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REPEATED SIMULTANEOUS CORTICAL ELECTROPHYSIOLOGICAL AND BEHAVIORAL RECORDING IN RATS EXPOSED TO MANGANESE-CONTAINING NANOPARTICLES

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Male Wistar rats wearing chronically implanted cortical electrodes were exposed to Mn-containing nanoparticles via the airways for 8 weeks following a 2-week pre-exposure period. The rats' cortical electrical activity and open field motility was recorded simultaneously, in weekly repetitions. It was supposed that this technique can provide better insight in the development of Mn-induced CNS damage. Decreased motility (less distance covered, longer periods of immobility) and increased total power of cortical electrical activity developed in parallel in the first 4–5 weeks of treatment but showed little change afterwards. Both the behavioral and the electrophysiological effect were in fair correlation with the rats' internal Mn exposure determined from brain samples. The results confirmed the non-linear dose- and time-dependence of Mn effects suggested by previous studies. Repeated simultaneous behavioral and electrophysiological recording during a longer treatment with neurotoxic metals (or other xenobiotics) seems to be a promising method.

Keywords: Manganese – rat – cortical electrical activity – open field motility – chronic recording

INTRODUCTION

Inhalation of Mn-containing airborne particles is a frequent occupational exposure, affecting first of all welders [3], but cases from other industrial branches (zinc-carbon battery manufacturing: [2]; Mn ore processing: [26]) also have been described.

Manganese is on one hand an essential micronutrient, but one of the known neurotoxic heavy metals on the other hand. The very first report on the CNS effects in Mn ore miners, called then manganese madness, was published by Couper in 1837 [6]. Today's terminology is manganism, which is a CNS disorder similar to Parkinson's disease, and develops in three stages [5, 18]. 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 typical Parkinson-like syndrome with its associated symptoms appears then in the third stage (however, the site of damage in manganism is in the striatum while in parkinsonism, in substantia nigra [7]).

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Mn-induced CNS disturbances are diagnosed and characterized mostly at the level of motor behavior and mental functions, and/or in pathological findings. There exists, however, a limited number of reports on electrophysiological alterations following chronic human Mn exposure. In young shipyard workers, EEG and visual evoked potential alterations were observed and blood Mn levels up to 14 ppb were measured [9] whereas in reference groups the blood Mn level is around 5–7 ppb [2]. EEG and evoked potential disturbances following occupational Mn exposure were reported several times (e.g. [19, 20]). A recent report on Mn-exposed welders [10] described increased abnormal theta and delta wave activity vs. controls and interpreted this as an indication of depression or fatigue.

In welding fumes and other industrial emissions responsible for the above-mentioned exposure, nanoparticles (NPs, components of the aerosol with at least one dimension smaller than 100 nm [15]) are necessarily present [1] even if they are not detected and analyzed separately. Due to their small size, high number concentration, and large specific surface area, NPs have greater biological activity per given mass than larger particles. On inhalation, they are deposited in the nasopharynx or in the alveoli [11] and reach target organs (including the brain) by different transfer routes and mechanisms, including transcytosis (by caveola formation) across epithelia of the respiratory tract into the interstitium [15] and axonal transport along the olfactory fibers directly into the CNS [4].

Chronic Mn exposure has been successfully modelled in animals (effects on motor behavior: [14, 23], general evaluation: [8]). In earlier works of our department [12, 25] electrophysiological (spontaneous and evoked cortical activity) and behavioral (open field, acoustic startle response etc.) effects were investigated by oral administration of the water-soluble $MnCl_2$ to rats for several weeks. In those works, however, cortical electrical activity was recorded in anaesthesia and hence could not be directly put in parallel with the behavioral effects. In the present work, electrophysiological and behavioral recording was performed simultaneously in awake rats wearing chronically implanted electrodes [21]. Also, a potentially more realistic way of exposure – Mn-containing NPs applied to the airways – was used. (Functional neurotoxicity of metal-containing NPs has been investigated, e.g. by Oszlánczi et al. [16].) It was supposed that the novel, combined recording technique detailed below can provide better insight in the development of Mn-induced CNS damage and in the relationships of its facets.

MATERIAL AND METHODS

Preparation and Mn exposure of the rats

Male Wistar rats of 11–12 weeks of age, with 350–400 g body weight, 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 anaesthesia (2–3 vol% in O_2, open system) down to the epidural space over the right and left frontal

