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Consequences of subacute intratracheal exposure of rats to cadmium oxide nanoparticles: Electrophysiological and toxicological effects

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Abstract

Cadmium (Cd) is a metal used in various industrial applications, thereby causing exposure to Cd-containing fumes. The submicron-sized particles in the fumes represent an extra risk due to their high mobility within the organism and high surface area. Toxicity of Cd on the liver, kidney and bones is well known, but there are less data on its neurotoxicity. Here, male Wistar rats were treated for 3 and 6 weeks by intratracheal instillation of cadmium oxide nanosuspension. The body weight gain in treated rats was significantly decreased, and in the rats treated with high dose (0.4 mg/kg Cd daily), there was a significant increase in the weight of lungs and thymus. In this group, the spectrum of spontaneous cortical electrical activity was shifted to higher frequencies, the latency of sensory-evoked potentials was lengthened, and the frequency following ability of the somatosensory evoked potential was impaired—even without detectable Cd deposition in the brain. The data support the role of the nano-sized Cd in the causation of nervous system damage and show the possibility of modeling human neurotoxic damage in rats.

Keywords

Cadmium, nanoparticle, neurotoxicity, electrophysiology, general toxicity

Introduction

Environmental conditions constitute one of the four major determinants of human health, and the medium causing the most direct exposure to harmful substances is air. Airborne particulate matter can be classified as sedimenting dust (>10 μm), suspended or fine dust (100 nm–10 μm; often called PM10) and ultrafine dust or nanoparticles (NPs, <100 nm).

NPs as pollutants arise mainly from combustion and other high-temperature processes (smelting, casting, welding of metals, etc.) (Antonini et al., 2003). Another potential source of exposure to NPs today is nanotechnology. Manufactured nanomaterials are present in numerous consumers' goods and in technical applications (Oberdörster et al., 2005). Quantum dots, novel nanotechnological materials (with application, among others, in biomedical research) often contain cadmium (more precisely, cadmium telluride) and show special toxicological properties (Rzigalinski and Strobl, 2009). Cd-containing metal dust and fumes, or paint spray, cause occupational airborne exposure in manufacturing and application of e.g. steel and other alloys, pigments and semiconductor materials (ATSDR, 2008).

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Inhaled NPs are either deposited in the nasopharynx or get down to the alveoli (ICRP, 1994). Once deposited, NPs translocate readily to other body parts and reach target organs by different transfer routes and mechanisms, including transcytosis (by caveola formation) across epithelia of the respiratory tract into the interstitium (Oberdörster et al., 2005) and axonal transport along the olfactory fibers directly into the central nervous system (CNS) (Calderon-Garciduenas et al., 2002). Due to their small size, number concentration and large specific surface area, NPs have greater biological activity per given mass than larger particles (Oberdörster et al., 2005), including oxidative stress induction (Li et al., 2003). In the target organs, the components of the NPs also exert their own toxic effects, after transport to these sites in whole or after being dissolved from the surface of the NPs (Lundborg et al., 1985). Cadmium, in airborne forms, is absorbed from the respiratory tract in 2–50%, depending primarily on particle size (Chaney et al., 2004). Its several target organs include the lungs, liver, kidney, testis, placenta, as well as the nervous system (ATSDR, 2008). Concerning the latter, the reported consequences of chronic Cd exposure include amyotrophic lateral sclerosis, optic nerve damage, striatal damage and peripheral polyneuropathy (Bar-Sela et al., 2001; Fern et al., 1996; O'Callaghan and Miller, 1986; Viaene et al., 1999). In children, a straight relationship between hair Cd and altered visual- or auditory (AUD)-evoked potential (EP) parameters was found (Thatcher et al., 1984), and school behavioral problems were also reported (Marlowe et al., 1985). Similar effects were observed in rats (Agar et al., 1999). In our previous works, oral application of Cd to rats for several weeks resulted in altered electrocorticogram (ECoG) power spectrum and in changes of cortical EPs and peripheral nerve action potentials (Institóris et al., 2002; Papp et al., 2003). In the present work, a potentially more realistic way of exposure—intratracheal application of cadmium oxide (CdO_2) NPs—was chosen, and the general toxicological and electrophysiological measurements were supplemented with some biochemical ones.

Materials and methods

Animals and treatment

Adult male Wistar rats of 320–350 g body weight (b.w.) were obtained from the breeding center of the university and were housed under standard conditions (22–24°C, 12-h light/dark cycle with light on at 06:00) with free access to tap water and standard pellet. The rats were divided into 4 groups of 20 animals each at start.

