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GENERAL AND NERVOUS SYSTEM EFFECTS OF LEAD APPLIED IN NANOPARTICULATE FORM INTO THE TRACHEA OF RATS

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ABSTRACT

Lead is a heavy metal notoriously harmful for human health and environment. In case of leaded petrol (still in use in certain regions involved in this symposium), and in lead processing and reprocessing industries, airborne particles are emitted, exposing people by inhalation. Nervous system is a primary target of lead, with known consequences like occupational neuropathy and delayed mental development of children. In inhalational exposure, the size of particles entering the airways is crucial. In this study, submicroscopic (mean diameter ca. 20 nm) PbO particles were suspended in distilled water and instilled into the trachea of male Wistar rats (2 and 4 mg/kg), 5 times a week for 3 and 6 weeks. The treated rats' body weight gain was significantly lower than in the controls from the 3rd week on, and the weight of their lungs was significantly increased. Spontaneous cortical activity, recorded in urethane anesthesia, was shifted to higher frequencies in the treated rats. The cortical sensory evoked potentials in the same rats had mostly increased latency, sometimes also increased duration, and decreased frequency following ability on rapid stimulation. Lead in nanosuspension form had access to the brain so the human effects of inhalation of lead nanoparticles can be modelled in rats this way.

1. INTRODUCTION

Exposure by lead-containing airborne particles is seen in occupational settings (smelting, processing and reprocessing of lead) and in the general environment in areas where leaded petrol is till in use. Lead is well absorbed from the alveoli [12] and from the intestines[5]. Airborne lead causes primarily exposure by the airways, where the size of the inhaled particles is a crucial factor. Grains of 10 µm or more are trapped in the upper airways while those of 1-10 µm are typically deposited in the alveoli. Even smaller particles – nanoparticles, ultrafine dust - have been newly recognized as having unique characteristics including pathogenicity. Such tiny particles, depositing either in the nasopharynx or in the alveoli [10] are highly mobile and can cross boundaries like the alveolar and capillary wall by mechanisms specific for this size range (transcytosis by caveola formation [20]). Axonal transport of such particles is also known.

Lead is a neurotoxic metal. In lead-exposed humans, various forms of central and peripheral evoked activity, namely sensory evoked potentials and nerve conduction velocity, were affected [1]. Impaired postural balance was seen in lead-exposed workers [28]. The deleterious effect of childhood lead exposure on IQ development and school performance has been amply documented [7,19]. In our previous studies lead, given orally in organic or inorganic form, altered the cortical electrical activity [17,18] and the memory performance [27] of rats.

In the present work - within the framework of the *Regional University Knowledge Centre for Environmental and Nanotechnology, Szeged, Hungary* - lead oxide (PbO) nanoparticles were produced, and their effects on general toxicological parameters and on the function of the central and peripheral nervous system were investigated in experimentally exposed rats.

2. MATERIALS AND METHODS

The experiments were carried out on adult male Wistar rats (280±20 g body weight at start), obtained at the university's breeding centre. They were housed in an air-conditioned room maintained at 22 °C with a 12-h light/dark cycle (light on at 06:00), and free access to tap water and standard pellet. There were 4 groups of 10 animals each: an untreated control group (Con), a vehicle control group (W), and a low dose (LD) and a high dose (HD) group. The doses applied were equivalent to 2 and 4 mg Pb / kg body weight, and were determined on the basis of data about the ventilation volume of rats [25]; and on published inhalation toxicity effects of lead in rats [6,23].

The PbO nanoparticles were synthesized at the Department of Applied Chemistry, University of Szeged in a dry procedure. Pb-acetate was milled with NaOH and the resulting hydroxide was calcined. Particle size (20 - 30 nm) was determined by X-ray diffraction and transmission electron microscopy. For administration, the nanoparticles were suspended in distilled water, and were instilled into the trachea of the treated rats 5 days a week, for 6 weeks. Before and during administration, the suspension was sonicated to prevent aggregation. The instilled volume was 1.0 ml/kg b.w., the vehicle control (W) group received distilled water. For intratracheal instillation, the animals were quickly anesthetized with diethyl ether in a glass jar with air-tight lid, then were suspended on a tilted (60°) board by hanging the upper incisor teeth in a wire loop which held the animal in place and its mouth open [21]. Focussed light was aimed transdermally on the trachea, the tongue was pulled forward with a pair of non-traumatic forceps, and a custom-made laryngoscope was used to gain access to the glottis. Intratracheal instillation was done by means of a 1 ml syringe and 1.2 mm OD plastic tubing, inserted between the vocal chords.

The rats' body weight was recorded weekly. Symptoms of general toxicity were also observed and noted.