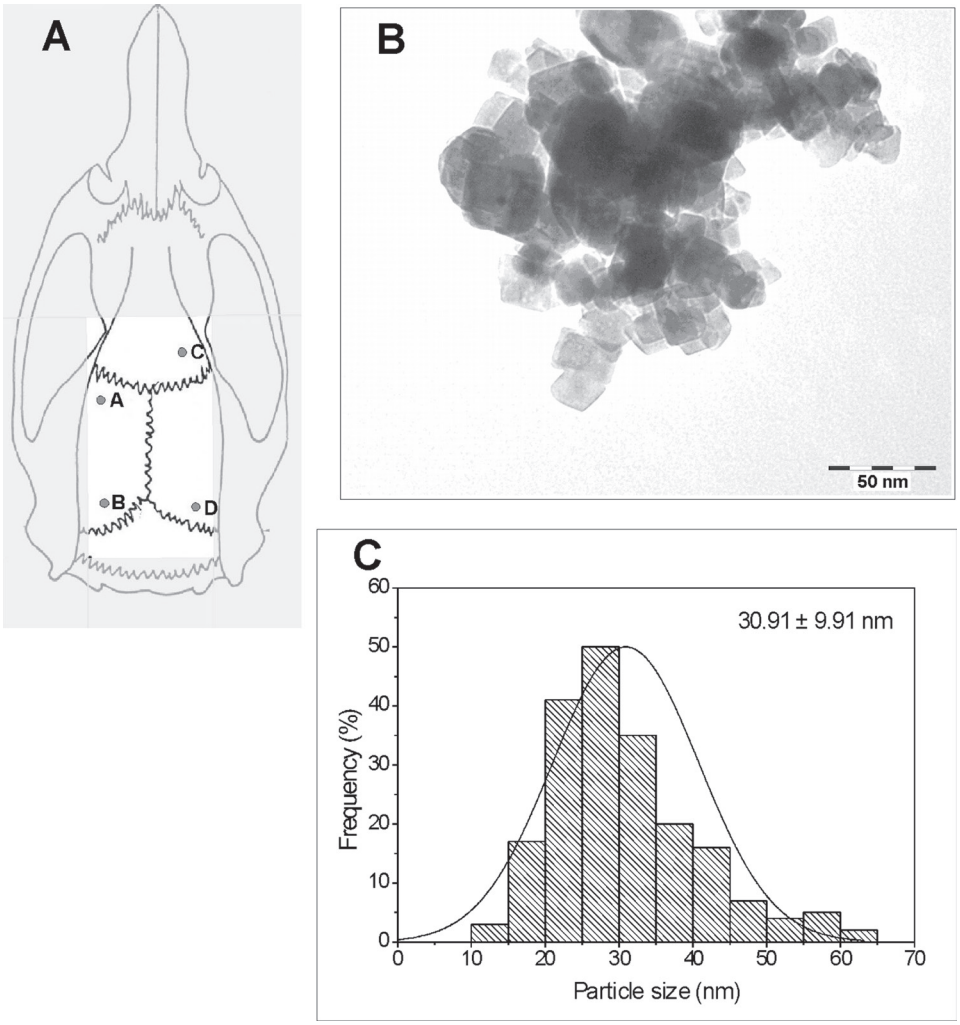

Fig. 1. A – Position of the holes drilled in the rat's skull; B – Electron microscopic image of the MnO_2 nanoparticles; C – Size histogram of the nanoparticles

and parietal lobe (Fig. 1A). Silver wire electrodes were placed in two holes (positions A and D in the figure) while the other two holes (B, C) received the fine steel screws which provided both electrical lead-off and fixation of the "crown" used for electrical connection onto the skull. 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 hours 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.

The experiment for each rat started with a 2-week pre-exposure control period. Then, 2 of the 11 crowned rats were further kept unexposed as parallel controls, for 6 rats Mn exposure was started, and the remaining 3 animals received the vehicle (see Table 1). This vehicle consisted of 1% hydroxyethyl cellulose (HEC) dissolved in phosphate-buffered saline (PBS; pH 7.4); it was physiologically neutral and slowed the aggregation of the NPs. The NPs suspended in the vehicle consisted of MnO_2, were approximately spherical with 30.9±9.9 nm diameter, and were synthesized at the Department of Applied and Environmental Chemistry, University of Szeged. The suspension was intensively sonicated as it was prepared, and was sonicated again before administration. It was directly instilled into the rat's trachea in brief anesthesia induced by isoflurane (5 vol%) in a glass jar with air-tight lid. For details of the treatment, and the manufacturing and properties of the NPs, see [16]. The NPs' electron microscopic image and size histogram is given in Fig. 1B and C.

The procedures applied were approved by the Ethical Committee for the Protection of Animals in Research of the University of Szeged. During the whole procedure, the principles (based on the EU-conform Hungarian law) of the Committee were strictly followed.

Recording

Weekly one recording session of 60 min duration was held with each animal, using a combined system (provided by Experimetria Ltd., Hungary) capable of recording motility and cortical electrical activity in parallel. The rat was in an open field (OF) box, detecting and analyzing its movements; while the electrocorticogram (ECoG) was recorded via a cable attached to the crown. The OF box was of 48×48×40 cm size, equipped with an array of infrared light gates at floor level, and the rat's movements were detected by the light beam interruptions.

Table 1
Groups of animals and the corresponding treatments used in the experiment

Group	Code	Treatment, weeks 3–10	Starting number of animals	Final number of animals	
				ECoG	OF
Untreated control	*UnC*	None	2	1	2
Vehicle treated	*VT*	Intratracheal instillation: vehicle (HEC) 1 ml/kg b.w.	3	2	3
Manganese treated	*MnT*	Intratracheal instillation: MnO_2 NPs 2.63 mg/kg b.w./1 ml/kg b.w.	6	4	6

The cortical electrical activity was monitored by a pre-amplifier 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 channel 2 – with one electrode in the parietoocciptial, and another in the frontal region (Fig. 1A) thus detecting the activity of nearly the complete right hemisphere – were used for analysis. 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.

From the 11 rats, 4 were discarded from the ECoG analysis due to wear-induced malfunction of the crown, causing distorted recordings, leaving 4 *MnT*, 2 *VT* and 1 *UnC* rat for final ECoG evaluation (Table 1). However, the OF records of each rat were usable and were evaluated.

Analysis

From the beam interruptions in the OF box, event counts and time of the basic activity forms (ambulation, local activity, immobility) and run length 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 during a time unit of 1 s was interpreted as horizontal activity (ambulation); less shift, as local activity; and no shift at all, as immobility. The calculation was done for the whole 60 min period. Vertical movements (rearing) were 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 and 49 Hz was generated, and the total power (the sum of the power in the 0.5 Hz wide bins) was calculated. The power spectra were calculated for the same periods which were used for OF activity analysis.

