EXPERIMENTAL NEUROPHYSIOLOGICAL ALTERATIONS CAUSED BY COMBINED NANO-MANGANESE EXPOSURE

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ABSTRACT

Manganese (Mn) is a well known heavy metal, causing central nervous system damage after chronic exposure. To model inhalational Mn exposure, we used an animal model system in which male Wistar rats were intratracheally instilled daily with $MnCl_2$ (2.5 or 5.0 mg/kg bw.) dissolved in distilled water; or with nanosuspension of MnO_2 (2.5 or 5.0 mg/kg bw.); or with the combination of 2.5 mg/kg solute $MnCl_2$ and 2.5 mg/kg nanosized MnO_2, administered with a short delay. After the 5 weeks treatment period open field activity of the rats was tested and electrophysiological investigation was performed; spontaneous and stimulus evoked action potentials were recorded from somatosensory, visual and auditory areas of the cortex. Mn could reach the CNS and caused significant alterations in the spectral distribution of the ECoG bands, increased the latency of the evoked potentials and influenced the locomotor activity of the rats. The observed effects showed a kind of additive effect in case of the combined exposure.
Keywords: manganese, nanoparticle, intratracheal instillation, evoked potentials, rat

INTRODUCTION

Manganese (Mn) is an essential micronutrient, cofactor of enzymes (Mn-SOD, GS), but has toxic effects after chronic overexposure. It is often used in industry: in dry cells, coated welding rods, steel alloys, chemical fertilizers or pesticides. Technical applications of manganese (Mn) often results in human exposure, mostly due to inhalation of metal dust and fumes (ATSDR, 2008) causing mitochondrial dysfunction and leading to oxidative stress and excitotoxicity in the neurons (Taylor et al., 2006). Inhibition of voltage-gated Ca-channels and disturbed release of neurotransmitters are also typical of Mn intoxication. Mn evolves its deteriorating effects manly on the central nervous system. Manganism, a chronic human neurological disorder resembling Parkinson's disease (Bowler et al., 2006) often occurs among welders and miners exposed to Mn-containing aerosols (Dobson et al., 2004). These dusts and fumes, arising from soldering and welding contain Mn nanoparticles as well.

Inhalational Mn exposure may result in massive internal doses, depending on the size of the inhaled particles. Microscopic particles cannot pass the blood-brain barrier, but submicroscopic particles (that is, nanoparticles, with dimension 100 nm and below) have high mobility within the organism, so these can have direct access to the CNS (Oberdörster et al., 2000, 2005). According to this, it is worth to pay more attention on the effects of inhaled Mn on the nervous system and its dependence on the different physicochemical forms. In this study we aimed to explore the alterations in the CNS caused by solute and nanosized and their combinations.

MATERIALS AND METHODS

Adult male Wistar rats obtained from the Breeding Centre of the University (300-320 g body weight at start) were housed in an air conditioned room maintained at 22 ºC, with 12-hour light/dark cycle (light on at 06:00) and free access to tap water and standard rodent chow.

Animals were divided into seven groups (with 8 animals each) and were treated intratracheally with $MnCl_2$ dissolved in distilled water (*LD*: 2.5 mg/kg b.w. $MnCl_2$; *HD*: 5 mg/kg b.w. $MnCl_2$) or with MnO_2 nanoparticles (*nLD*: 2.5 mg/kg b.w. MnO_2; *nHD*: 5 mg/kg b.w. MnO_2). In *Comb* group, rats received 2.5 mg/kg b.w. $MnCl_2$ and the same dose of MnO_2 with a short delay. The MnO_2 nanoparticles (NPs) were synthesized by a technique combining ultrasonic and hydrothermal treatment (for details, see Sárközi et al., 2009) at the Department of Applied Chemistry, University of Szeged Faculty of Science and Informatics. MnO_2 NPs were suspended in hydroxyethyl cellulose (swollen in phosphate buffered saline) which was used as vehicle and that the rats in vehicle control (*VC*) group were treated with. An untreated control (*Cont*) group was also applied.

Intratracheal instillation (1 ml/kg b. w.) was carried out in brief diethyl ether anaesthesia. The treatment lasted for 5 weeks, performed once a day, 5 days per week.

Before the beginning of the treatment period and on the day following the last instillation, the rats were put into an open field (OF) box to test their spontaneous horizontal and vertical motor activity in 10 min sessions.

