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ARTICLE

Subacute exposure of rats by metal oxide nanoparticles through the airways: general toxicity and neuro-functional effects

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ABSTRACT In order to create an animal model of human inhalational exposure by industrial metal fumes, nanoparticulate metal oxides (MnO_2, CdO_2, PbO) were synthesized and instilled into the trachea of rats 5 times a week for 6 weeks (metal doses per kg b.w.: 2.63 and 5.26 mg Mn; 0.04 and 0.4 mg Cd; 2 and 4 mg Pb). At the end, the rats' body weight gain during the treatment was determined, the animals had an open field session to investigate their spontaneous motility, and finally spontaneous and stimulus-evoked cortical activity was recorded in urethane anaesthesia. Mn caused decrease of open field ambulation and rearing, Cd had no effect, whereas Pb caused decreased rearing and increased ambulation. Spontaneous cortical activity was shifted to higher frequencies with each metal. Cortical evoked potentials had lengthened latency, mainly with Mn and Cd; and increased frequency dependence with Cd and Pb but hardly with Mn. The effects proved indirectly that the metal content of the nanoparticles had access form the airways to the CNS. Our method seems suitable for modelling human nervous system damage due to inhaled nanoparticles.
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KEY WORDS

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The nervous system is a target for various environmental xenobiotics including the heavy metals manganese, lead and cadmium (ATSDR, 1999a, 1999b, 2000). These metals, as well as their alloys and compounds, have a number of applications including a lot of industrial procedures which emit metal, metal oxide etc. particles. Inhalation of airborne metal dusts or fumes is a major way of occupational exposure, causing acute and chronic diseases such as metal fume fever and COPD. In inhalational exposure situations, the size of the particles is crucial, whereby the importance of submicroscopic particles – also called nanoparticles (NPs) – has been recognized only recently. Particles of <100 nm diameter, found typically in metal fumes, have a huge surface area relative to their mass, and can penetrate across tissue boundaries such as the alveolar wall and the blood-brain barrier, which possibly results in direct access to the CNS (Obedörster et al. 2005; Oszlánczi et al. 2010).

Manganese-containing fumes are generated in welding and other high-temperature industrial operations. Inhalation of Mn fumes can cause manganism (a chronic neurological syndrome resembling Parkinson's disease: Bowler et al. 2006) and other neurological manifestations. Deposition of Mn following chronic exposure in the human brain (Yamada et al. 1986) and the resulting damages to the dopaminergic system (Shinotoh et al. 1997) have been reported.

Occupational exposure by cadmium metal fumes resulted in reduced visuomotor performance, and difficulties of concentration and postural balance (Viaene et al. 2000). Exposed children showed straight relationship between hair Cd levels and altered sensory evoked potential parameters (Thatcher et al. 1984).

Lead processing and recycling, and the use of leaded petrol in the past (but in certain countries also at present) have been the main sources of airborne exposure by lead. Whatever the physicochemical form of the absorbed lead is, it is deposited in the central nervous system, first of all in the cortex and hippocampus (Grandjean 1978). In lead-exposed workers, sensory evoked potentials and nerve conduction velocity were affected (Araki et al. 2000). There exists a well-known relationship between blood or dental lead level and IQ loss or behavioural abnormalities in exposed children (Fergusson et al. 1997; – whereby it is noteworthy that children's lead exposure also can come from scaling-off old lead paint in the home environment: ATSDR, 1999b).

In this study, exposure by airborne NPs was modelled by intratracheal instillation of a nanosuspension of MnO_2, CdO_2 or PbO to rats, and the resulting behavioural and electrophysiological alterations were investigated.

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Table 1. Treatment groups and doses of the metal oxide nanoparticles.