Due to the small data pool, a simple statistical evaluation was done. Corresponding data of one rat group in the pre-exposure and the treatment period, or data of the different groups (*UnC*, *VC*, *Mn*) in the treatment period, were taken as one data set each and were compared by means of one-way ANOVA. Significance was accepted at $p < 0.05$. Linear regression between different data (including tissue Mn level, see below) was calculated by the "linear fit" function of MS Excel.

Manganese level determination

After the last recording session at the end of the 8 weeks treatment period, the animals were sacrificed by an overdose of urethane. Organs (brain, lungs, heart, liver, kidneys and adrenals) were removed and weighed to obtain data for general toxicity of the

treatment. For Mn level determination, whole brains and lungs, a ca. 1 g slice from the liver, and 2–5 ml blood samples were taken and stored at –20 °C. For measurement, the samples were dried at 80 °C to constant weight, and were digested in 5 ml 65% HNO_3 at 90 °C for 90 minutes. Mn determination was finally done by inductively coupled plasma mass spectrometry (at the laboratory of the MOL Hungarian Oil and Gas Company).

RESULTS

ECoG power

The total ECoG power in the 1–49 Hz range was increased in the Mn-treated rats. The change started after ca. 2 weeks Mn exposure and went on in the following 4–5 weeks. Then there was some decrease but the ECoG power remained above the level of control up to the last recording from each rat (that is, up to week 10, Fig. 2). The

Table 2
Significance of the differences in the variables investigated

Variable	Difference: weeks 1–2 vs. weeks 3–10 within a treatment group	Significance	Difference: weeks 3–10, between treatment groups	Significance
ECoG total power	*UnC*	n.s.	*VT* vs. *UnC*	$p<0.05$
	VT	n.s.	*MnT* vs. *UnC*	$p<0.001$
	MnT	$p<0.01$	*MnT* vs. *VT*	$p<0.01$
Ambulation distance	*UnC*	n.s.	*VT* vs. *UnC*	n.s.
	VT	$p<0.001$	*MnT* vs. *UnC*	$p<0.001$
	MnT	$p<0.001$	*MnT* vs. *VT*	$p<0.01$
Ambulation time	*UnC*	n.s.	*VT* vs. *UnC*	n.s.
	VT	$p<0.001$	*MnT* vs. *UnC*	$p<0.001$
	MnT	$p<0.001$	*MnT* vs. *VT*	$p<0.001$
Ambulation count	*UnC*	n.s.	*VT* vs. *UnC*	n.s.
	VT	n.s.	*MnT* vs. *UnC*	$p<0.001$
	MnT	$p<0.001$	*MnT* vs. *VT*	$p<0.01$
Immobility time	*UnC*	n.s.	*VT* vs. *UnC*	$p<0.01$
	VT	$p<0.05$	*MnT* vs. *UnC*	$p<0.001$
	MnT	$p<0.001$	*MnT* vs. *VT*	$p<0.05$
Immobility count	*UnC*	n.s.	*VT* vs. *UnC*	$p<0.05$
	VT	n.s.	*MnT* vs. *UnC*	$p<0.001$
	MnT	n.s.	*MnT* vs. *VT*	$p<0.05$

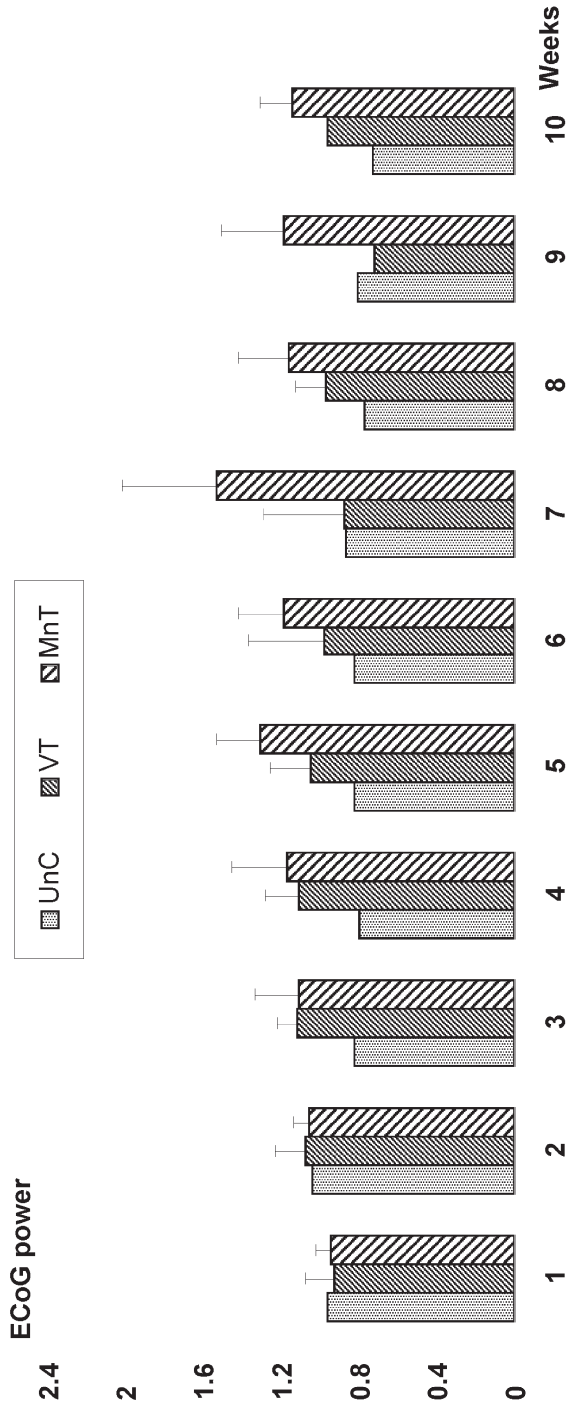

Fig. 2. Time course of the ECoG total power in control and Mn-treated rats over the 10 weeks of the experiment. Mean+SD. For group codes, see Table 1, for significance, see Table 3

