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RESEARCH ARTICLE

Nervous system effects in rats on subacute exposure by lead-containing nanoparticles via the airways

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Abstract

Context and objective: Lead (Pb) is a heavy metal harmful for human health and environment. From leaded gasoline (still used in certain countries), and in Pb processing and reprocessing industries, airborne particles are emitted which can be inhaled. In such exposure, the size of particles entering the airways is crucial. The nervous system is a primary target for Pb, and consequences like occupational neuropathy and delayed mental development of children are well-known. The aim of this work was to investigate the neurotoxicity of Pb nanoparticles (NPs) applied into the airways of rats.

Methods: Nano-sized lead oxide particles (mean diameter ca. 20 nm) were suspended in distilled water and instilled into the trachea of adult male Wistar rats (in doses equivalent to 2 and 4 mg/kg Pb), 5 times a week for 3 and 6 weeks. At the end, open field motility was tested, then central and peripheral nervous activity was recorded in urethane anesthesia.

Results and conclusion: The treated rats' body weight gain was significantly lower than that of the controls from the 3rd week onwards, and the weight of their lungs was significantly increased. Horizontal motility increased while vertical motility decreased. Spontaneous cortical activity was shifted to higher frequencies. The somatosensory cortical evoked potential showed increased latency and decreased frequency-following ability, and similar alterations were seen in the tail nerve. Significant Pb deposition was measured in blood, brain, lung and liver samples of the treated rats. The experiments performed seem to constitute an adequate model of the human effects of inhaled Pb NPs.

Keywords: Nanoparticle, lead, neurotoxicity, motility, electrical activity, rat

Introduction

Exposure by lead (Pb)-containing airborne particles is seen in occupational settings (smelting, processing and reprocessing of Pb) and in the general environment in areas where leaded petrol is still in use. Pb in humans is absorbed from the alveoli in ca. 50% and in 10–15% (but in children 50%) from the intestines (Järup, 2003). Airborne Pb causes exposure primarily by inhalation, leading to significant internal exposure both in humans and in experimental animals (Griffin et al., 1975a, 1975b). The size of the inhaled particles is a crucial factor in cases of exposure via the airways. Submicron particles—nanoparticles (NPs), ultrafine dust—have been newly recognized as having unique characteristics including pathogenicity. Such tiny particles, depositing either in the nasopharynx or in the alveoli (ICRP, 1994) are highly mobile within the human or animal organism and can cross boundaries like the alveolar and capillary wall by mechanisms specific for this size range (transcytosis by caveola formation; Oberdörster et al., 2005).

The blood-brain barrier in healthy adults prevents most of the ionic Pb content of the blood from entering the brain (Bradbury and Deane, 1993). NPs of various compositions were, however, detected in the brain after application to the airways of rats (Kreyling et al., 2006).

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Transport along nerve fibers of such particles is also known (Oberdörster et al., 2005).

In Pb-exposed humans, neurotoxicity is manifested in alterations of central and peripheral evoked activity, namely sensory evoked potentials (EPs) and nerve conduction velocity (Araki et al., 2000). Impaired postural balance was also seen in Pb-exposed workers (Yokoyama et al., 2002). In schoolchildren, Otto et al. (1985) found characteristic auditory EP alterations after several years of exposure to Pb. In our previous studies, Pb, given orally in organic or inorganic form to rats, altered the cortical electrical activity (Nagymajtényi et al., 1997) and the memory performance (Vezér et al., 2000).

In the present work, lead oxide (PbO) NPs were synthesized and instilled into the trachea of rats. Functional alterations in the central and peripheral nervous system, as well as some general toxicological effects, were investigated.

Materials and methods

Animals and treatment

The experiments were carried out on adult male Wistar rats (280 ± 20 g body weight at start), obtained at the university's breeding centre. The rats were kept in a GLP-rated animal house (22 ± 1 °C, 30–60% relative humidity, 12-h light/dark cycle with light on at 06:00) and had free access to tap water and standard pellet. There were 4 groups of 20 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 (Table 1) were determined by indirect estimation because no directly comparable experiments were found in the literature. Among the physiological data published by Strohl et al. (1997), ventilation minute values of different rat strains were ca. 24–32 ml/100 g b.w. Taking 30 ml, the total inhaled volume for 24 h was estimated as nearly 0.5 m^3. Coffigny et al. (1994) and Pinon-Lataillade et al. (1993) obtained significant internal Pb exposure in rats (blood levels 500–700 ppb) by continuous exposure to air containing 5 mg/m^3 PbO dust. Combining the two data would give about 2.5 mg/kg b.w. daily, but—taking the considerable uncertainties involved into account—we chose one dose below, and another dose above, this level.