Cadmium dioxide NPs were synthesized at the Department of Applied Chemistry by a dry process. Stoichiometric amount of $CdCl_2$ and Na_2CO_3 were put, in NaCl matrix, in the drum of a planetary ball mill and rotated with stainless steel mill balls at 400 rpm for 4 h (reaction 1: $CdCl_2 + Na_2CO_3 \rightarrow CdCO_3 + 2\ NaCl$). The mixture milled this way was then calcined at 480°C for 4 h in air (reaction 2: $CdCO_3 + ½\ O_2 \rightarrow CdO_2 + CO_2$). After calcination, the synthesis mixture was filtered (0.45 μm polytetrafluoroethylene membrane filter) and washed with 80°C preheated water to remove any unreacted starting material and the soluble NaCl matrix. The precipitate was dried at 100°C for 1 h and characterized with X-ray diffraction and transmission electron microscopy. The size distribution and electron micrograph of the CdO_2 NPs is shown in Figure 1.

The synthesized NPs were suspended in distilled water and were instilled into the rats' trachea, in daily doses shown in Table 1, 5 days a week (Monday to Friday). The volume instilled was 1 ml/kg b.w. Treatment was continued for 3 and 6 weeks, whereby 10 rats from each group were killed after 3 weeks of treatment and the remaining 10 after 6 weeks. There was an untreated control group (Con), and a vehicle control group (W). The choice of doses was influenced by literature data and by the technically possible concentration of the NPs in the distilled water medium. Calculating with ca. 0.5 m^3/kg b.w. the daily breathing volume for the rats (based on data by Strohl et al., 1997), our lower dose is comparable to that reported from industrial settings (ca. 30 μg/m^3, indoors in car body repair shops: Vitayavirasuk et al., 2005; or 1–19 μg/m^3, outdoors in bridge maintenance: Conroy et al., 1995), and the higher one, to the 550 μg/m^3 used by Takenaka et al. (2004) in a rat inhalation experiment. A more direct comparison is, however, impossible because of the unknown retention fractions.

Intratracheal instillation was done in diethyl ether anesthesia, with the rat suspended on a 60° inclined board so that its upper incisors were held by a wire loop to keep the animal's mouth open. The trachea was illuminated transdermally. The tongue was pulled forward with a pair of nontraumatic forceps, and a custom-made laryngoscope was used to visualize the glottis. The nanosuspension (or distilled water for

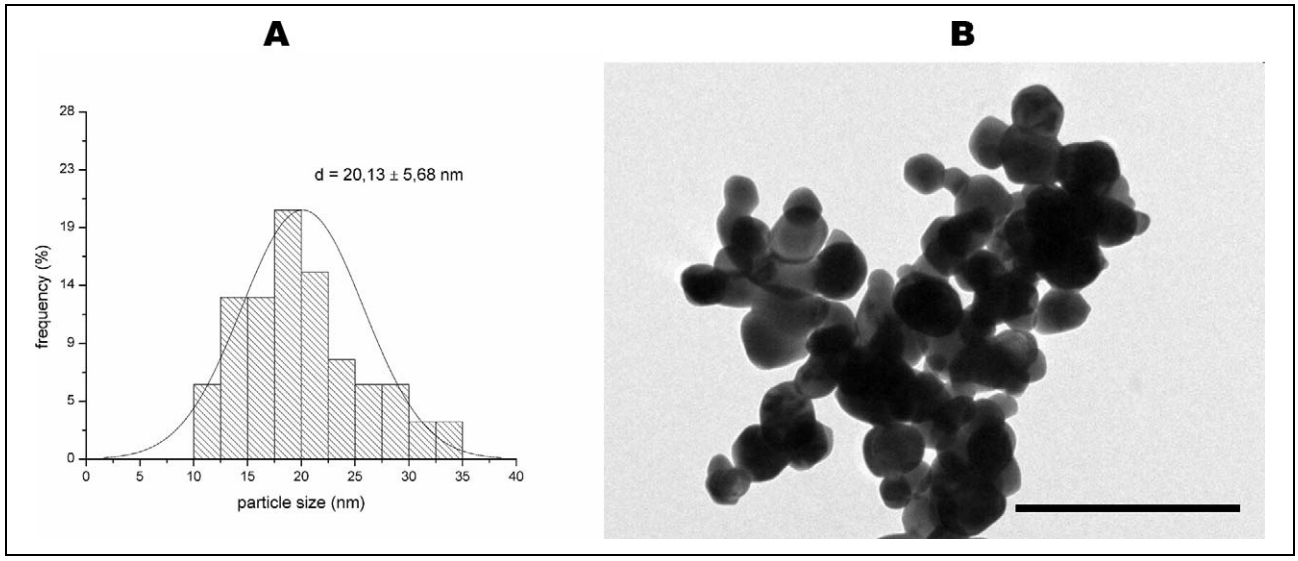

Figure 1. Size distribution histogram (a) and electron micrograph (b) of the CdO_2 nanoparticles. Scale bar: 100 nm.

Table 1. Treatment groups and doses

Group	Code	Treatment and dose	Duration
Untreated control	*Con*	–	3 and 6 weeks[a]
Vehicle control	*W*	Distilled water 1 ml/kg b.w.	
Low dose	*LD*	CdO_2 nanosuspension, 0.04 mg Cd/kg b.w.; 1 ml/kg b.w.	
High dose	*HD*	CdO_2 nanosuspension, 0.4 mg Mn/kg b.w.; 1 ml/kg b.w.	