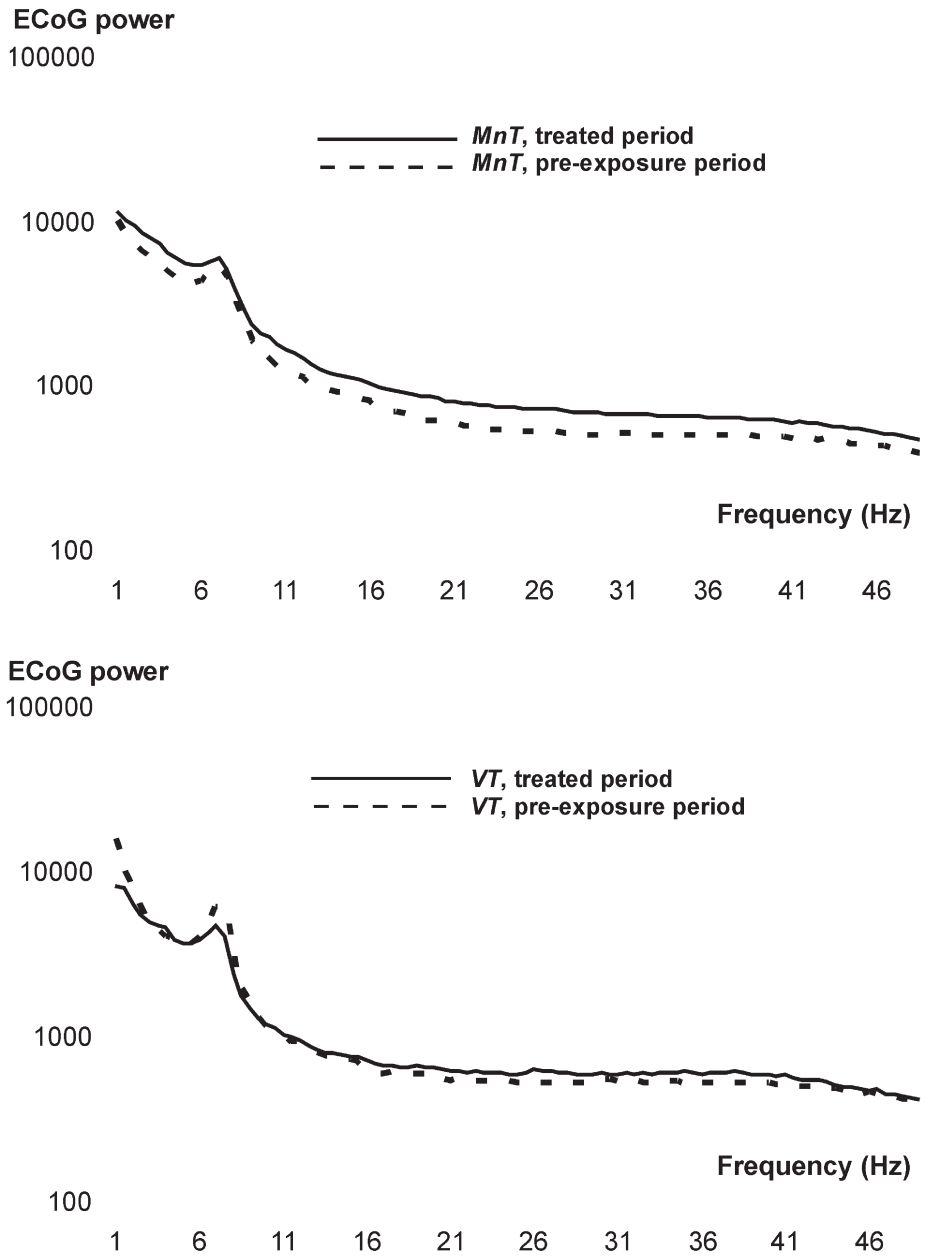

Fig. 3. Electrocorticogram spectra of the Mn-treated (*MnT*, top) and vehicle-treated (*VT*, bottom) rats in the control (group mean for weeks 1 and 2) and treatment (group mean for weeks 3–10) periods