Of each group, 10 rats were used after 3, and the other 10 after 6, weeks of exposure. The PbO NPs 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 (19.5 ± 3.6 nm) was determined by X-ray diffraction and transmission electron microscopy (Figure 1).

For administration, the NPs were suspended in distilled water, and were instilled into the trachea of the treated rats 5 days a week. The instilled volume was 1.0 ml/kg b.w., the W group received pure distilled water. Before and during administration, the suspension was sonicated to counteract aggregation. For intratracheal instillation, the animals were quickly anesthetized with diethyl ether and were suspended on a tilted board (60° to horizontal) by hanging the upper incisor teeth in a wire loop which held the animal in place and its mouth open. Focused light was

Table 1. Treatment groups and doses.

Group	Code	Treatment and dose	Duration
Untreated control	Con	—	3 and 6 weeks
Vehicle control	W	Distilled water 1 ml/kg b.w.	
Low dose	LD	PbO nanosuspension, 2.0 mg Pb/kg b.w.; 1 ml/kg b.w.	
High dose	HD	PbO nanosuspension, 4.0 mg Pb/kg b.w.; 1 ml/kg b.w	

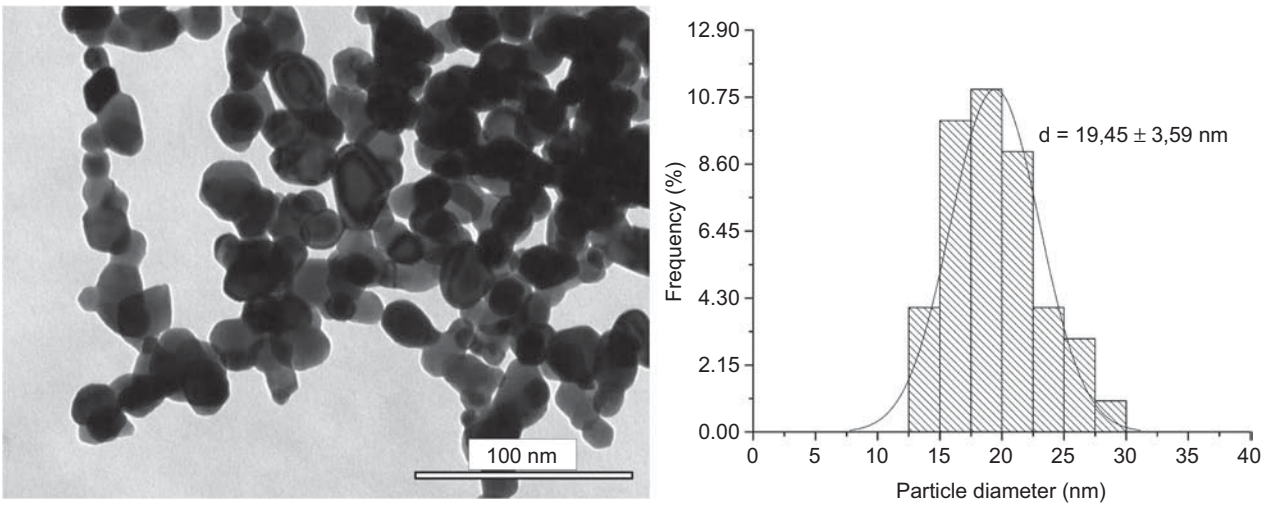

Figure 1. The PbO nanoparticles used for intratracheal instillation to the rats. Left, transmission electron micrograph. Right: size histogram.

aimed transdermally on the trachea 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 (for further details, see Oszlánczi et al., 2010). The rats' body weight was recorded weekly. Symptoms of general toxicity were also observed and noted.