[a]There were 20 rats in each group at start. Ten of them were processed and killed after 3 weeks of treatment, and the another 10, after 6 weeks of treatment.

group W) was instilled into the trachea by means of a syringe and 1.2 mm outer diameter plastic tubing, inserted between the vocal chords. The group *Con* had neither ether anesthesia nor instillation, while the water control (W) group was anesthetized and instilled with distilled water. The nanosuspension was vigorously sonicated before, and repeatedly during, administration to prevent agglomeration.

General toxicological and biochemical measurements

Body and organ weights were the end points for general toxic effect of the CdO_2 NPs. The rats' b.w. was measured each workday during the treatment period, and the mean b.w. of the groups was plotted against time to see the course of weight gain. Following electrophysiology (see below), the rats were killed by an overdose of urethane, dissected, and the organ weight of the brain, liver, lungs, heart, kidneys, spleen, thymus and adrenals was measured. Relative weights were calculated by relating organ weights to brain weight. To reduce costs, 5 of the 10 rats from each group were randomly assigned for chemical measurements. Of these, blood, brain, lung and liver samples were taken and stored at −22°C.

Metal level was determined from ca. 1 g of the samples, dried at 80°C to constant weight and digested in 5 ml of 65% HNO_3 at 90°C for 90 min. After filtration and dilution, metal level was determined by inductively coupled plasma mass spectrometry (at the laboratory of the MOL Hungarian Oil and Gas Company).

For biochemical measurements, another 1 g of the samples was homogenized with 4 ml saline and centrifuged under cooling for 10 min at 5000 rpm. The supernatant was centrifuged again for 20 min at 14,000 rpm.

From the supernatant, protein content was measured according to Lowry et al. (1951). As oxidative stress indicators, reduced glutathione (GSH) measured by the method of Sedlak and Lindsay (1968),

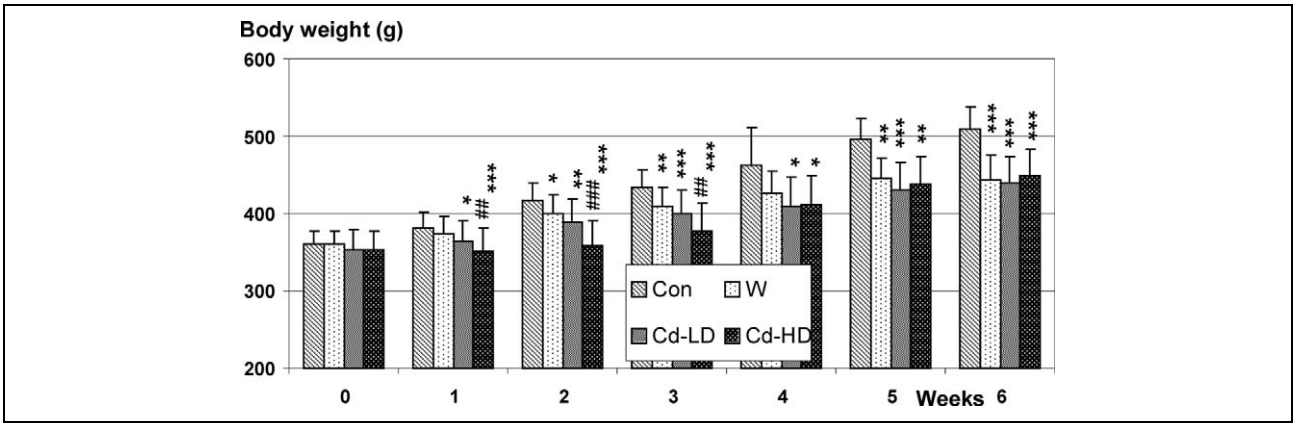

Figure 2. Body weight gain of the treated and control rats over the 6 weeks of treatment period. Always the data from the first workday of the corresponding week are plotted. Mean + SD, $n = 10$. Insert: group codes, see Table 1 for explanation. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$ vs. untreated control group (Con); ##$p < 0.01$, ###$p < 0.001$ vs. vehicle control group (W).

based on the reaction of nonprotein-bound SH groups with the Ellman reagent (5, 5′-dithio-bis(2-nitrobenzoic acid)), was used. Another oxidative stress parameter, superoxide dismutase (SOD) activity, was measured by the method of Misra and Fridovich (1972), modified by Matkovics et al. (1982), based on inhibition of the spontaneous adrenaline-adrenochrome transformation.