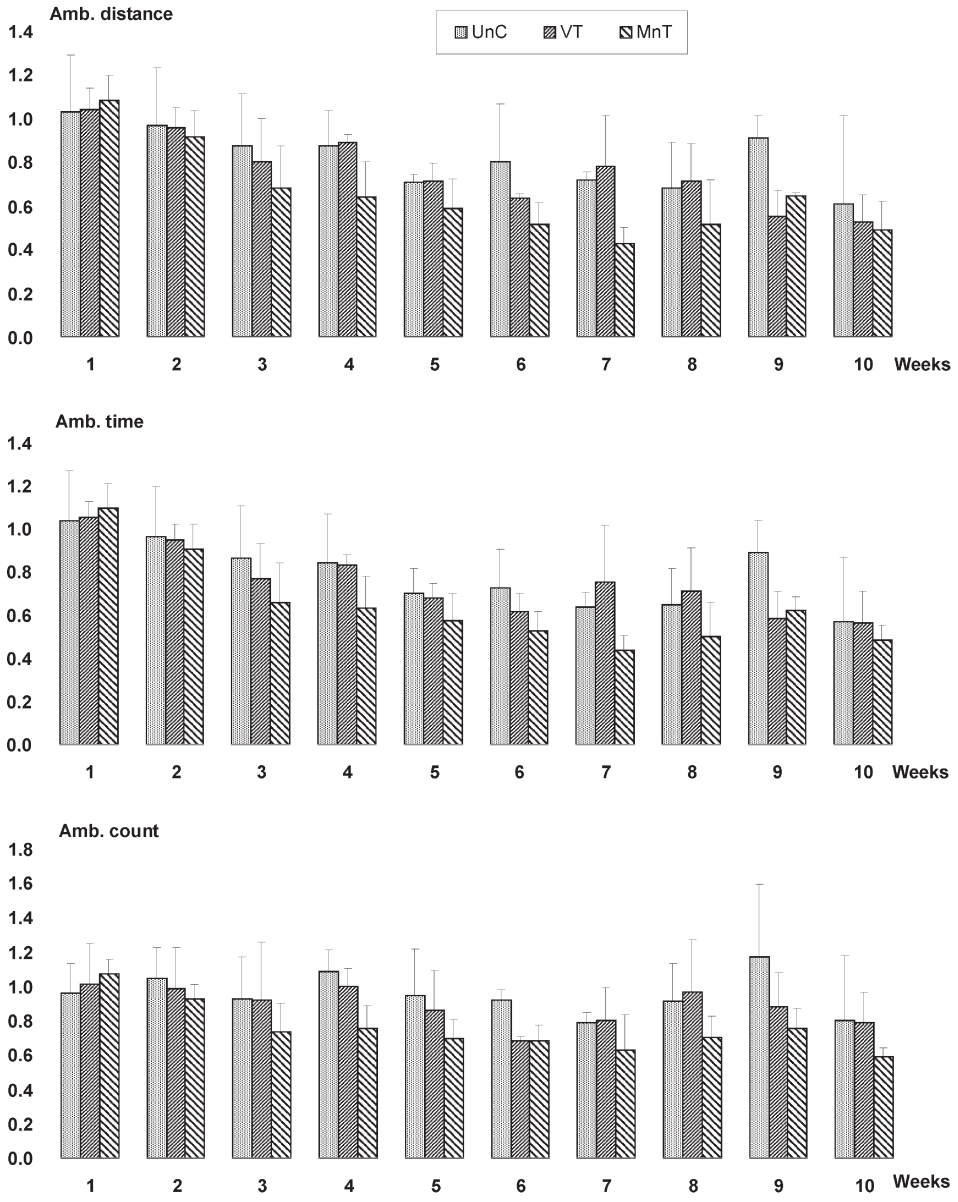

Fig. 4. Time course of ambulatory activity in the open field in control and Mn-treated rats over the 10 weeks of the experiment. Mean+SD. For group codes, see Table 1, for significance, see Table 3

significance data in Table 2 show that the difference of the treatment vs. pre-treatment period was significant in the *MnT* but not in the *UnC* and *VT* groups and, accordingly, the *MnT* vs. *VT* or *MnT* vs. *UnC* difference was highly significant all along the treatment period.

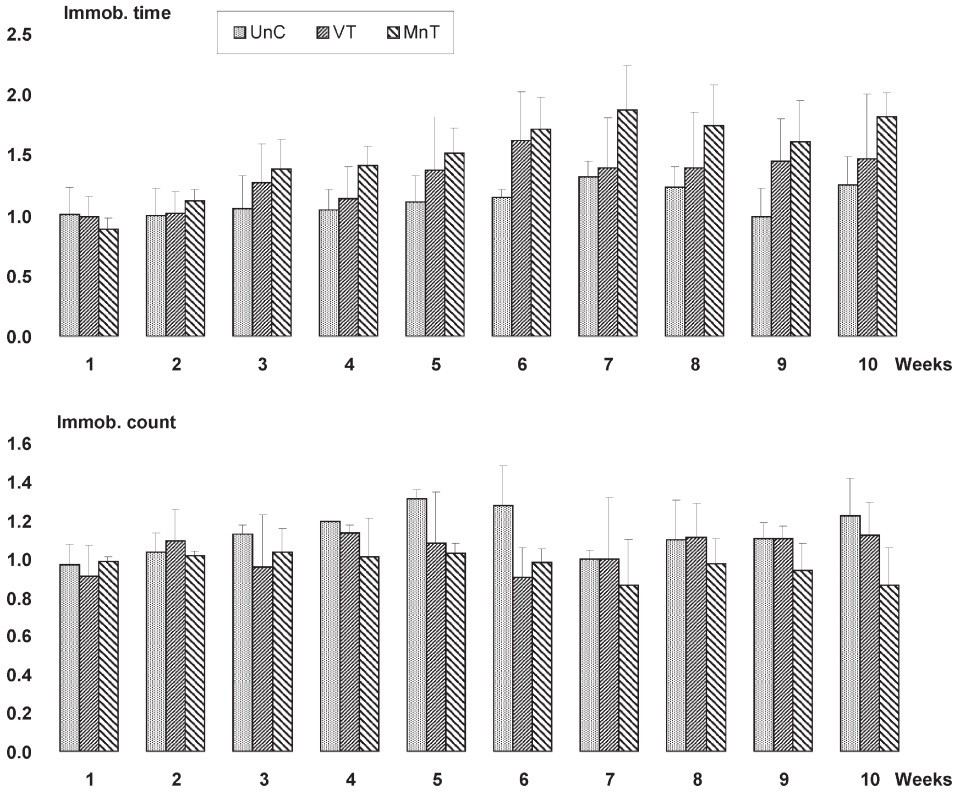

Fig. 5. Time course of immobility in the open field in control and Mn-treated rats over the 10 weeks of the experiment. Mean + SD. For group codes, see Table 1, for significance, see Table 3

Table 3
Manganese levels measured in the tissue samples of Mn- and vehicle-treated rats

Groups	Mn level (μg/kg dry weight), mean±SD			
	Brain	Blood	Lungs	Liver
MnT (n=6)	10776±1368**	1023±418*	4845768±1048254***	12816±1444
VT (n=2)	1918±231	209±131	2481±1407	6829±2236

*, **, ***: $p<0.05$, 0.01, 0.001 *MNT* vs. *VT*.