Behavioral investigation

One or two days after the last instillation, the animals were tested for spontaneous motility; using an open field (OF) box of 48 × 48 × 40 cm size, equipped with two arrays of infrared light gates at floor level and in 12 cm height (Conducta 1.0 System, Experimetria, Budapest). The animals were placed, after 20–30 min accommodation in the testing room, individually into the center of the box. The system recorded their horizontal and vertical motor activity in 10-min sessions, based on the interruptions of the infrared beams. From these data, event counts and summed time of the basic activity forms (ambulation, local activity, rearing, and immobility), as well as run length of ambulation, were computed as follows: More than 40 mm shift in the location of interrupted beams at the floor level during a time unit of 1 s was interpreted as horizontal activity; less shift, as local activity; and no shift at all, as immobility. Rearing was recorded if beams at floor level and at the higher level were interrupted simultaneously. In earlier works (Vezér et al., 2005, 2007), this method was found sensitive to metal-induced changes of motor activity.

Electrophysiological investigation

On the day following the OF test, the rats were prepared for electrophysiological recording. In urethane anesthesia (1000 mg/kg b.w i.p.), 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 min recovery, a silver electrode was placed on the primary somatosensory (SS) projection area of the whiskers ("barrel field": Waite, 2004). Electrocorticogram (ECoG) was taken for 6 min and 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 determined. From the relative band power data, the so-called "ECoG index" was calculated with the formula: ([δ]+[θ])/([β1]+[β2]). This proved to be a handy (though simplifying) single-figure descriptor of the ECoG spectrum in earlier works (e.g. Nagymajtényi et al., 1997).

Then, sensory cortical EPs were recorded. The contralateral whisker pad was stimulated with square electric pulses (3–4 V; 0.05 ms; 1, 2 and 10 Hz). One train of 50 stimuli was applied with each frequency, and the recorded EPs were averaged. After averaging, latency and duration of the EPs was measured manually (for details, see Papp et al., 2004). 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. Conduction velocity of the nerve was calculated from the latency of the response and the distance between the site of stimulation and recording. The change in the latency of the SS EP, and in the latency and amplitude of the tail nerve action potential, on increasing the frequency of stimulation was also investigated as an indicator of the action of the treatment on the state of the nervous system (Papp et al., 2004). The complete electrophysiological recording and analysis was done by means of the Neurosys 1.11 software (Experimetria, Budapest).

General toxicology and Pb level determination

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 weight of the brain was calculated. (Brain weight was chosen because it was much less affected by the treatment than body weight. For methodological rationale, see Schärer, 1977.)

For Pb level determination (and subsequent biochemical analyses not dealt with here), whole brains, lungs and liver, and ca. 1.5 ml of blood were stored at –20°C in self-sealing polyethylene bags and microcentrifugation vials, respectively. Five animals from the groups W, LD and HD were randomly chosen for this. Samples of ca. 1 g weight were dried at 80°C to constant weight, and were digested in 5 ml 65% HNO_3 at 90°C for 90 min. The digested matter was washed quantitatively into 100 ml measuring flasks, and metal determination was done by inductively coupled plasma mass spectrometry.

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

Data processing and significance testing

From all data, group mean and standard deviation were calculated and compared. Significance was tested with one-way analysis of variance, and the post hoc analysis was done by Scheffe's test. The relationship of the investigated toxicological and physiological parameters to the Pb levels in blood and brain samples was tested by means of correlation diagrams. For this purpose, all parameters of the rats chosen for Pb determination (see above) were normalized to the mean of the five values of the same parameter from group W, and one set of these normalized data was plotted against the blood and brain Pb levels normalized the same way. Trend line was fitted and significance was tested by the "linear fit" function of MS Excel.

Results

General toxicology: body and organ weights

Treatment by Pb NPs caused significant retardation in the rats' body weight gain from the 2nd treatment week on. The difference between group Con and W was, however, moderate, indicating that the procedure of repeated ether anesthesia and instillation in itself had no significant negative effect (Figure 2).

Among all organs, only the lungs showed significant change of relative weight after 6 weeks instillation of PbO NPs (Table 2). In the HD group, the lungs had also a strongly emphysematous appearance. There was some decrease in the relative weight of the liver and increase in that of the kidneys, but these remained below significance as did all organ weight changes after 3 weeks exposure only (data not shown). The absolute brain weight, used as calculation basis, was not fully stable (Con: 2.164 ± 0.088 g; W: 2.086 ± 0.068 g; LD: 2.092 ± 0.159 g; HD: 2.058 ± 0.062 g, $P < 0.05$ vs. Con) but was much less affected than body weight. Moreover, it indicated absence of brain edema.

Behavioral effects

There was a weak tendency of more motility of the treated rats after 3 weeks NP exposure. After 6 weeks, both LD and HD treated rats spent significantly more time with ambulation than the controls (Con and W). The decrease in rearing, and even more the increase in local activity, was less characteristic (significant vs. Con but not vs. W; Figure 3).