Group	Code	Substance	Dose (mg metal / kg b.w.)
Untreated control	Con	--	--
Vehicle control	W	Distilled water	--
Manganese low dose	LD-Mn	MnO_2 nanosuspension	2.63
Manganese high dose	HD-Mn		5.26
Cadmium low dose	LD-Cd	CdO_2 nanosuspension	0.04
Cadmium high dose	HD-Cd		0.4
Lead low dose	LD-Pb	PbO nanosuspension	2
Lead high dose	HD-Pb		4

Materials and Methods

Adult male Wistar rats (300-350 g body weight) were used. The experiments with the three metal oxide NPs were carried out separately. In each of them, there was an untreated control group (Con), a vehicle control group (W), and a low dose (LD) and a high dose (HD) group; with 10 rats each. For doses and group coding, see Table 1. The rats were kept under normal conditions in a GLP-certified animal house. They were weighed weekly, and the weight gain during the 6 weeks was calculated.

The nanoparticulate metal oxides (mean diameter: MnO_2, 23.2±3.3 nm; CdO_2, 20.1±5.7; PbO, 19.5±3.6 nm – determined by X-ray diffraction) were synthesized at the Department of Applied Chemistry, University of Szeged. The NPs were made up in distilled water to a dilute suspension, which was sonicated to prevent aggregation and was instilled into the rats' trachea 5 days a week for 6 weeks under brief ether anesthesia, using a syringe and 1.2 mm OD plastic tubing, inserted between the vocal chords (for details, see Sárközi et al. 2009).

One or two days after the last instillation, the animals had a 10 min session in an open field (OF) box to investigate spontaneous motility. The box was equipped with two arrays of infrared beam gates at floor level and at 12 cm height (Conducta 1.0 System, Experimetria Ltd., Budapest), for automatic detection of the beam interruptions caused by the moving animal during the 10 min session. The software of the system computed these to data of counts, time and run length of the basic activity forms (ambulation, local activity, rearing, immobility).

The rats were finally anesthetized with urethane ip. (1000 mg/kg b.w. ip.), and the left hemisphere was exposed by removing the most of the left parietal bone and the above lying soft tissues. For recording cortical electrical activity, silver electrodes were placed over the primary somatosensory (SS), visual (VIS) and auditory (AUD) area, and electrocorticogram (ECoG) was recorded for 6 min. From the records, the relative spectral power of the frequency bands (delta, theta, alpha, beta1, beta2, gamma; standard human EEG bands as described in Kandel and Schwartz 1985) was calculated. After recording ECoG, sensory stimulation was applied and the cortical evoked potentials (EPs) were recorded from the same points. The SS stimuli were square electric pulses (3-4 V, 0.05 ms) delivered to the contralateral whisker pad of the rat by means of a pair of needle electrodes at 1, 2 and 10 Hz frequency. The contralateral eye (for VIS EPs) was stimulated by flashes of a white LED, and the ear with clicks from a mini-speaker, at 1 Hz. After averaging the EPs, onset latency was measured manually. All electrophysiological recording and analysis was done by means of the Neurosys 1.11 software (Experimetria Ltd, Budapest, Hungary).

From the individual data, group means were calculated and compared by means of one-way ANOVA using the SPSS 17.0 software. During the whole study, the principles of the Ethical Committee for the Protection of Animals in Research of the University were strictly followed.

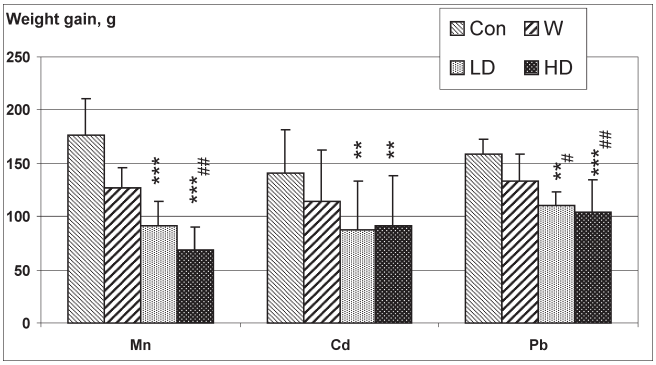

Figure 1. Weight gain of the control and treated rats during the 6 weeks treatment period. The data (mean + SD, n=10) represent the difference in the rats' body weight measured on week 0 and week 6. The metals and treatment groups are indicated on the abscissa and in the insert, respectively (see Table 1 for explanation). **, ***: p<0.01, 0.001 vs. Con; ##: p<0.01 vs. W.