Electrophysiological measurements

Electrophysiological recording was done 1–3 days after the last instillation. In urethane anesthesia, the animal's head was fixed and the sensory areas of left hemisphere were exposed. The wounds were sprayed with 10% lidocaine, and a thin layer of petroleum jelly was applied on the dura to prevent drying. After 30 min recovery, silver electrodes were placed on the primary somatosensory (SS), visual (VIS) and AUD areas. ECoG was recorded from these areas for 6 min, and the relative spectral power of the frequency bands (delta, theta, alpha, beta1, beta2, gamma; standard human EEG bands) was determined. Then, sensory EPs were recorded by the same electrodes. For SS stimulation, two needles were inserted into the contralateral whiskery skin to deliver square electric pulses (3–4 V, 0.05 ms, 1–10 Hz). VIS stimulation was produced by a high-luminance white light-emitting diode aimed directly at the rat's right eye, driven by 0.2 ms pulses at 1 Hz. The AUD stimuli were clicks (1 Hz, 40 dB) guided from a miniature earphone into the animal's right ear via the hollow ear bar. Fifty stimuli of each modality per rat were applied and the EPs recorded. After averaging, latency and duration of the EPs was measured manually (for details, see Lukács and Szabó, 2007). The change in latency of the SS EP with increasing stimulation frequency was also investigated as a possible indicator of the action of the treatment on the state of the cortex. All electrophysiological recording and analysis was done by means of the Neurosys 1.11 software (Experimetria Ltd, Budapest, Hungary). The study was approved by the Ethical Committee for the Protection of Animals in Research of the University. During the whole procedure, the principles of the Committee (based on the EU-conform Hungarian law) were strictly followed.

Data processing

From the data, group means (±SD) were calculated. The results were tested for significance with one-way analysis of variance and the post hoc analysis was done by Scheffe's test.

Results

Body and organ weights

Intratracheal exposure by the nanoparticulate CdO_2 had marked effect on the b.w. gain in rats. As shown in Figure 2, the untreated controls' (Con) weight gain was undisturbed. In the vehicle control (W) group (anesthesia and instillation but no CdO_2 NPs), the weight gain was lower, and with the advance of time became more and more similar to that seen in the treated rats. In the high-dose (HD) group, there was hardly any weight increase in the first 2 weeks.

Table 2. Relative organ weights after 6 weeks of exposure to Cd nanoparticles[a]

Groups organs	Con	W	Pb-LD	Pb-HD
Lungs	0.787 ± 0.067	0.692 ± 0.084	0.831 ± 0.088	1.359 ± 0.254[b,c]
Liver	7.323 ± 0.718	7.198 ± 1.071	6.549 ± 0.502[d]	6.198 ± 0.595
Kidney	1.407 ± 0.070	1.350 ± 0.089	1.311 ± 0.172	1.379 ± 0.146
Heart	0.583 ± 0.031	0.542 ± 0.043	0.558 ± 0.039	0.556 ± 0.066
Spleen	0.468 ± 0.062	0.358 ± 0.040	0.334 ± 0.056[b]	0.387 ± 0.041[e]
Thymus	0.213 ± 0.026	0.194 ± 0.033	0.207 ± 0.017	0.277 ± 0.056[e,f]
Adrenals	0.028 ± 0.008	0.027 ± 0.007	0.029 ± 0.008	0.029 ± 0.008

Con: untreated control group; HD: high-dose group; LD: low-dose group, W: vehicle control group.
[a]Mean ± SD, $n = 10$.
[b]$p < 0.001$ vs. Con.
[c]$p < 0.001$ vs. W.
[d]$p < 0.05$ vs. Con.
[e]$p < 0.01$ vs. Con.
[f]$p < 0.01$ vs. W.

Table 3. Cd deposition and reduced glutathione level in tissue samples of rats after 6 weeks of exposure by CdO_2 NPs[a]

	Treatment groups		
	W	Cd-LD	Cd-HD
Cd level (μg/kg)			
Brain	0	0	0
Liver	26 ± 26	683 ± 271[b]	9986 ± 4171[b,c]
Lung	1707 ± 1391	43,020 ± 23,904[d]	268,399 ± 199,844[d,e]
GSH (μM)			
Brain	0.0895 ± 0.0037	0.0914 ± 0.0075	0.1071 ± 0.0035[f,g]
Liver	0.0729 ± 0.0040	0.0806 ± 0.0065	0.1062 ± 0.0020
Lung	0.2080 ± 0.1780	0.2398 ± 0.1335	0.1681 ± 0.1620[f,g]

HD: high-dose group; LD: low-dose group, NPs: nanoparticles; W: vehicle control group.
[a]Mean ± SD, $n = 5$.
[b]$p < 0.01$ vs. W.
[c]$p < 0.01$ vs. Cd-LD.
[d]$p < 0.05$ vs. W.
[e]$p < 0.05$ vs. Cd-LD.
[f]$p < 0.001$ vs. W.
[g]$p < 0.001$ vs. Cd-LD.

Then, some compensation seemed to take effect and the weight gain was similar to that seen in the low-dose (LD) group and approached that of the vehicle control (W).

The relative weight of the lungs was significantly higher in the HD group vs. Con after 6 weeks of exposure (Table 2). In the W and LD groups, there was no noteworthy increase. There was also significant increase in the relative thymus weight in the HD group, and decrease in the relative spleen and liver weight in the treated groups. After only 3 weeks of treatment (not shown), the trends were similar but less expressed.