ECoG spectrum

The power increase in the treated rats did not result from a change in the power spectrum, but from a general increase mainly between the 7 Hz peak and ca. 30 Hz. In the *VT* rats, there was no such change (Fig. 3).

Open field motility

MnT rats showed hypomotility which appeared earlier than the manifest change in ECoG total power. As seen in Fig. 4, ambulation distance and time was lower than in either of the controls – untreated and vehicle-treated – already in week 3 (that, is, after only 5 administrations) and the difference evolved later similarly to the time course of the ECoG power. Distance, time and count of ambulation changed in parallel which meant that horizontal motor activity decreased as a whole, without significant change in its pattern (the calculated speed of locomotion – distance/time – and the length of single events – time/count – was not altered). The clear increase in immobility (the very opposite of ambulation) of *MnT* rats resulted, on the contrary, mostly from increased time length but nearly unchanged number of events of immobility (Fig. 5).

Mn levels

The measured Mn levels in the *MnT* rats' tissue samples (Table 3) indicate massive metal deposition first of all in the brain, but also in other organs. The extreme levels in the lung samples were due to local deposition of the NPs after being instilled.

Correlations

Repeated recording during the treatment period enabled us to test to what extent the changes of OF motility and cortical electrical activity evolved in parallel. The points in Fig. 6A and B represent data pairs of ECoG and OF group mean values, each point belonging to one week of the experiment. The trend lines and R^2 values indicate a fair correlation between motility and cortical electrical activity of the *MnT*, but not of the *VT*, rats; in full accordance with the bar graphs of Figs 2, 4 and 5.

Brain Mn level was determined post mortem, which did not allow the investigation of its temporal relationship to functional parameters. So, the causal role of the internal Mn dose in the observed electrophysiological and behavioral changes was tested by relating the individual rats' Mn levels to the relative change of ambulation, immobility, and ECoG total power (Fig. 6C–E). The strength of correlation supported the role of Mn and indicated that such a comparison might be useful in detecting metal-induced neurotoxicity.