On the day following the last instillation, the animals were prepared for electrophysiological recording. In urethane anesthesia (1000 mg/kg b.w ip.), the animal's head was fixed in a stereotaxic frame, and the left hemisphere was exposed by opening the bony skull. Lidocaine (10%) was sprayed on the wounds, and the exposed dura was protected by a thin layer of petroleum jelly. After 30 minutes recovery, silver electrodes were placed on the primary somatosensory (SS), visual (VIS) and auditory (AUD) areas. Electrocorticogram (ECoG) was taken from these areas for 6 minutes and the relative spectral power of the frequency bands (delta, theta, alpha, beta1, beta2, gamma; standard human EEG bands as described in [11]) was determined. Then, sensory cortical evoked potentials (EPs) were recorded. Somatosensory stimulation was done with square electric pulses (3-4 V, 0.05 ms, 1, 2 and 10 Hz) delivered to the contralateral whisker pad of the rat. For visual stimulation, flashes of a high-luminance white LED (driven by 0.2 ms pulses at 1 Hz) were aimed directly at the rat's right eye. The acoustic stimuli were clicks (1 Hz, 40 dB) from a small earphone, guided into the animal's right ear via the hollow ear bar. Fifty stimuli of each modality per rat were applied, and the recorded EPs were averaged. After averaging, latency and duration of the evoked responses was measured manually (for details, see [22]). Finally, compound action potential was recorded form the rat's tail nerve. Two stimulating needles (delivering 4-5 V, 0.05 ms pulses at 1, 20 and 50 Hz) were inserted into the tail base; and another two, for recording, 50 mm distally. From the records, the conduction velocity of the nerve was calculated. The change of the latency of the somatosensory EP, and latency and amplitude of the nerve action potential, with increasing stimulation frequency was also investigated as an indicator of the action of the treatment on the state of the nervous system [22]. The complete electrophysiological recording and analysis was done by means of the Neurosys 1.11 software (Experimetria Ltd, Budapest, Hungary). Following electrophysiology, the rats were sacrificed by an overdose of urethane, dissected, and the relative organ weight of the lungs, liver, heart, kidneys, spleen, thymus and adrenals, related to the 1/100-th of body weight, was calculated. The results were tested for significance with one-way ANOVA and the post hoc analysis was done by Scheffe's test.

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

3. RESULTS

Lead treatment caused significant retardation in the rats' body weight gain. The difference between group Con and W was moderate (Fig. 1).

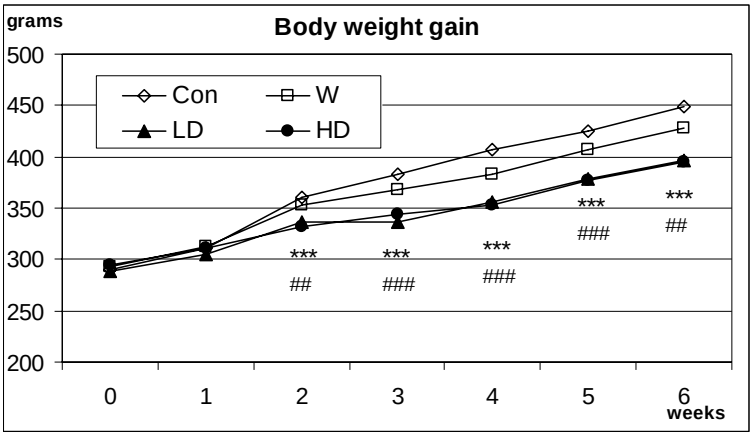

Figure 1. Body weight gain of the rat groups (see insert) during the 6 weeks of treatment. Group means, n=10.
*** : p<0.001 vs. Con; ##, ###: p<0.01, 0.001 vs. W.

The organ weights measured during final dissection indicted massive increase of the lungs and kidneys in both treated groups , and less severe effect on the brain and liver weight. In the HD groups, the lungs had a strongly emphysematous appearance.

Table 1. Relative organ weights after 6 weeks treatment.

	Treatment groups			
Organs	**Con**	**W**	**LD**	**HD**
Heart	0.255±0.018	0.264±0.020	0.282±0.032*	0.267±0.016
Spleen	0.188±0.025	0.176±0.026	0.199±0.034	0.187±0.021
Thymus	0.090±0.011	0.095±0.026	0.103±0.021	0.104±0.023
Adrenals	0.010±0.003	0.012±0.004	0.013±0.005	0.013±0.004
Liver	3.111±0.208	3.201±0.333	3.342±0.367	3.324±0.164*
Kidney	0.605±0.037	0.618±0.038	0.721±0.097**###	0.686±0.038******###
Lung	0.334±0.036	0.343±0.028	0.515±0.076******###	0.583±0.059******###
Brain	0.474±0.029	0.480±0.022	0.529±0.056*#	0.520±0.048*#

Mean±SD, n=10. Calculation: [organ weight]/[0.01 × body weight]
*,**,***: p<0.05, 0.01, 0.001 vs. Con; #, ##, ###: p<0.05, 0.01, 0.001 vs. W.