Brain weight itself was little influenced by the Cd NP treatment (after 6 weeks of exposure: Con, 1.278 ± 0.054 g; W, 1.156 ± 0.91 g; Cd-LD, 1.169 ± 0.091 g; Cd-HD, 1.189 ± 0.134 g)—so the relative organ weights were not biased.

Cadmium levels and oxidative stress indicators

A shown in Table 3, most of the Cd content of the instilled NPs was located in the lungs but a significant amount was absorbed and deposited in the liver in a dose-dependent manner. In the brain (and blood), however, no Cd was detected.

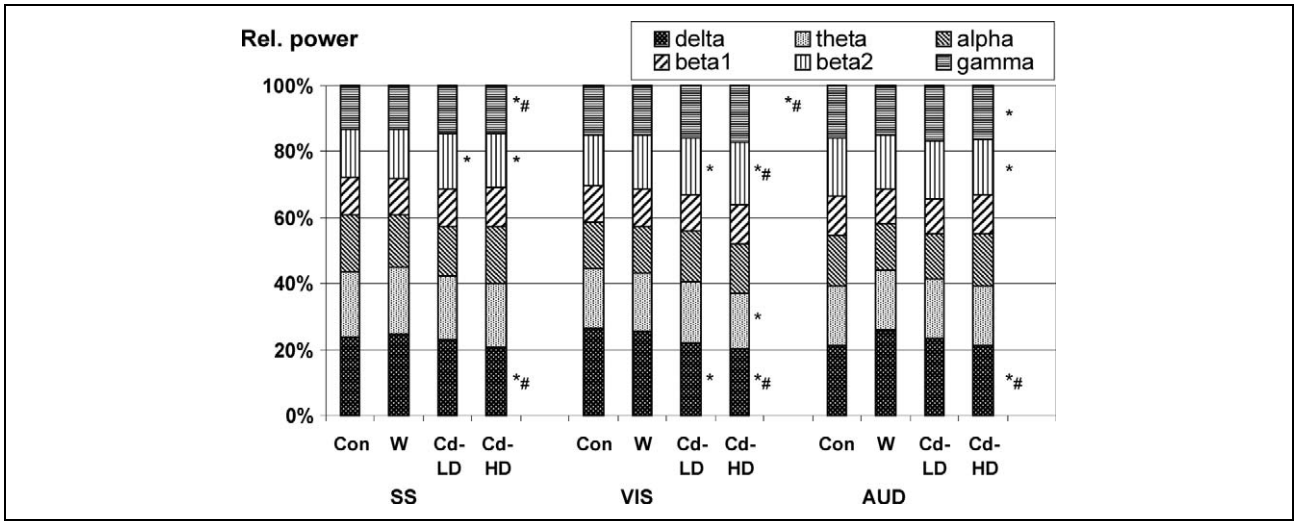

Figure 3. Band power spectrum (delta to gamma, see insert) of the rats' electrocorticogram after 6 weeks of exposure. Abscissa: group codes. SS: somatosensory area; VIS: visual area; AUD: auditory area. *$p < 0.05$ vs. untreated control group (Con); #$p < 0.05$ vs. vehicle control group (W).

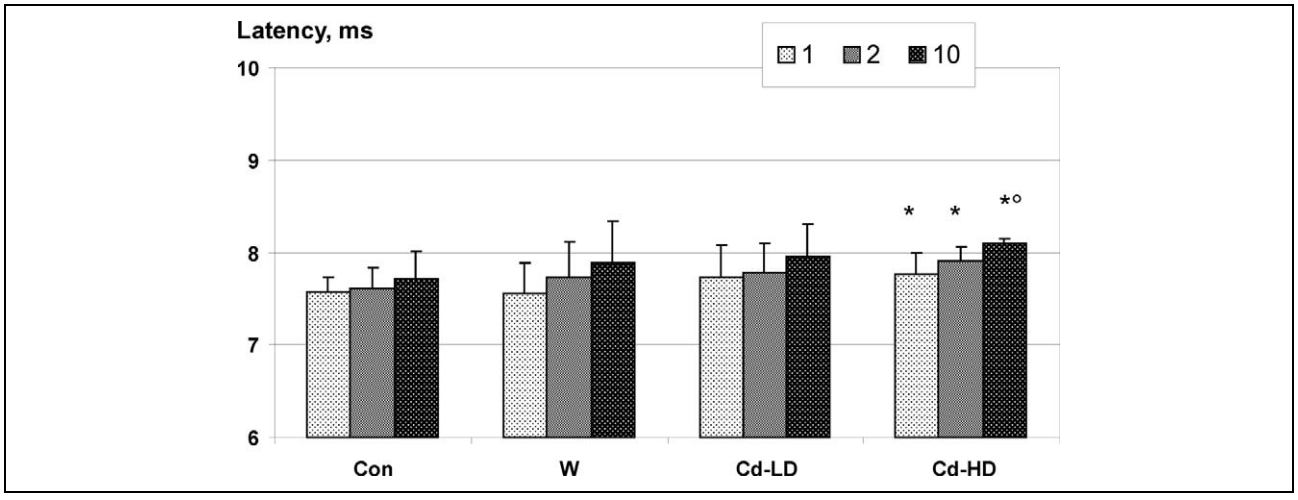

Figure 4. Latency of the somatosensory-evoked potential after 6 weeks of exposure. Abscissa: group codes. Mean $\pm$ SD, $n = 10$. Insert: stimulation frequency. *$p < 0.05$ vs. untreated control group (Con); °$p < 0.05$ vs. 1 Hz stimulation within the same treatment group.