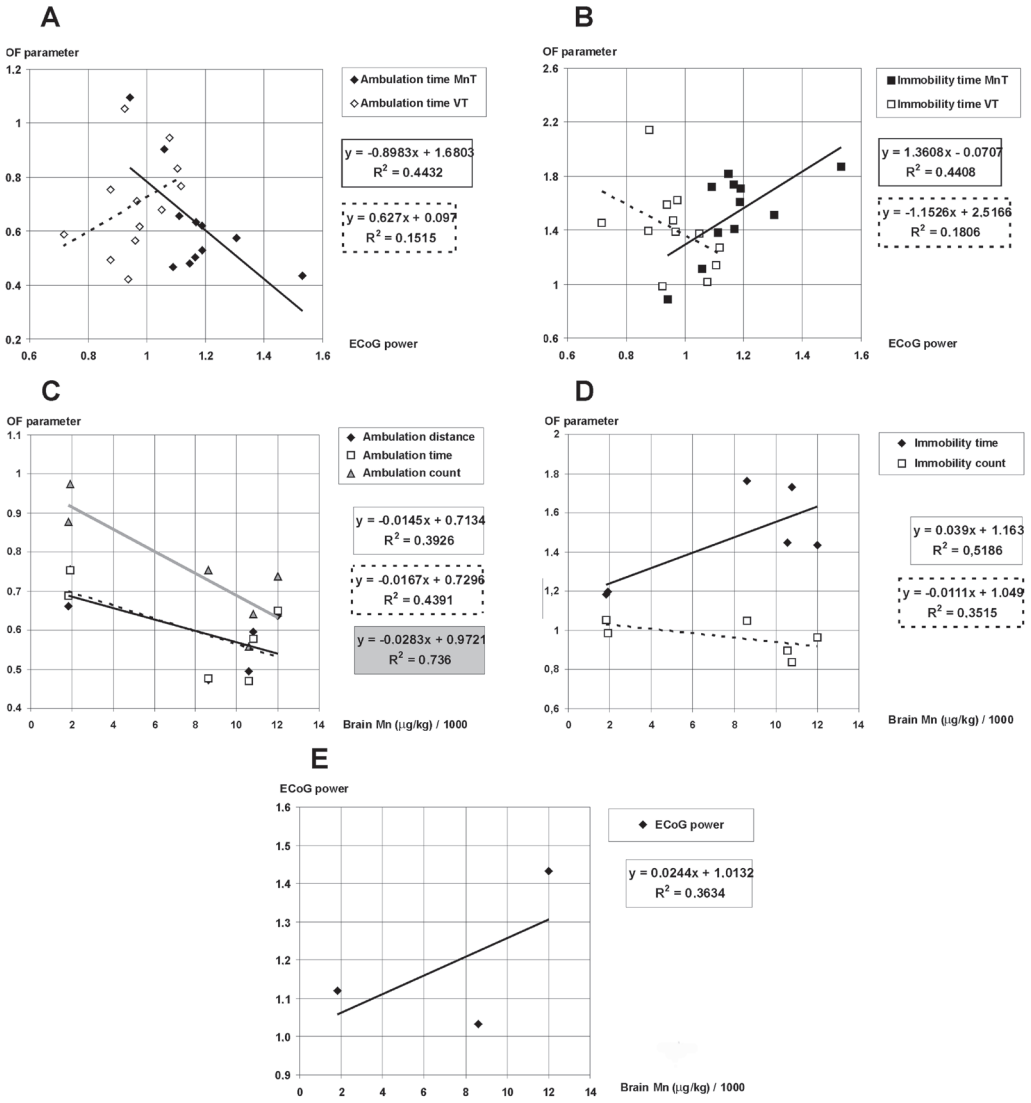

Fig. 6. Correlation diagrams. A, B – Relationship of the time spent in ambulation and in immobility to the ECoG power during the whole experiment. Each of the 10 points per group represents data pairs of group mean values for *MnT* (filled symbols) and *VT* (light symbols) on a given week; C, D, E – Relationship of the open field parameters (C, D) and the ECoG total power (E) to Mn content (abscissa) of the individual rat's brains (in C–E, Mn content was divided by 1000 in order to obtain slope coefficients between 0 and 1). The number of points in the graphs was determined by the number of evaluable animals, see Table 1. Trend lines fitted and R^2 calculated by Mn Excel. Corresponding trend lines and equation boxes have the same line style

DISCUSSION

Intratracheal application of the MnO_2 NPs resulted in massive internal exposure, evidenced by the measured Mn levels in the treated rats' organs. The recording method applied revealed that the neuro-functional changes developed over the 8 weeks of NP application in a non-monotonous way; the roughly linear change in the first 4 or 5 weeks of application stalled later and was followed by some drop – all the same, the effect did not vanish as shown by Figs 2, 4 and 5. Assuming the causal role of the inner Mn dose, this time course was possibly due to a similar time course of Mn accumulation. In an earlier work by Vezér et al. [24] the Mn levels in cerebral cortex, liver and blood samples of rats after 10 weeks oral $MnCl_2$ application were much less than twice of the levels measured after 5 weeks application. A saturation-like disproportionality was seen in the brain Mn concentration also when MnO_2 NPs were applied to rats for 6 and 9 weeks with the dose used in this work and a twice higher one [16], that is, the brain Mn level in the higher dose rats was only slightly higher than in the lower dose group, and the levels after 9 weeks were even slightly lower than after 6 weeks.

To see the relationship between brain Mn level and neuro-functional parameters, the relative change of ambulation, immobility, and ECoG total power was calculated for each rat, by taking the average value of the given parameter for the 8 treatment weeks and dividing it by the mean of the 2 pre-exposure weeks. This self-controlled calculation for each rat separately was enabled by the repeated recording from the animals, and the level of correlation displayed in Fig. 6C–E indicated the role of inner Mn load in determining the changes of OF motility and ECoG power. Similar relationship between Mn load and neuropsychological effects of exposure were also reported in humans [3].

One possible cause of the supposed time profile of Mn deposition could be that elimination of Mn from the brain mass, normally a slow diffusion process [22], might have been increased in case of high Mn loads [13], such an effect was suggested also in our previous work referred to above [24]. A secondary effect of the local tissue damage in the lungs, fibrosis and scar formation, to impede the transport of either the MnO_2 NPs or Mn ions dissolved from their surface and so to decrease the daily internal dose, cannot be ruled out either.

The general effect of Mn treatment on motor behavior was hypomotility, in line with own previous experiences [24, 25] and numerous literature data (summarized e.g. in [17]). Bradykinesia is a leading feature of human manganism [5, 18]. The 8 weeks treatment used in the present study may correspond, based on the average life span of rats and humans, to 5–6 years of human exposure which is more than enough to develop manganism in case of massive inhalational exposure (e.g. in bridge welders [3]).

Electrophysiological alterations in human subjects suffering from Mn-induced CNS damage have been scarcely mentioned in the literature. In shipyard workers exposed to inhalable Mn, EEG alterations were correlated to internal (blood Mn) and external (cumulative exposure index) load [9]. In another study, exposed welders

showed decreased beta and increased theta activity compared to controls [10]. In our *MnT* rats, increase of ECoG power was seen mostly in the range corresponding to beta- and gamma-waves, which could be likened to the band spectrum changes observed in earlier experiments in anesthetized rats [16, 24] after several weeks exposure to nanoparticulate or dissolved Mn.

The results of the present experiment, obtained with a novel methodology, confirmed some observations of previous studies, since the alterations in OF motility and ECoG at the end of the Mn treatment period were similar to those seen in experiments in which the functional tests were done only at the end. This time, by means of the repeated simultaneous behavioral and electrophysiological recording during the treatment period, the time course of the Mn effects could be described, and the relationship of its facets (electrical activity and motility) to each other and the brain Mn level could be characterized.