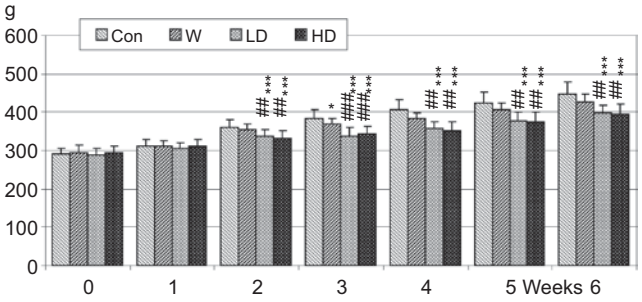

Figure 2. Change of body weight of the control and treated rats during the 6 weeks of PbO nanoparticle exposure. Abscissa, weeks; ordinate, body weight in grams. Mean ± SD, $n = 20$ or 10 (see Materials and methods section). The groups are coded as given in Table 1, for the bar patterns see insert. *, ***: $P < 0.01$, 0.001 vs. Con; ##, ###: $P < 0.01$, 0.001 vs. W.

Electrophysiological effects

The general trend of the ECoG was activity decrease in the low and increase in the high-frequency bands. As seen in Figure 4, the change was, except in the β2 band, significant 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 (Figure 5). In the LD group, there was only minor lengthening of latency but the frequency-dependent increase (with 10 vs. 1 Hz stimulation) became significant. In the HD group, significant latency increase was seen and the frequency-dependent increase was also more pronounced.

In line with the lengthened cortical latencies, the conduction velocity of the tail nerve was reduced in the treated groups (Figure 6A). The effect of frequent stimulation (20 and 50 Hz instead of 1 Hz) was also tested here. The relative changes in the latency and amplitude of the nerve action potential indicated the decreased ability of the nerve to follow high stimulation rates in the treated rats (Figure 6B and 6C).

Tissue Pb levels and correlations with other measured parameters

After 6 weeks of treatment, massive deposition of Pb was measured in the blood, brain, liver and lung samples (Table 3), whereby the tissue Pb levels were approximately proportional to the doses.

Body weight gain during the treatment period, as well as several of the physiological parameters, was in fair correlation with the measured brain and blood Pb levels (Figure 7). The correlation with brain Pb was stronger (steeper line, higher R^2) except for SS EP.

Discussion

The electrophysiological changes in this study were similar to those observed earlier (Nagymajtényi et al., 1997) in rats treated orally with aqueous solution of Pb-acetate. This, and the elevated Pb level measured in the treated rats' brains (the levels found in the present study were similar to those obtained earlier with oral

Table 2. Relative organ weights after 6 weeks treatment.

	Treatment groups			
Organs	Con	W	LD	HD
Heart	0.541 ± 0.054	0.551 ± 0.043	0.536 ± 0.055	0.517 ± 0.049
Spleen	0.396 ± 0.059	0.367 ± 0.062	0.379 ± 0.085	0.361 ± 0.041
Thymus	0.191 ± 0.023	0.199 ± 0.054	0.196 ± 0.043	0.199 ± 0.040
Adrenals	0.020 ± 0.006	0.026 ± 0.008	0.025 ± 0.008	0.024 ± 0.007
Liver	6.599 ± 0.740	6.680 ± 0.798	6.353 ± 0.719	6.446 ± 0.667
Kidney	1.281 ± 0.112	1.288 ± 0.081	1.366 ±. 0.118	1.328 ± 0.120
Lungs	0.707 ± 0.080	0.714 ± 0.061	0.976 ± 0.113*** ###	1.128 ± 0.125*** ###

Mean ± SD, $n = 10$. Calculation: [organ weight, g]/[brain weight, g].
***: $P < 0.001$ vs. Con; ###: $P < 0.001$ vs. W.