SOD activity was affected neither in the brain nor in the lung and liver (in which organs Cd deposition was detected). The level of GSH was, on the contrary, dose-dependently influenced and the HD vs. W difference was significant in the lungs and the brain.

Electrophysiological effects

The alterations in the spontaneous cortical activity (ECoG) were alike in all three cortical areas. There was a dose- and time-dependent shift from slower to faster waves which became significant in the HD group after 6 weeks of exposure (Figure 3). After 3 weeks only, no significant changes were seen.

The SS EP showed significant increase in latency in the HD group vs. Con at each stimulation frequency (Figure 4). The slight dependence of the latency on the frequency of stimulation, seen in Con and W, was more expressed in the treated groups, up to the significant difference between the latencies obtained with 1 and 10 Hz stimulation in the HD group. The latency of the VIS EP, and to a lesser extent of the AUD EP, also increased in the treated groups vs. Con (Figure 5).

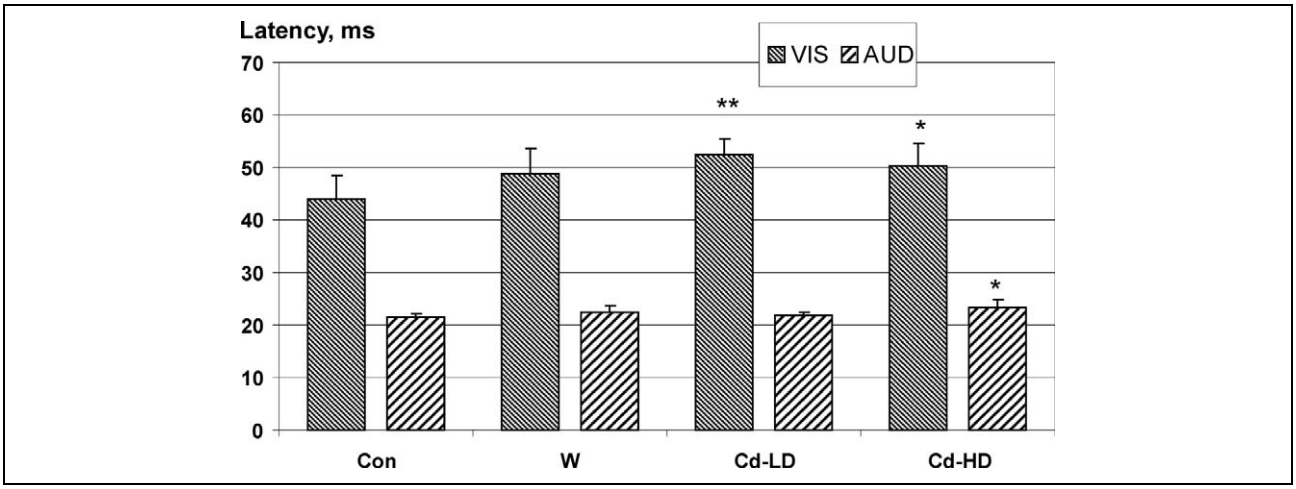

Figure 5. Latency of the visual- and auditory (see insert)-evoked potential after 6 weeks of exposure. Abscissa: group codes. Mean $\pm$ SD, $n = 10$. *$p < 0.05$, **$p < 0.01$ vs. untreated control group (Con).

Discussion

The metal levels, as well as the electrophysiological and biochemical changes, indicated that Cd instilled into the rats' trachea in the form of CdO_2 NPs was in fact absorbed from the airways and unfolded its toxicity. The time trend of body weights and the increase in alterations after 6 vs. 3 weeks suggested a gradual buildup of Cd. Absorption of the metal via the airways has been described repeatedly. Takenaka et al. (2004) detected Cd in the blood, liver and kidney of rats after inhalation CdO_2 NPs—in an experiment with much shorter duration, and faster tissue sampling after exposure than it was in our work, which may explain the main difference viz. the absence of detectable Cd level in the blood of treated rats. In bulk, CdO_2 is hardly water soluble, but its absorption from the lung is rather good (Oberdörster, 1979). No detectable blood Cd level in our work probably meant that the absorbed amount was promptly sequestered in the liver where it was detected in fact. In the study by Dill et al. (1994), the blood Cd level after ca. 3 months inhalation exposure by CdO particles of about 1 μm diameter was 10^3 times lower than in the kidney, the other organ known to accumulate Cd in the organism. The absence of noteworthy amounts of Cd in the blood was, logically, the reason for not detecting Cd in our brain samples. Beyond that, Cd is known to have low permeability across the blood–brain barrier (ATSDR, 2008) and no transneuronal movement from the periphery to the brain (Tjälve et al., 1996).