Results

The rats' body weight gain during the 6 weeks of treatment (Fig. 1) showed some difference between the untreated (Con) and vehicle-treated (W) groups. This effect was, however, mild and did not obscure the weight gain reducing effect of the instilled NPs, indicating substantial general toxicity of the instilled NPs.

The results of the OF tests demonstrated in contrast that on the investigated central nervous functions the treatment procedure itself (ether anesthesia and instillation of distilled water) had no major effect. Figure 2 shows the time (in seconds) spent by the rats in the various forms of motor activity during the 600 s of the OF session. High dose Mn caused significantly decreased ambulation and rearing. In the HD-Pb group, increased ambulation and local activity was seen, while the applied doses of Cd had no noteworthy effect.

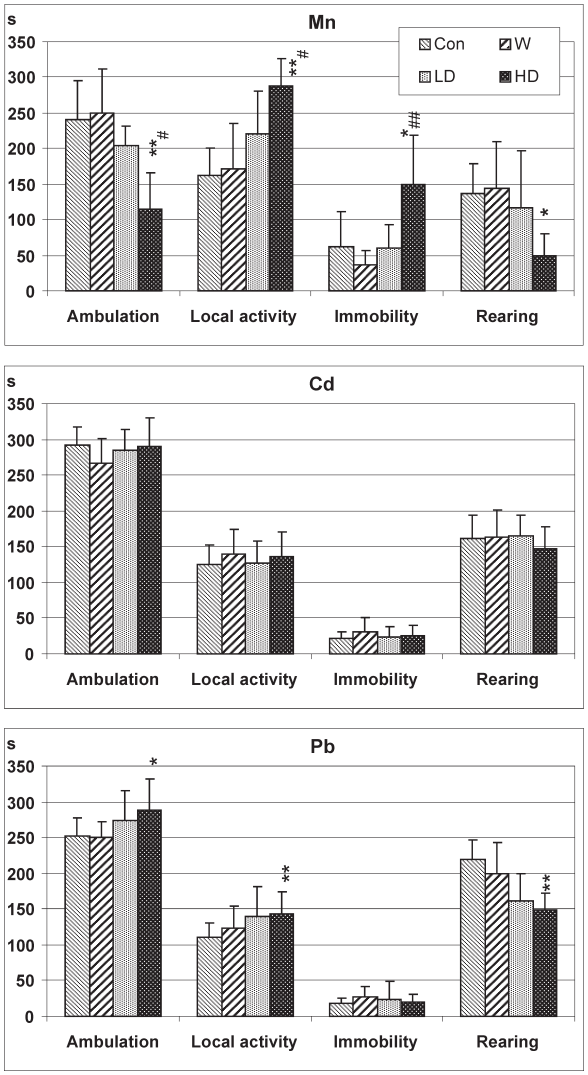

Figure 2. Open field activity after 6 weeks treatment with Mn, Cd and Pb containing nanoparticles (top, middle and bottom panel, respectively). The data (mean + SD, n=10) represent the time spent with the activity forms given on the abscissa. For control and treated groups, see insert in the top panel. *, **: p<0.05, 0.01 vs. Con; #, ##: p<0.05, 0.01 vs. W.