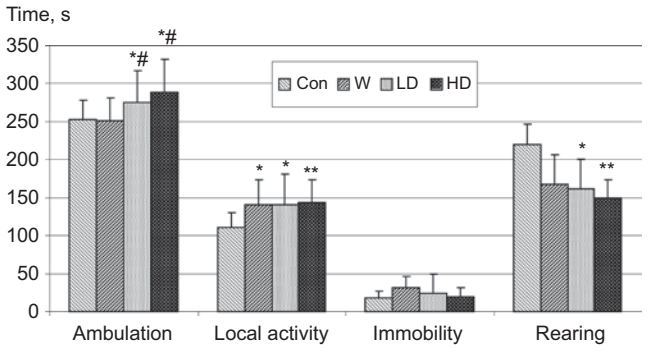

Figure 3. Time spent in the four basic forms of open field activity (ambulation, local activity, immobility, and rearing) by the control and treated rats after 6 weeks of exposure to PbO nanoparticles. Mean ± SD, $n = 10$. *, **: $P < 0.05$, 0.01 vs. Con; #: $P < 0.05$ vs. W.

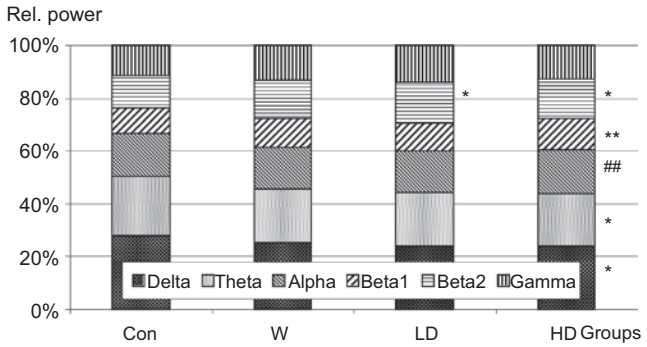

Figure 4. Band power spectrum of the electrocorticogram recorded from the rats' somatosensory cortex after 6 weeks treatment with PbO nanoparticles. Ordinate: relative power of the ECoG bands (see insert). *, **: $P < 0.05$, 0.01 vs. Con; ##: $P < 0.05$, 0.01 vs. W, always between identical bands.

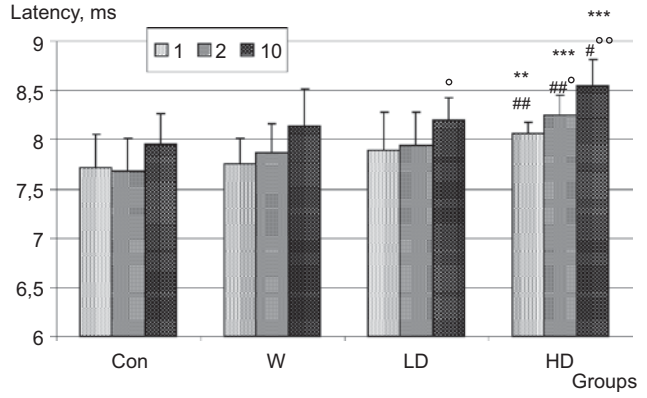

Figure 5. Latency of the somatosensory evoked potentials obtained with stimulation of the whiskers at different rates (insert shows stimulation frequency in Hz) in the control and treated rats after 6 weeks. Mean ± SD, $n = 10$. **, ***: $P < 0.01$, 0.001 vs. Con; #, ##: $P < 0.05$, 0.01 vs. W, always within the same frequency. °, °°: $P < 0.05$, 0.01 on stimulation at 2 or 10 Hz vs. 1 Hz, always within the same treatment group.

Pb-acetate; Vezér, unpublished data), indicated that the NPs not only were absorbed from the airways but either were transported to the brain in whole or Pb ions were dissolved from their surface and entered the brain. Intact NPs have in fact the capacity to cross the blood-brain barrier (Wang et al., 2008); alternatively, after phagocytosis, the acidic local environment within the phagosomes (Lundborg et al., 1985) may release metal ions, so neither mechanism can be excluded. As to Pb^{2+} ions, these are known to damage the blood-brain barrier (Goldstein et al., 1974) possibly increasing the chance of both intact NPs and Pb^{2+} ions to enter the brain. The comparison of the present results to the earlier ones referred to above suggests that the instillation of NPs was much more efficient in bringing Pb to the brain than oral application of Pb-acetate solution had been (PbO NPs, 4.0 mg/kg into the trachea for 6 weeks, resulted in ca. 1870 ppb brain Pb level, whereas 500 mg/kg Pb-acetate given by gavage for 10 weeks resulted in ca. 2500 ppb).

Blood Pb level in the vehicle-treated rats (group W in Table 3) was similar to that of identically treated control rats from another experiments from us and corresponded to the upper part of the range (0–260 ppb) given for unexposed workers (i.e. human background) in the meta-analysis published by Goodman et al. (2002), indicating the adequacy of our model, namely that our unexposed rats also represented background Pb exposure.

