro-functional alterations, the generally used chemical biomarkers are not ideal, so the development of markers based on electrophysiological recording may be a promising field of investigation. In the present work, Wistar rats were implanted chronically with epidural electrodes to record spontaneous cortical electrical activity (electrocorticogram, ECoG) in awake animals, simultaneously with the recording of motor behaviour in an open field box. It was tested how the measured data and their correlations could be used as an indicator of toxic damage and its progression.

The test substance chosen was manganese. Numerous data testify its effect at the molecular and neuronal level, and the late consequences on human exposure, but less is known about the development of the functional alterations. Mn itself is a trace element essential in a number of biological functions e.g. as cofactor of enzymes (ATSDR, 2008). Excess Mn alters the activity of the mitochondrial superoxide dismutase and can inhibit the removal of glutamate by glia-specific glutamine synthetase (Normandin and Hazell, 2002), which leads to excitotoxicity and oxidative stress in the brain. This, together with mitochondrial dysfunction (Zhang et al., 2003) affects first of all dopaminergic structures. In the long run this results in manganism, an occupational disease resembling Parkinson's disease. In the present work, Mn was applied via the rats' drinking water, modelling cases of human nervous system damage due to high-Mn drinking water (e.g. in Greece: Kondakis et al., 1989). In the affected Greek population, motor symptoms and hair Mn levels were strongly correlated in persons over 50 years of age.

Electrophysiological (spontaneous and evoked cortical activity) and behavioural (open field, acoustic startle response etc.) effects of subacute oral Mn exposure have already been investigated at our Department on rats, e.g. by Vezér et al. (2005) – with the shortcoming, however, that cortical electrical activity was recorded in anaesthesia and hence could not be directly put in parallel with the behavioural effects. With the method described here, the two can be recorded simultaneously in wake state.

METHODS

Male Wistar rats of ca. 350 g body weight were prepared for repeated ECoG recording by means of chronically implanted electrodes: Four small holes were drilled in the skull in isoflurane anaesthesia (2-3vol% in O_2, open system) down to the epidural space over the right and left frontal and parietal lobe. Two holes received fine steel screws which served as electrodes and fixed the "crown" used for electrical connection, while in the other two holes, silver wire electrodes were placed. The screws and the silver wires were electrically 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 11-14 days before the first recording. Before and after surgery, sufficient analgesic and antibiotic treatment was given. Altogether 5 such animals were prepared and used (see Takács and Papp, 2010 for more details).

Weekly one recording session of 60 min duration was held with each animal. After the first two weeks as control period, four of the five rats had 7.5 mg/ml manganese chloride ($MnCl_2 \cdot 4H_2O$ analytical grade, Reanal, Hungary) in their drinking water while the fifth had normal tap water and served as parallel control (the natural Mn level of the local tap water was 0.03 μg/ml). The intended length of the Mn exposure period was eight weeks. The procedures applied were approved by the Ethical Committee for the Protection of Animals in Research of the University.

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 analysing its movements by means of a grid of infrared light gates. From the light beam interruptions, counts, time and run length of the basic activity forms (ambulation, local activity, immobility) were computed by the software of the OF box (Conducta 1.3, Experimetria, Hungary).

Cortical electrical activity was recorded via a cable connected to the crown. The two electrodes above the left and right hemisphere 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 pre-amplified signals were fed via swivel contact in the

main amplifier. Overall amplification was 10^4x 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. Off-line analysis (using a purpose-developed software by Experimetria) provided the power spectrum between adjustable limits with 0.5 Hz resolution. The complete spectrum between 5.5 and 49 Hz was generated, and the total power (the sum of the power in the 0.5 Hz wide bins) was calculated. Mn level in the blood and brain of the treated rats was determined by inductively coupled plasma mass spectrometry after acidic digestion.
Due to the small data pool, no statistical evaluation was done, and it is planned to repeat the experiment with more animals. Linear regression between different data was calculated, and its significance tested, by the "linear fit" function of MS Excel.

RESULTS

Oral Mn exposure resulted in substantial accumulation of Mn in the brain, but not blood, of the treated rats (brain: 5113±1025 vs. 1931±476 ppm; blood: 502±115 vs. 514±217 ppm; untreated controls from another experiment). The effect on the ECoG appeared in two distinct phases. The total power (Fig. 1A) was massively increased in ca. the first 4 weeks of exposure, compared to the control animal, but later this change turned to the opposite. The changes in the spectrum curve were small (first increase in most part of the range, then decrease above 30 Hz) and were in concordance with the data of total power.