Electrophysiological measurement was done after anesthetizing the animals with urethane (1000 mg/kg b. w., i.p.). The left hemisphere was exposed, the animals were put in a stereotaxic device, and silver electrodes were placed on the primary somatosensory (SS), visual (VIS) and auditory (AUD) areas. Spontaneous electrical activity (electrocorticogram, ECoG) was

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recorded from these sites simultaneously for 6 min, and the relative spectral power of the frequency bands was determined (Kandel and Schwartz, 1985).

Stimulus-evoked activity was then recorded via the same electrodes. Somatosensory stimulation was done by electric pulses given trough a pair of needles inserted into the whiskery skin (3-4 V; 1000, 500, 100 ms repetition time). Visual stimulation was performed by flashes (1 Hz) delivered by a high-luminescence white LED directly into the contralateral eye of the rat. For acoustic stimulation, clicks (1 Hz, 40 dB), were applied into the ear of the rat. Fifty stimuli of each modality per rat were applied and the evoked activity recorded. After averaging, latency and duration of the evoked responses was measured.

The body weight of the animals was regularly measured during the 5 weeks of the experiment.

Statistical analysis was done by two-sample t-test.

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

RESULTS

Mn administration caused significantly slowed body weight gain in all treated groups (vs. *Cont*), from the first week on (Fig. 1).

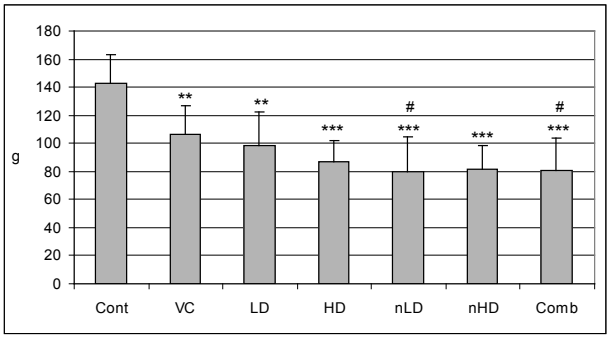
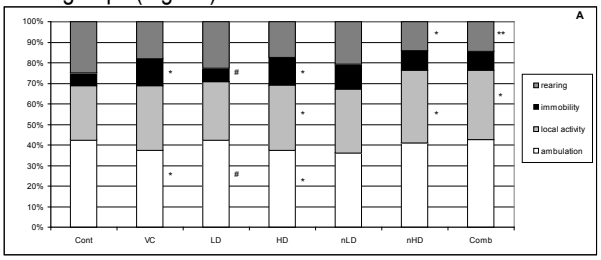


Fig. 1 Body weight gain. *,**,*** p<0.05, 0.01, 0.001 vs. *Cont*; #, ##, ### p<0.05, 0.01, 0.001 vs. *VC*.

The open field test after the 5 weeks treatment period showed decreased motor activity in all the treated rats. Increased immobility and decreased rearing was observed both in the $MnCl_2$ and MnO_2 treated groups. The time spent with local activity increased and showed mild significance in *HD*, *nHD* and *Comb* groups (Fig. 2a). Changes in activity counts were similar to the above mentioned alterations, confirming that the OF activity of the rats shifted to hypomotility, especially in $MnCl_2$ treated and *Comb* groups (Fig. 2b).



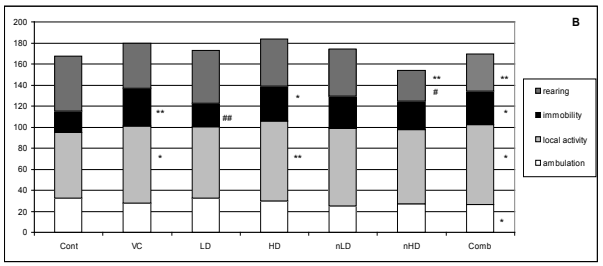


Fig. 2 Effect of $MnCl_2$ and MnO_2 treatment on the OF activity of the rats after the treatment. (A:time B:count) *,**,*** p<0.05, 0.01, 0.001 vs. *Cont*; #, ##, ### p<0.05, 0.01, 0.001 vs. *VC*.

Intratracheal instillation of $MnCl_2$ and MnO_2 significantly altered the spectral distribution of the electrocorticogram. The general trend of the ECoG band spectrum change was different in each modality depending on the treatment type (solute or nano-sized Mn). $MnCl_2$ treatment had a strong effect on theta band and the increase was significant in each cortical area (vs. *VC*). Effect of high dose MnO_2 treatment was mainly seen on the VIS and AUD ECoGs. On the VIS ECoG, the proportion of the fast frequency bands increased and that of the slow bands decreased. On the contrary, spectral distribution of the AUD ECoG showed decrease in fast bands, and increase in slow bands. These changes were significant in case of gamma and delta bands (Fig. 3).