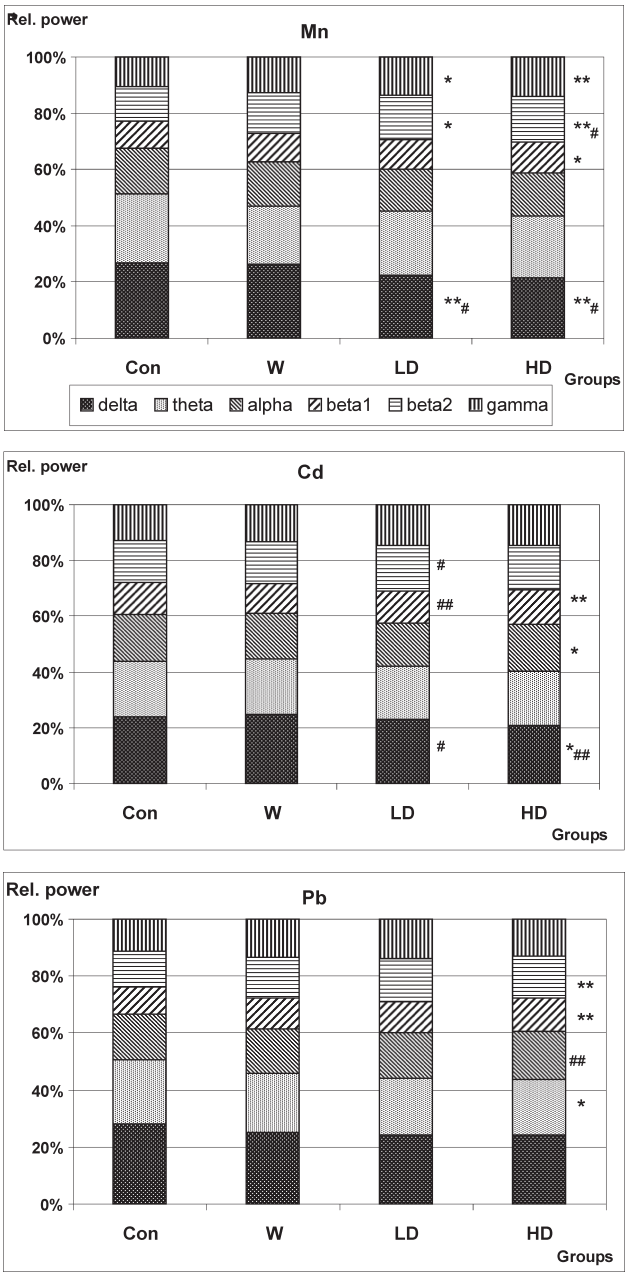

Figure 3. Band spectrum of the electrocorticogram recorded from the somatosensory cortex of the rats after 6 weeks treatment with Mn, Cd and Pb (top, middle and bottom panel, respectively). Abscissa: groups. Ordinate: relative power of the ECoG bands (for the bar filling pattern of the bands, see insert in the top panel). *, **: p<0.05, 0.01 vs. Con; #, ##: p<0.05, 0.01 vs. W, always between identical bands.

The spontaneous cortical electrical activity, recorded in urethane anaesthesia, was shifted to higher frequencies by all three metal oxides. There was no qualitative difference between the changes seen in the three cortical areas; hence, only the activity spectra from the SS area are shown in Figure 3. The change was, similarly to that of the open field activity, apparently dose-dependent; and the difference between the Con and W groups was also here minimal.

The most typical change in the cortical EPs was latency lengthening. The SS EPs had significantly longer latency in the HD groups than in Con or W with each metal (Fig. 4). With Mn, the effect of the low dose was also significant. The dependence of latency on the frequency of stimulation was also more pronounced in the treated rats, but the extra increase of latency at faster (2 and 10 Hz) stimulation vs. 1 Hz was significant only with Pb and Cd. The increase of latency of the VIS and AUD EPs was also significant in all HD groups