The general trend of the ECoG was activity decrease in the low and increase in the high frequency bands. As seen in Fig. 2, this trend was present in all three cortical areas but the change was significant only in the SS and VIS area and only in the HD group.

The latency of the SS EP was nearly identical in the Con and W groups, and noteworthy frequency-dependent increase was seen only with 10 Hz stimulation (Fig. 3). In the LD group, there was only minor latency increase but the frequency-dependent increase (10 vs. 1 Hz) significant. In the HD group, significant latency increase was seen and the frequency-dependent increase was more pronounced.

In line with the lengthened cortical latencies, the conduction velocity of the tail nerve was reduced in the treated groups (Fig. 4 left). Faster stimulation (50 and 20 ms period time instead of 1 s)was also applied to the tail, and the relative difference of the nerve action potential amplitude and latency, compared to the values obtained with 1 s period, was calculated. As seen in Fig. 4 (right), amplitude decrease on fast stimulation was present in both treated groups while latency increases only in the HD group.

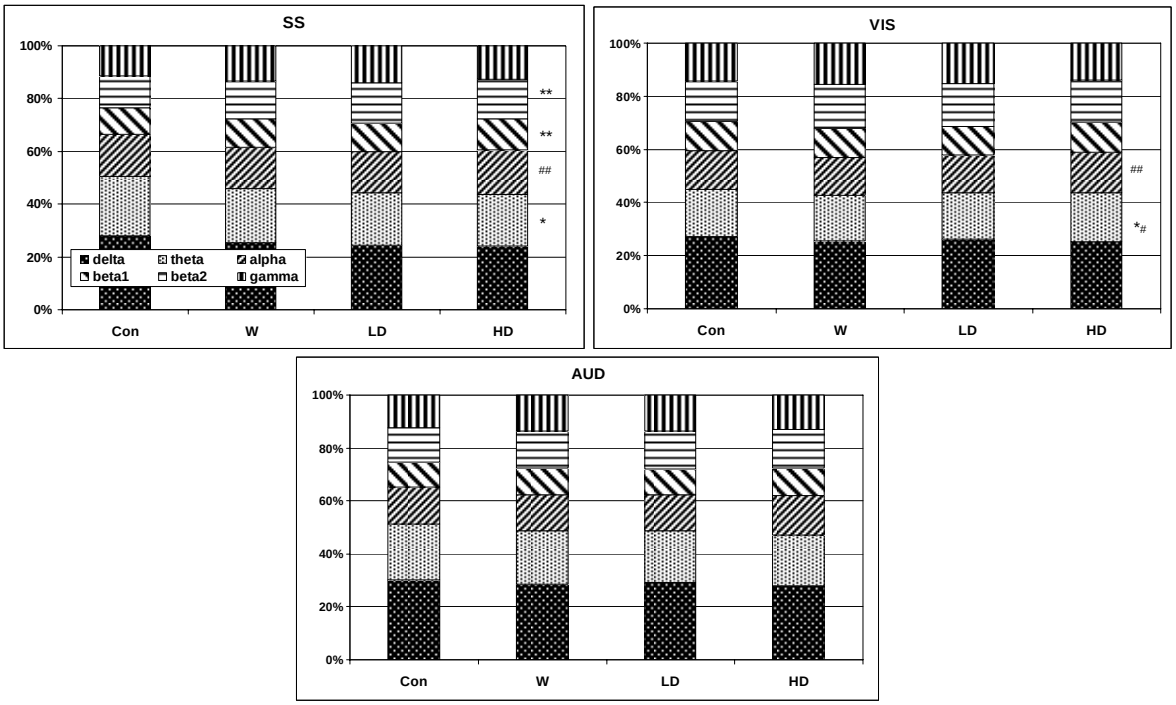

Figure 2. Band spectrum of the spontaneous cortical activity. Abscissa, groups; ordinate, relative ECoG power of the bands indicated in the insert (top left).
Group means, n=10. *,**: p<0.05, 0.01 vs. Con; #, ##: p<.05, 0.01 vs. W.