In terms of external and internal exposure, the adequacy of the model is a more complicated question. The exposure level applied by Coffigny et al. (1994) and Pinon-Lataillade et al. (1993), 5 mg/m^3 airborne PbO, is much higher than what can be found in the literature for occupational settings. Jiang et al. (2008) measured 1.06 mg/m^3 Pb in "fume" in a Pb battery workshop (fume includes fine and possibly ultrafine dust but the actual particle size is unknown) and 635 ppb Pb in the exposed workers' blood (controls: 87 ppb). Lormphongs et al. (2004) found up to 0.6 mg/m^3 Pb in the atmosphere of a Pb battery plant, and up to 500 ppb Pb in the exposed workers' blood. These two data would have given, with the calculation described in Materials and methods section, ca. 0.5 and 0.3 mg/kg daily doses, respectively. On the other hand, it is noteworthy that the group of Coffigny and Pinon-Lataillade obtained, after twice as long continuous inhalation as the time span of our experiment, 3–8 times lower blood Pb level in the rats than we did. Intratracheal instillation (being more feasible technically and more accurate in applied dose than inhalation) seems to be also more efficient in bringing the metal content of the suspended particles into the treated rats' organism, and the effect of particle size (which was more or less unknown in the reports referred to) is probably also important. So, lower doses of metal NPs may be sufficient in future experiments to create internal doses in rats comparable to those seen in exposed humans.

The nervous system effects of Pb^{2+} ions result in many cases from its chemical similarity to Ca^{2+}. The mild but significant change in the band spectrum of ECoG (more high-frequency and less low-frequency activity) after exposure might result from increased spontaneous release of ACh from nerve endings of the ascending reticular activation system (due to the presynaptic effect of Pb^{2+}) and/or increased activation of the ascending fibers via

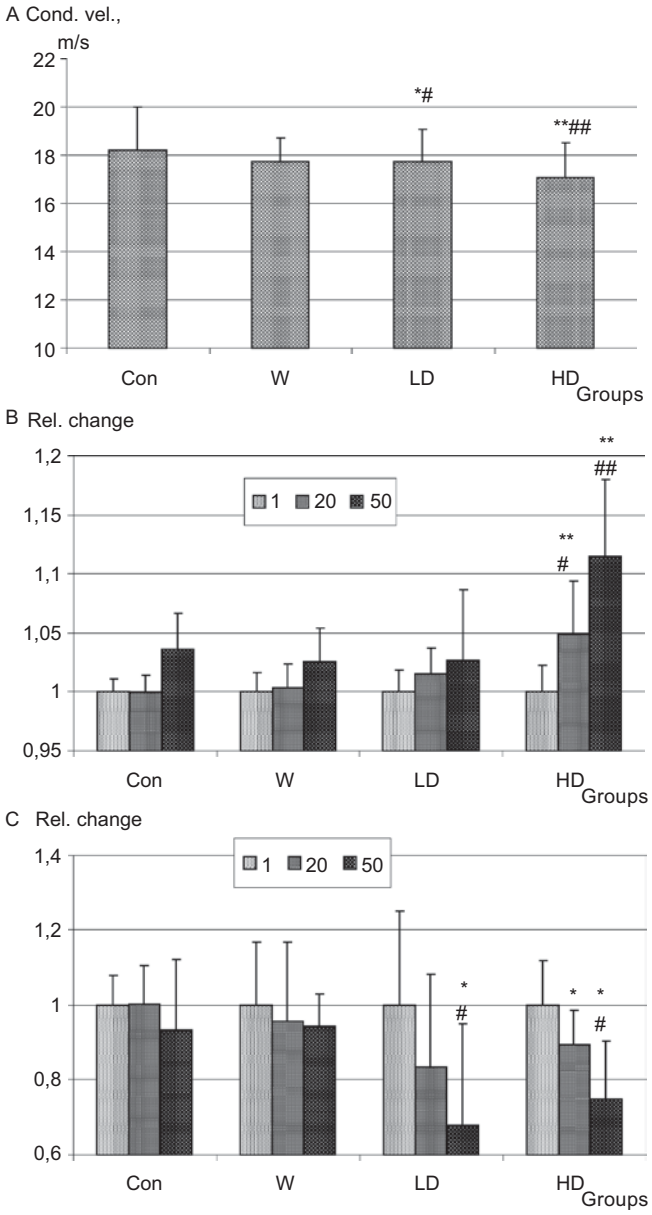

Figure 6. Effects of the PbO nanoparticles on the tail nerve action potential. (A) Conduction velocity of the nerve in the control and treated rats. (B and C) Relative change of the nerve action potential latency (B) and amplitude (C) on stimulation at 20 or 50 Hz vs. 1 Hz. Mean ± SD, $n = 10$. *, **: $P < 0.05$, 0.01 vs. Con; #, ##: $P < 0.05$, 0.01 on stimulation at 20 or 50 Hz vs. 1 Hz, always within the same treatment group.