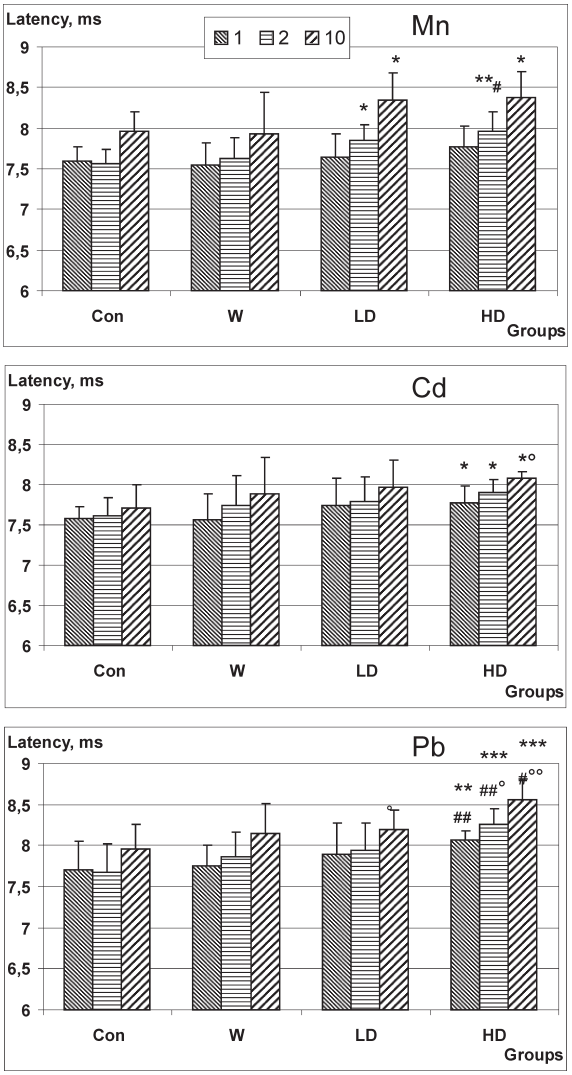

Figure 4. Latency of the somatosensory evoked potentials obtained with stimulation frequency of 1, 2 and 10 Hz (see insert in the top panel) in Mn, Cd and Pb treated rats. Ordinate: treatment groups. Mean + SD, n=10. *, **, ***: p<0.05, 0.01, 0.001 vs. Con; #, ##: p<0.05, 0.01 vs. W; °: p<0.05 vs. 1 Hz stimulation within the same group.

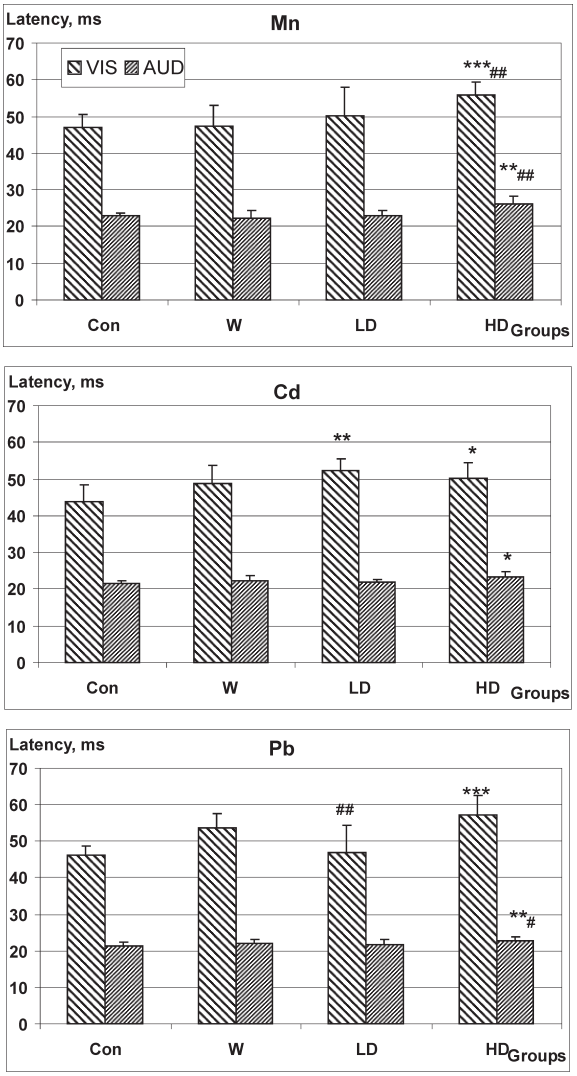

Figure 5. Latency of the visual and auditory evoked potentials (see insert in the top panel) in Mn, Cd and Pb treated rats. Ordinate: treatment groups. Mean + SD, n=10. *, **, ***: p<0.05, 0.01, 0.001 vs. Con; #, ##: p<0.05, 0.01 vs. W.