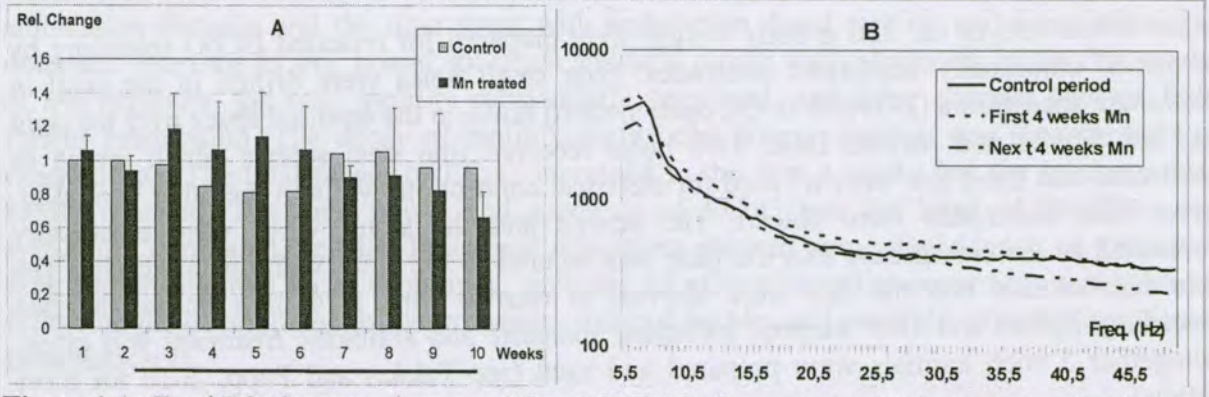
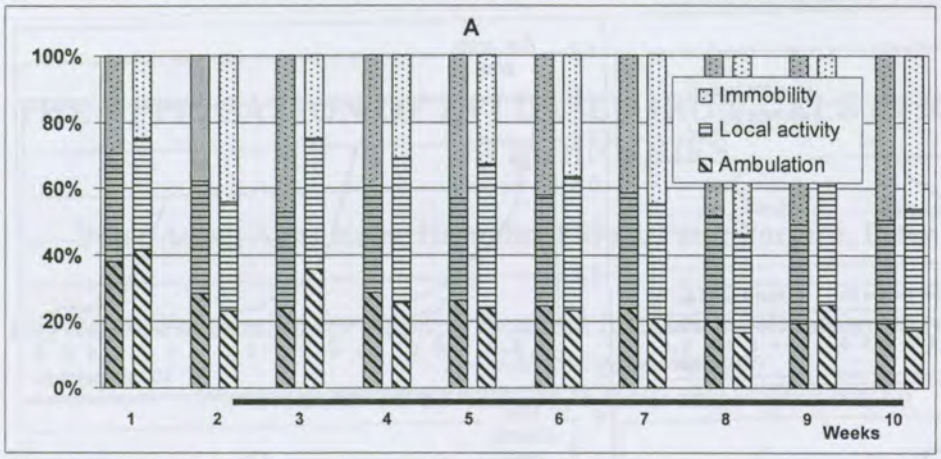


Figure 1 A: Total ECoG power (between 5.5 and 49 Hz) in the control and Mn-treated period (n= 4 treated and 1 control); relative data on the basis of the two control weeks. The dark line below the graph signalizes treatment period. **B:** ECoG power spectrum of the treated rats, averaged from data of the 4 treated animals.

In the OF activity, oral exposure by Mn caused decreased motility in the treated vs. control rats (Fig. 2A). This effect was also more pronounced in the first 4 weeks of exposure. The best indicator was the time spent in immobility, the course of which in the pre-treatment and treatment period is shown in Fig. 2B.
Comparison of the graphs in Fig. 1A and 2B suggested that ECoG activity and motility change in parallel and are possibly in causal relationship. To test that in more detail, the 60-min records were analyzed in 10-min sections and the outcome was separately averaged for the 2 pre-treatment weeks and the 4+4 treatment weeks.



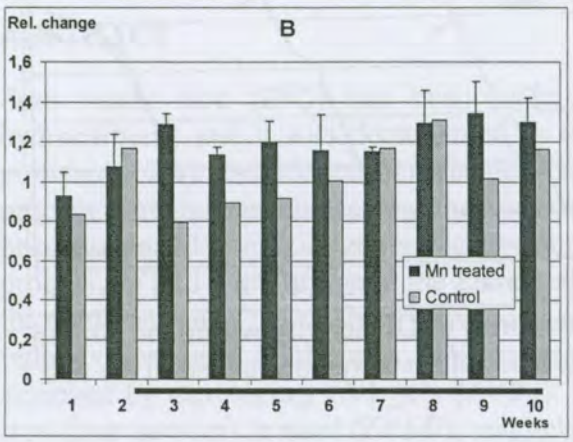


Figure 2
A: Time shares of the three forms of OF activity in the treated (grey bars) and untreated (white bars) rats in the control and treatment period.
B: Time course of the times spent by immobility in the OF. The same display as in Fig. 1B.