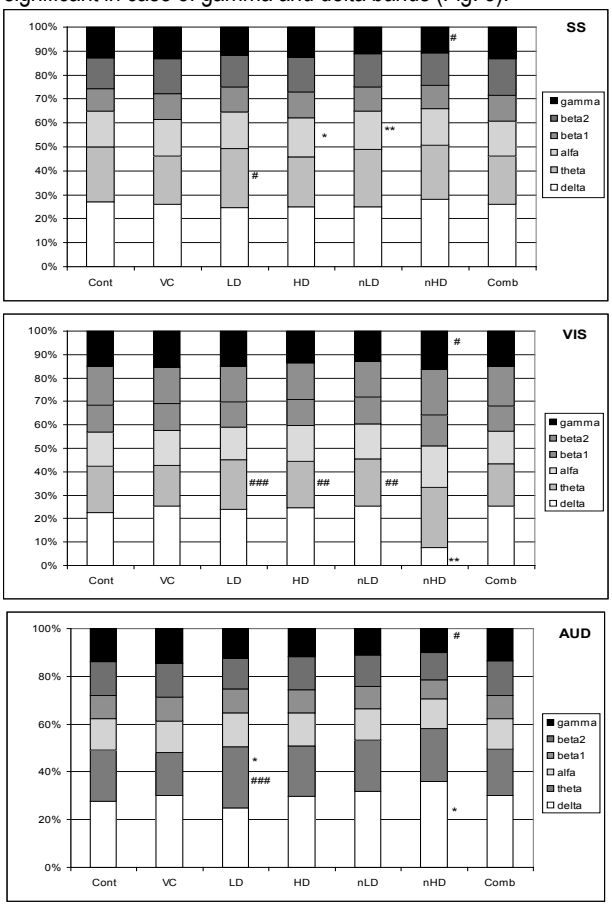


Fig. 3 Frequency spectrum of the spontaneous cortical activity. (SS-somato-sensory, VIS-visual, AUD-auditory area) *,**,*** p<0.05, 0.01, 0.001 vs. *Cont*; #, ##, ### p<0.05, 0.01, 0.001 vs. *VC*.

From the parameters of the cortical evoked potentials, lengthening of the SS latency, caused by $MnCl_2$ treatment, was the most prominent. MnO_2 treatment did not significantly affect the SS latency, but in *Comb* group a combined effect of the two forms of Mn was seen (lower, but significant increase; Fig. 4). SS amplitude was only affected in *nLD* group, where the increase was significant. In case of fast stimulation (10 Hz), the same trend was discovered, but the effect of the combined treatment – strong decrease – appeared to be significant (Fig. 5). Between VIS and AUD latency there was an opposite alteration, similarly to the ECoG findings (Fig. 6).

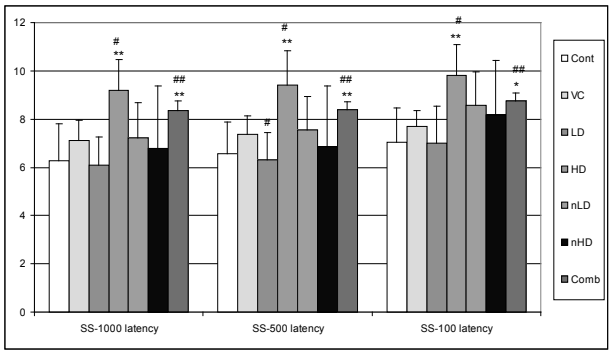


Fig. 4 Comparison of the latency of somatosensory evoked potentials of the treated groups. *,**;*** p<0.05, 0.01, 0.001 vs. *Cont*; #, ##, ### p<0.05, 0.01, 0.001 vs. *VC*.