In spite of the latter, neurotoxicity of Cd in humans has been reported repeatedly (see Introduction section). In exposed workers, elevated urine Cd level was associated with reduced visuomotor performance and difficulties of concentration and stance (Viaene et al., 2000) and with peripheral neuropathy (Viaene et al., 1999). In children, the exposure indicator was hair Cd, and the outcomes, cortical EPs (Thatcher et al., 1984) and behavior (Marlowe et al., 1985). The significant change in the latency of cortical EPs in our work, without detectable Cd deposition in the CNS, was probably due to secondary effects. Along the peripheral part of the afferent pathways, Cd^{2+} ions, if present, could interfere with ion channels (primarily Ca channels: Viarengo and Nicotera, 1991), and with mitochondrial energy production (López et al., 2006), resulting in delayed arrival of the excitation to the subcortical and cortical centers, and so to lengthened cortical EP latency. Cd-induced liver damage could affect the substrate supply for synthesis of monoamine transmitters (Yourdaydin et al., 1990), the abnormal activity of which is known to alter cortical electrical activity (Sebban et al., 1999). The ECoG shift in the present study was similar to that found with oral application of dissolved Cd for 12 weeks (Papp et al., 2003).

The oxidative stress inducing effect of Cd is indirect; due, among others, to depletion of GSH (Valko et al., 2005) and to mitochondrial damage (López et al., 2006). The GSH level in the lungs of treated rats (the organ having the highest Cd load) was in fact significantly reduced. The increase in the brain samples was possibly of compensatory nature. Others, e.g.

Tandon et al. (2003), found depletion of GSH in Cd-exposed rats but in that experiment Cd was applied per os in dissolved form, in higher dose (1.5 mg/kg b.w.) and was detected in brain samples after 5 days treatment.

In spite of some disagreements with others' findings, it can be stated that the data, presented above, emphasize the role of the nano-sized fraction of Cd-containing industrial fumes in the causation of nervous system damage, and show that it is possible to model the human neurotoxic damage caused by inhalational Cd exposure in rats.

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Conflict of interest

The authors declared no conflicts of interest.

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References

Agar A, Yargicoglu P, Edremitlioglu M, Kara C, and Oguz Y (1999) The effect of cadmium on somatosensory evoked potentials (SEP) and conduction velocity in alloxane-induced diabetic rats: relation to lipid peroxidation. *Journal of Basic and Clinical Physiology and Pharmacology* 10(1): 41–56.

Antonini JM, Lewis AB, Roberts JR, and Whaley DA (2003) Pulmonary effects of welding fumes: review of worker and experimental animal studies. *American Journal of Industrial Medicine* 43(4): 350–360.

ATSDR (2008) *Toxicological Profile for Cadmium, Draft for Public Comment (Update)*. GA, USA: Public Health Service, US Department of Health and Human Services.

Bar-Sela S, Reingold S, and Richter ED (2001) Amyotrophic lateral sclerosis in a battery-factory worker exposed to cadmium. *International Journal of Occupational and Environmental Health* 7(2): 109–112.

Calderon-Garciduenas L, Azzarelli B, Acuna H, et al. (2002) Air pollution and brain damage. *Toxicological Pathology* 3(3): 373–389.

Chaney RL, Reeves PG, Ryan JA, Simmons RW, Welch RM, and Angle JS (2004) An improved understanding of soil Cd risk to humans and low cost methods to phytoextract Cd from contaminated soils to prevent soil Cd risks. *Biometals* 17(5): 549–553.

Conroy LM, Lindsay RM, and Sullivan PM (1995) Lead, chromium and cadmium emission factors during abrasive blasting operations by bridge painters. *American Industrial Hygienic Association Journal* 56(3): 266–271.

Dill JA, Greenspan B, Mellinger KH, Roycroft JH, and Dunnick J (1994) Disposition of inhaled cadmium oxide aerosol in the rat. *Inhalation Toxicology* 6: 379–393.

Fern R, Black JA, Ransom BR, and Waxman SG (1996) Cd(2+)-induced injury in CNS white matter. *Journal of Neurophysiology* 76(5): 3264–3273.

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, Oxford: Pergamon Press.

Institóris L, Papp A, Siroki O, Banerjee BD, and Dési I (2002) Immuno- and neurotoxicological investigation of combined subacute exposure with the carbamate pesticide propoxur and cadmium in rats. *Toxicology* 178(2): 161–173.

Li N, Sioutas C, Cho A, et al. (2003) Ultrafine particulate pollutants induce oxidative stress and mitochondrial damage. *Environmental Health Perspectives* 111(4): 455–460.

López E, Arce C, Oset-Gasque MJ, Cañadas S, and González MP (2006) Cadmium induces reactive oxygen species generation and lipid peroxidation in cortical neurons in culture. *Free Radicals in Biology and Medicine* 40(6): 940–951.