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REFERENCES

1. Antonini, J. M., Lewis, A. B., Roberts, J. R., Whaley, D. A. (2003) Pulmonary effects of welding fumes: review of worker and experimental animal studies. *Am. J. Ind. Med. 43,* 350–360.
2. Bader, M., Dietz, M. C., Ihring, A., Triebig, G. (1999) Biomonitoring of manganese in blood, urine and axillary hair following low-dose exposure during the manufacture of dry cell batteries. *Int. Arch. Occup. Environ. Health 72,* 521–527.
3. Bowler, R., Koller, W., Schultz, P. E. (2006) Parkinsonism due to manganism in a welder: Neurological and neuropsychological sequelae. *Neurotoxicology 27,* 327–332.
4. Calderon-Garciduenas, L., Azzarelli, B., Acuna, H. Garcia, R., Gambling, T. M., Osnaya, N., Monroy, S., Tizapantzi M. D. R., Carson, J. L., Villarreal-Calderon, A., Rewcastle, B. (2002) Air pollution and brain damage. *Toxicol. Pathol. 3,* 373–389.
5. Calne, D. B., Chu, N. S., Huang, C. C., Lu, C. S., Olanow, W. (1994) Manganism and idiopathic Parkinsonism: Similarities and differences. *Neurology 44,* 1583–1586.
6. Couper, J. (1837) On the effects of black oxide of manganese when inhaled into the lungs. *Br. Ann. Med. Pharm. Vit. Stat. Gen. Sci. 1,* 41–42.
7. Erikson, K. M., Aschner, M. (2003) Manganese neurotoxicity and glutamate-GABA interaction. *Neurochem. Int. 43,* 475–480.
8. Gwiazda, R., Lucchini, R., Smith, D. (2007) Adequacy and consistency of animal studies to evaluate the neurotoxicology of chronic low-level manganese exposure in humans. *J. Toxicol. Environ. Health 70,* 594–605.
9. Halatek, T., Sinczuk-Walczak, H., Szymcsak, M., Rydzynski, K. (2005) Neurological and respiratory symptoms in shipyard welders exposed to manganase. *Int. J. Occup. Med. Environ. Health 18,* 265–274.
10. He, S. C., Niu, Q. (2004) Subclinical neurophysiological effects of manganese in welding workers. *Int. J. Immunopathol. Pharmacol. 17,* 11–16.

11. ICRP (1994) Human respiratory tract model for radiological protection. A report of a task group of the ICRP. *Annals of the International Commission on Radiation Protection, ICRP Publication 66*, Pergamon Press, Oxford
12. Máté, Zs., Szabó, A., Oszlánczi, G., Papp, A. (2011) Modelling per os Mn exposure and examining its functional nervous system effects in rats. *Egészségtudomány 55,* 71–81 (in Hungarian).
13. Mena, I., Marin, O., Fuenzalida, S., Cotzias, G. C. (1967) Chronic manganese poisoning: clinical picture and manganese turnover. *Neurology 17,* 128–136.
14. Normandin, L., Beaupré, L. A., Salehi, F., St-Pierre, A., Kennedy, G., Mergler, D., Butterworth, R. F., Philippe, S., Zayed, J. (2004) Manganese distribution in the brain and neurobehavioral changes following inhalation exposure of rats to three chemical forms of manganese. *Neurotoxicology 25,* 411–433.
15. Oberdörster, G., Oberdörster, E., Oberdörster, J. (2005) Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environ. Health Persp. 7,* 823–839.
16. Oszlánczi, G., Vezér, T., Sárközi, L., Horváth, E., Kónya, Z., Papp, A. (2010) Functional neurotoxicity of Mn-containing nanoparticles in rats. *Ecotoxicol. Environ. Saf. 73,* 2004–2009.
17. Santamaria, A. B., Cushing, C. A., Antonini, J. M., Finley, B. L., Mowat, F. S. (2007) State-of-the-science review: Does manganese exposure during welding pose a neurological risk? *J. Toxicol. Environ. Health, Part B 10,* 417–465.
18. Saric, M., Markicevic, A., Hrustic, O. (1977) Occupational exposure to manganese. *Br. J. Ind. Med. 34,* 114–118.
19. Sinczuk-Walczak, H., Jakubowski, M., Matczak, W. (2001) Neurological and neurophysiological examinations of workers occupationally exposed to manganese. *Int. J. Occup. Med. Environ. Health 14,* 329–337.
20. Sjögren, B., Iregren, A., Frech, W., Hagman, M., Johansson, L., Tesarz, M., Wennberg, A. (1996) Effects of the nervous system among welders exposed to aluminium and manganese. *Occup. Environ. Med. 53,* 32–40.
21. Takács, Sz., Papp, A. (2010) Effects of antiepileptics and an anesthetic on basal cortical activity and spontaneous motility in an epilepsy-prone rat strain. *Acta Physiol. Hung. 97,* 480–481.
22. Takeda, A., Sawashita, J., Okada, S. (1995) Biological half-lives of zinc and manganese in rat brain. *Brain Res. 695,* 53–58.
23. Tapin, D., Kennedy, G., Lambert, J., Zayed, J. (2006) Bioaccumulation and locomotor effects of manganese sulphate in Sprague-Dawley rats following subchronic (90 days) inhalation exposure. *Toxicol. Appl. Pharmacol. 211,* 166–174.
24. Vezér, T., Kurunczi, Á., Náray, M, Papp, A., Nagymajtényi, L. (2007) Behavioral effects of subchronic inorganic manganese exposure in rats. *Amer. J. Ind. Med. 50,* 841–852.
25. Vezér, T., Papp, A., Hoyk, Z., Varga, C., Náray, M., Nagymajtényi, L. (2005) Behavioral and neurotoxicological effects of subchronic manganese exposure in rats. *Env. Toxicol. Pharmacol. 19,* 797–810.
26. Young, T., Myers, J. E., Thompson, M. L. (2005) The nervous system effects of occupational exposure to manganese – measured as respirable dust – in a South African manganese smelter. *Neurotoxicology 26,* 993–1000.