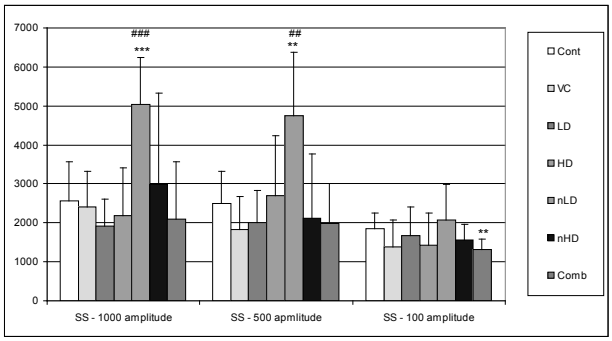


Fig. 5 Comparison of the amplitude of somatosensory evoked potentials of the treated groups. *,**;*** p<0.05, 0.01, 0.001 vs. *Cont*; #, ##, ### p<0.05, 0.01, 0.001 vs. *VC*.

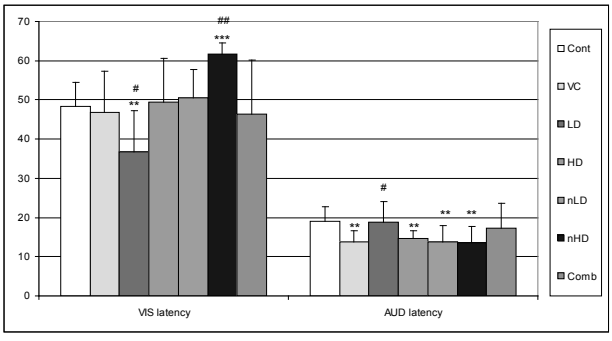


Fig. 6 Comparison of the latency of visual and auditory evoked potentials of the treated groups. *,**;*** p<0.05, 0.01, 0.001 vs. *Cont*; #, ##, ### p<0.05, 0.01, 0.001 vs. *VC*.

DISCUSSION

According to the observed changes in the electrophysiological parameters the applied animal model proved to be suitable for intratracheal Mn intoxication follow up. As it was indicated in previous experiments (9), Mn had access to the CNS and the observed electrophysiological alterations were caused by Mn accumulation in the brain.

In the regulation of OF activity, which is known to be affected by Mn (Normandin et al., 2004), dopaminergic structures play important role (Alexander et al., 1990). Alterations in cortical evoked activity due to Mn can be explained by Mn-dependent inhibition of astrocytic glutamine synthetase (10). Desensitization and slowed action of the thalamocortical afferents (due to enhanced glutamatergic transmission) might cause lengthening of the latency of the cortical evoked potentials. On the other side, nerve conduction can be damaged as well. Mn might also interfere with Ca channels (11) and with mitochondrial energy production (12).

Decrease of slow (delta, theta) and increase of fast (beta, gamma) bands of the ECoG (or the opposite in VIS-AUD ECoG) in the same rats might have resulted from impaired collateral input of the glutamatergic afferents. Altered EEG and event-realated potentials, which was found in this experiment in VIS and AUD recordings, were also described in case of human occupational Mn exposure.

The results further suggested that electrophysiological tests might be more sensitive to the effects of Mn than general toxicological or neurobehavioral ones, which is potentially relevant both in experimental work and in hygienic toxicology.

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ALTERARI NEUROFIZIOLOGICE EXPERIMENTALE DETERMINATE DE EXPUNEREA COMBINATA LA NANOPARTICULE SI MANGAN

REZUMAT

Manganul (Mn) este un metal greu bine cunoscut, care determina injurii la nivelul sistemului nervos dupa expunerea cronica. Pentru a crea un model de expunere inhalatorie la Mn, am folosit un model animal, in care sobolanii masculi Wistar au fost instilati zilnic intra-traheal cu $MnCl_2$ (2,5 sau 5,0 mg/kg corp), dizolvata in apa distilata, sau cu nanosuspensie de MnO_2 (2,5 sau 5,0 mg/kg corp); au fost folosite si combinatii de 2,5 mg/kg solutie $MnCl_2$ si 2,5 mg/kg MnO_2 in suspensie de nanoparticule, care au fost administrate consecutive, intr-un interval de timp scurt. Dupa o perioada de 5 saptamani de tratament, animalele au fost testate din punct de vedere al activitatii si s-au efectuat investigatii electrofiziologice; au fost inregistrate potentiale de actiune evocate si spontane la nivelul ariilor corticale somato-senzoriale, vizuale si auditorii. Mn a ajuns la nivelul SNC si a determinat alterarea semnificativa a spectrului de distributie al benzilor ECoG, a indus latenta potentialelor evocate si a influentat activitatea locomotorie la sobolani. Efectele observate au aratat un efect aditiv in cazul expunerii combinate la ambele solutii.
Cuvinte cheie: mangan, nanoparticlule, instilare intra-traheala, potentiale evocate, sobolan