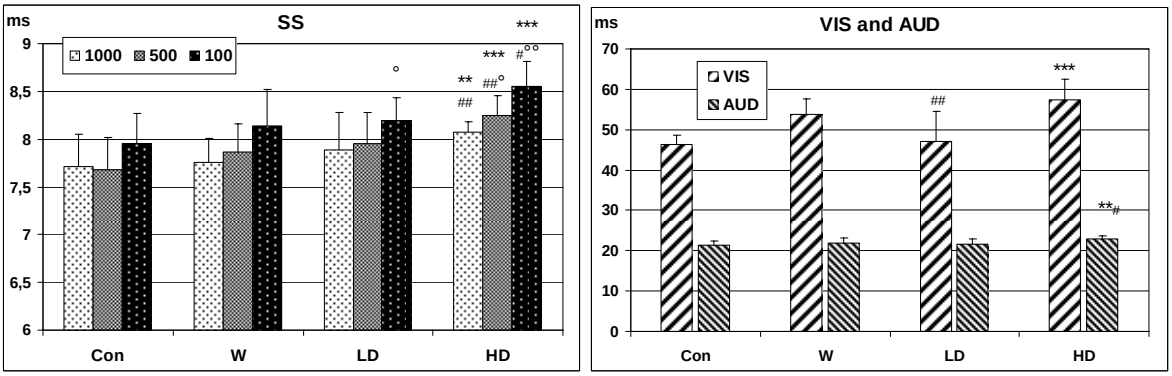

Figure 3. Left: latency of the somatosensory evoked potentials obtained with the stimulation period times given in the insert in ms (corresponding to 1, 2 and 10 Hz frequency).
Right: latency of the visual and auditory evoked potential.
Mean+SD, n=10. Significance marking as before

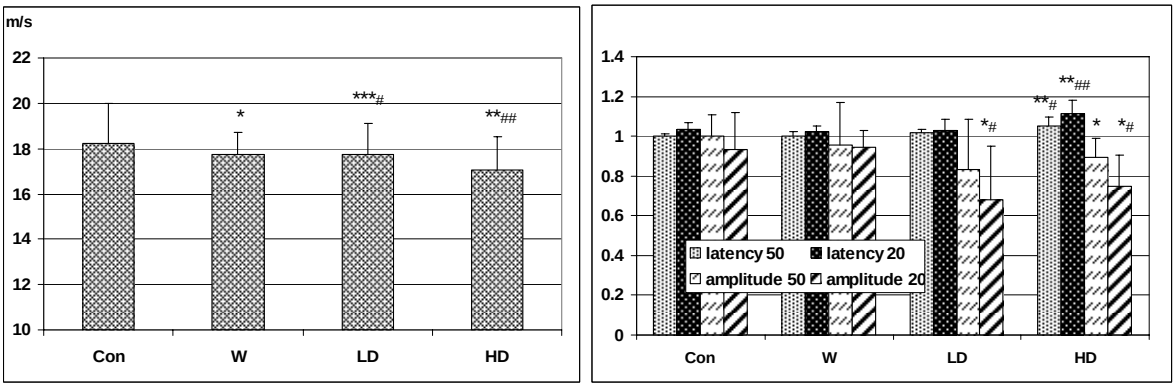

Figure 4. Left: conduction velocity of the tail nerve (ordinate, m/s) in the control and reated group.
Right: relative change of the latency and amplitude of the tail nerve action potential obtained with 50 and 20 ms period time (see insert).
Mean+SD, n=10. Significance marking as before

4. DISCUSSION

The electrophysiological changes in this study were similar to those observed earlier [17,18] in rats treated orally with a dissolved form of Pb. This indicates that, beyond causing lung inflammation and emphysema [16], the nanoparticulate metal was most probably absorbed from the airways and was present in the rats' brain. Intact nanoparticles have the capacity to cross the blood-brain barrier [20]. Or, after phagocytosis, the acidic local environment within the phagosomes [14] may set free Pb^{2+} ions [9] known to cross the blood-brain barrier [3] and even to damage it [8].

The nervous system effects of Pb^{2+} ions may be explained by its chemical similarity to Ca^{2+}. Stimulus-evoked release of ACh was reduced (but spontaneous release increased) by Pb^{2+} [26]. This possibly led to increased ascending cholinergic cortical activation and higher typical ECoG frequencies [13,17]. Reduced release of glutamate can, on the other hand, explain the slowed nerve pulse conduction and longer latencies [4] and decreased sensitivity of its cortical receptors [15]. Beyond that, Pb^{2+}, by acting on voltage-dependent Ca^{2+} and Ca^{2+}-activated K^{+}-channels [2,24], could slow down the propagation of action potential, resulting in the observed effects on the peripheral nerve and contributing to the increased latency of the cortical response.

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