glutamatergic collaterals of the specific afferent pathways (due to decreased inactivation of glutamate, see below). At the presynaptic endings, Pb^{2+} blocks the voltage-gated Ca-channels, impeding this way intracellular Ca^{2+} rise and depolarization on arrival of an axonal discharge, and preventing synchronous emptying of vesicles. At the same time, Pb^{2+} ions can also partially pass to the intracellular side through the channel. There they activate a number of Ca-dependent processes including those involved in exocytosis, so random liberation of the transmitter will be more likely (Suszkiw, 2004). Altered cortical activation may have contributed also to the increased EP latency, because a more activated state of the cortex is typically concomitant with less intense evoked responses (Herz et al., 1967). Besides that, the action of Pb^{2+} on voltage-dependent Ca^{2+} channels (Reuveny and Narahashi, 1991) could also slow down the propagation of the action potential, resulting in the observed effects on the peripheral nerve and contributing to the increased latency of the cortical response.

The expression of the astrocyte-specific glutamate transporter GLT-1 is decreased on exposure to Pb^{2+} (Struzynska et al., 2005), resulting in diminished clearance of glutamate from the synaptic cleft, which in turn may lead on one hand to receptor desensitization along the specific afferent pathways (and reduced stimulus-evoked activity, see above), and on the other hand to excitotoxicity (Coyle and Puttfarcken, 1993) due to NMDA receptor activation and excessive Ca^{2+} inflow (but the situation is more complicated because Pb^{2+} is also an NMDA blocker: Gavazzo et al., 2008). At the same time, increased glutamatergic activity can also contribute

to the above-mentioned increased cortical activation (Fournier et al., 2004).

Human EP studies are numerous, both in children and in exposed adults (the latter are more appropriately modeled in our work). Jeyaratnam et al. (1985) saw delayed somatosensory EP and slowed motor nerve conduction in workers exposed to Pb. Araki et al. (2000) stated that such alterations appear at 300–500 ppm blood Pb and indicate subclinical Pb poisoning. Changes of the EEG, on the contrary, have seldom been reported in human cases of Pb exposure. In 9-year-old children, increased theta activity was associated with blood Pb level (Poblano et al., 2001). Benignus et al. (1981) described increased asymmetry of the synchronous EEG amplitude between two parietal points (P3 and P4) in preschool children with elevated blood Pb.

Spontaneous locomotion observed in the OF is influenced less by external stimuli and more by the animal's inner drives and is dependent on the mesolimbic and nigrostrial dopaminergic systems (Fink and Smith, 1980). Vertical motility in particular is a sensitive indicator of striatal dopaminergic activity (Sedelis et al., 2001). Dopaminergic neurons themselves are especially vulnerable to oxidative stress due to the auto-oxidizing tendency of dopamine and to the presence of monoamine oxidase producing hydrogen peroxide (Alexi et al., 2000); and increased oxidative stress is a known consequence of Pb treatment *in vitro* (Naarala et al., 1995) and *in vivo* (Adonaylo and Oteiza, 1999). Increased horizontal motility, seen also in the present work, was reported by Ma et al. (1999) in rats following Pb exposure during intra- and extrauterine

Table 3. Pb levels (in ppb) in the tissue samples from vehicle-treated and nano-Pb treated rats.

	Treatment groups		
Organs	W	LD	HD
Blood	226.4 ± 124.6	1694.2 ± 289.5***	4011.6 ± 1030.7***##
Brain	174.5 ± 48.6	1056.1 ± 209.1***	1870.0 ± 442.9***##
Liver	80.1 ± 11.7	2166.4 ± 695.5***	4689.9 ± 695.4***###
Lung	5418.2 ± 4481.6	2,778,249.2 ± 1,232,587.1**	4,369,691.1 ± 2,060,069.1**

Mean ± SD, $n = 5$.
,*: $P < 0.01$, 0.001 vs. W; ##, ###: $P < 0.01$, 0.001 HD vs. LD.