(Fig. 5) The VIS EP latency was significantly longer also in LD-Cd and LD-Pb.

Discussion

The similarity of the three metals' effects suggested some common mechanism, oxidative stress being a likely candidate (Mn: McNeilly et al. 2004; Cd: Zhang et al. 2007; Pb: Adonaylo and Oteiza, 1999). The reduced body weight gain observed in the treated animals also might be due to this, more exactly to the metabolic disturbance caused by the presence of free radicals (Merry 2002). Systemic oxidative stress is present also in the brain (Mn: Zhang et al. 2009; Cd: Kumar et al. 1996; Pb: Patra et al. 2001) resulting in membrane lipid peroxidation (Mn: Ávila et al. 2008; Cd and Pb: Zanchi et al. 2010; Pb: Ahamed and Siddiqui 2007). This in turn results in altered neuronal membrane functions, and in a disturbance in a number of events depending on that, including all receptor-bound phenomena such as synaptic transmission.

In ionic form, each of the studied metals is known to affect Ca homeostasis (Büsselberg 1995). Considering the role of Ca influx in presynaptic transmitter release, interference by the metals (Mn: Takeda 2003; Cd: Soliakov and Wonnacott 1996; Pb: Sandhir and Gill 1993) can explain the lengthening of EP latency (together with the membrane damage mentioned above). Whether the applied metal was in fact present in the rats' brain after the 6 weeks of intratracheal NP exposure, has been proven by us in case of Mn only (Oszlánczi et al.

2010) and that analysis did not discriminate among forms of Mn. It may be generally true, however, that the surface of the NPs, consisting of a normally water-insoluble metal oxide, releases free metal ions in the acidic environment of the phagolysosomes of alveolar macrophages (Lundborg et al. 1985) which then escape and reach the brain via the bloodstream. Alternatively, complete NPs can be transported to the CNS (Elder et al. 2006; Wang et al. 2008) and can themselves act on the neurons or can be phagocyted by the microglia and release metals in a process mentioned above.

The increase of high-frequency cortical spontaneous activity was probably due to increased reticular ascending cholinergic activation in case of Cd and Pb (Carageorgiou et al. 2004; Suszkiw et al. 1984). In case of Mn, effects on the cholinergic system are also known (Finkelstein et al. 2007) but decreased inactivation of glutamate (inhibition of astrocytic glutamin synthetase by Mn: Normandin and Hazell 2002) probably contributed to the more intense input into the reticular ascending fibres.

The changes in the OF motor behavior of the treated rats are not sufficiently explained by the possible effects of the metal NPs mentioned so far. Here, damages to the dopaminergic influence on the cortex exerted by mid-brain structures like the ventral tegmental area may be of importance. Mn is well-known to damage dopaminergic structures in the brain (Verity 1999; Takeda 2003), and the decreased motility of rats can be set in analogy to the symptoms observed in Mn-exposed welders. Decrease of dopaminergic cortical receptors and hypermotility in rats was reported also after Pb exposure in rats (Ma et al. 1999). In this case an analogy may exist between the hypermotility seen in rats and the "attention deficit hyperactivity disease" found to be more frequent among children with elevated blood Pb (Needleman and Gatsonis 1990).

Our results showed that metal oxide nanoparticles can cause significant alterations in certain indicators of the CNS activity in the treated rats. The changes of the electrophysiological phenomena were similar to our earlier results obtained by oral application of Mn, Cd and Pb in water-soluble form (Nagymajtényi et al. 1997; Papp et al. 2003; Vezér et al. 2005) which indicated that the metal content of the instilled nanoparticles most probably had access to the brain. Experiments of the type presented here may constitute a suitable model of the nervous system effects of nanoparticle inhalation.

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