Lowry OH, Rosebrough EA, and Farr AL (1951) Protein measurement with Folin phenol reagent. *Journal of Biological Chemistry* 193(1): 265–275.

Lukács A, Szabó A (2007) Functional neurotoxic effects of heavy metal combinations given to rats during pre- and postnatal development. *Central European Journal of Occupational and Environmental Medicine* 13: 299–304.

Lundborg M, Eklund A, Lind DB, and Camner P (1985) Dissolution of metals by human and rabbit alveolar macrophages. *British Journal of Industrial Medicine* 42(9): 642–645.

Marlowe M, Cossairt A, Moon C, et al. (1985) Main and interaction effects of metallic toxins on classroom behavior. *Journal of Abnormal Child Psychology* 13(2): 185–198.

Matkovics B, László A, and Szabó L (1982) A comparative study of superoxide dismutase, catalase and lipid peroxidation in red blood cells from muscular dystrophy patients and normal controls. *Clinica Chimica Acta* 118(2-3): 289–292.

Misra HP, Fridovich I (1972) A role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. *Journal of Biological Chemistry* 247(10): 3170–3175.

Oberdörster G (1979) The clearance of cadmium aerosols after inhalation exposure. *America Industrial Hygienic Association Journal* 40(6): 443–450.

Oberdörster G, Oberdörster E, and Oberdörster J (2005) Nanotoxicology: An emerging discipline evolving from studies of ultrafine particles. *Environmental Health Perspectives* 113(7): 823–839.

O'Callaghan JP, Miller D (1986) Diethyldithiocarbamate increases distribution of cadmium to brain but prevents cadmium-induced neurotoxicity. *Brain Research* 370(2): 354–358.

Papp A, Nagymajtényi L, and Dési I (2003) A study on electrophysiological effects of subchronic cadmium treatment in rats. *Environmental Toxicology and Pharmacology* 13(3): 181–186.

Rzigalinski BA, Strobl JS (2009) Cadmium-containing nanoparticles: Perspectives on pharmacology and toxicology of quantum dots. *Toxicology and Applied Pharmacology* 238(3): 280–288.

Sebban C, Zhang XQ, Tesolin-Decros B, Millan MJ, and Spedding M (1999) Changes in EEG spectral power in the prefrontal cortex of conscious rats elicited by drugs interacting with dopaminergic and noradrenergic transmission. *British Journal of Pharmacology* 128(5): 1045–1054.

Sedlak J, Lindsay RH (1968) Estimation of total protein-bound and nonprotein sulfhydryl groups in tissue with Ellman's reagent. *Analytical Biochemistry* 25(1): 192–205.

Strohl KP, Thomas AJ, St Jean P, Schlenker EH, Koletsky RJ, and Schork NJ (1997) Ventilation and metabolism among rat strains. *Journal of Applied Physiology* 82(1): 317–323.

Takenaka DS, Karg E, Kreyling WG, et al. (2004) Fate and toxic effects of inhaled ultrafine cadmium oxide particles in the rat lung. *Inhalation Toxicology* 16(suppl 1): 83–92.

Tandon SK, Singh S, Prasad S, et al. (2003) Reversal of cadmium induced oxidative stress by chelating agent, antioxidant or their combination in rat. *Toxicology Letters* 145(3): 211–217.

Thatcher RW, McAlaster R, and Lester ML (1984) Evoked potentials related to hair cadmium and lead in children. *Annals of the New York Academy of Science* 425: 384–390.

Tjälve H, Henriksson J, Tallkvist J, Larsson BS, and Lindquist NG (1996) Uptake of manganese and cadmium from the nasal mucosa into the central nervous system via olfactory pathways in rats. *Pharmacology and Toxicology* 79(6): 347–356.

Valko M, Morris H, and Cronin MTD (2005) Metals, toxicity and oxidative stress. *Current Medicinal Chemistry* 12(10): 1161–1208.

Viaene MK, Masschelein R, Leenders J, De Groof M, Swerts LJ, and Roels HA (2000) Neurobehavioural effects of occupational exposure to cadmium: A cross sectional epidemiological study. *Occupational and Environmental Medicine* 57(1): 19–27.

Viaene MK, Roels HA, Leenders J, et al. (1999) Cadmium. A possible etiological factor in peripheral polyneuropathy. *Neurotoxicology* 20(1): 7–16.

Viarengo AS, Nicotera P (1991) Possible role of Ca2+ in the heavy metal cytotoxicity. *Comparative Biochemistry and Physiology C* 100(1-2): 81–84.

Vitayavirasuk B, Junhom S, and Tantisaeranee P (2005) Exposure to lead, cadmium and chromium among spray painters in automobile body repair shops. *Journal of Occupational Health* 47(6): 516–522.

Yourdaydin C, Hörtnagl H, Steindl P, et al. (1990) Increased serotoninergic and noradrenergic activity in hepatic encephalopathy in rats with thio-acetamide-induced acute liver failure. *Hepatology* 12(4 pt 1): 695–700.