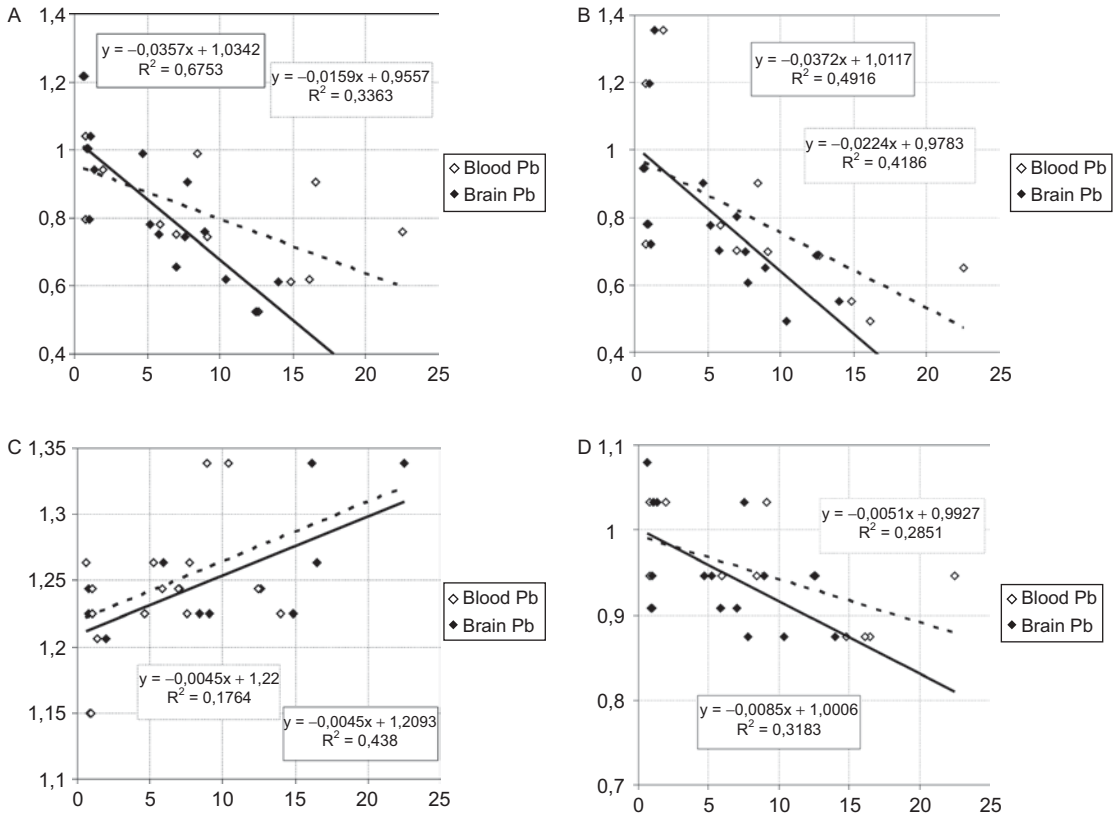

Figure 7. Correlation diagrams showing the relationship of body weight gain (A), ECoG index (B), evoked potential latency (C) and tail nerve conduction velocity (D) to the lead levels in blood and brain. The values plotted were normalized to the mean of the vehicle controls (W), lead level (of blood and brain, see insert) is always on the abscissa. The solid line was fitted to the dark, and the dotted line to the light, points, and the inserts with the R^2 values are marked accordingly.

development and was explained by decreased cortical D2 receptor level.

In this work, several alterations of cortical and peripheral nervous activity were found, which were dependent on Pb exposure (inner dose) and were mechanistically conceivable. Of these, the frequency dependence of the SS EP latency and of the tail nerve action potential latency and amplitude were significantly and dose-dependently altered by PbO NPs. Based on earlier experience, these parameters were proposed as potential functional biomarkers of nervous system damage (Papp et al., 2004), which proposal has been verified in case of Mn (Oszlánczi et al., 2010). The results altogether allow the conclusion that the methods of treatment and recording we applied constitute a valid animal model of inhalational Pb exposure and can be used to study the effects of metal NPs as sources of health hazard.

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Declaration of interest

The authors declare no interest. The authors alone are responsible for the content and writing of the article.

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