This kind of analysis also showed that increasing ECoG power was concomittant with decreased motility (Fig. 3). The best correlation existed (also suggested by the lines in the graphs) between the ECoG power and the time spent in immobility. For the first 4 treatment weeks, $R^2=0.386$ for the treated and $R^2<<0.1$ for the control; for all 8 treatment weeks the relationship was less clear, in accordance with the time course of the electrical and motor activity shown in Fig. 1 and 2.

CONCLUSION

The treated rats' decreased motility can be likened to adult human manganism, and most probably results from the effects of Mn on the metabolism of transmitters in the CNS, such as glutamate, dopamine or GABA. 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. High cortical electrical activity together with total or subtotal immobility is typical for some forms of epilepsy (petit mal). Mn intoxication is known to induce epileptic activity mainly in children (Hernandez et al. 2003) but also in young adults (Ono et al., 2002). The method described is apparently suitable for modelling and following-up neuro-functional alterations of environmental origin, but could be used in any case of purposeful or accidental external chemical influence on the central nervous system.

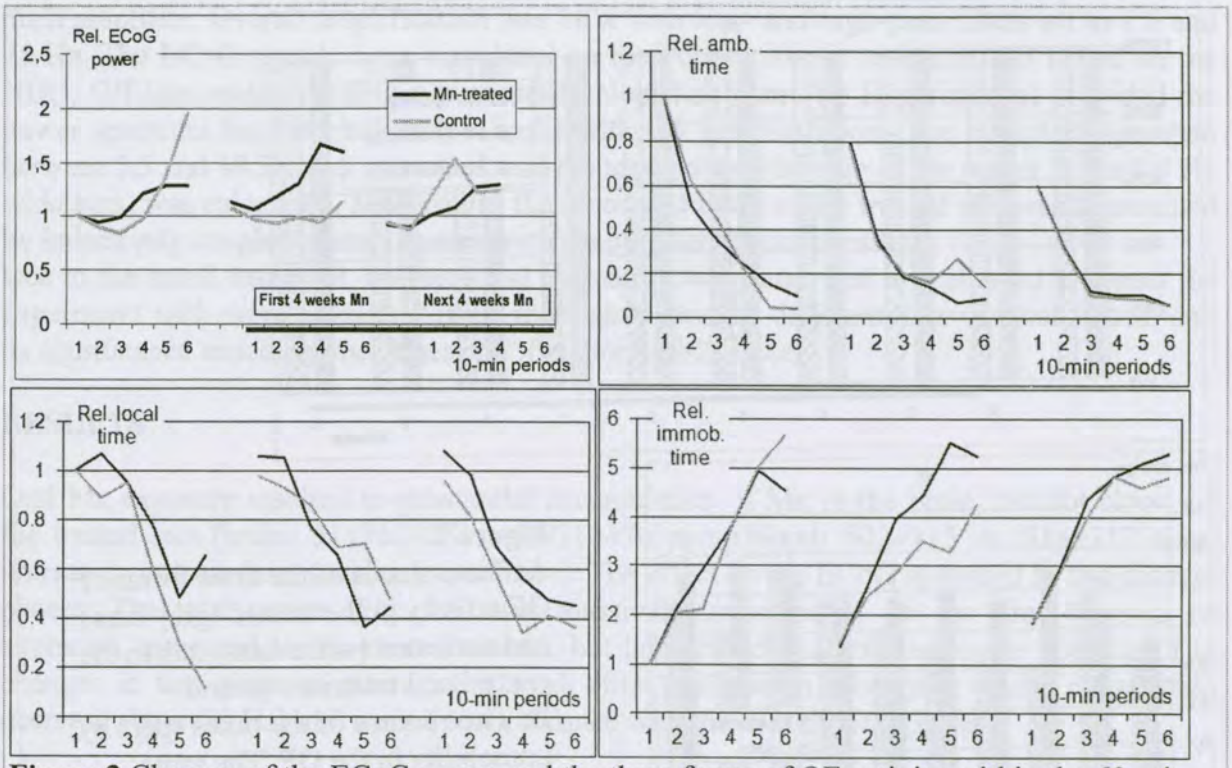


Figure 3 Changes of the ECoG power and the three forms of OF activity within the 60-min recording sessions before and during Mn exposure. The values were normalized to the first 10-min period of the pre-treatment weeks.

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