BEHAVIOURAL AND ELECTROPHYSIOLOGICAL CHANGES CAUSED BY SUBCHRONIC LEAD EXPOSURE IN RATS

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ABSTRACT: Male Wistar rats were treated *per os* through gavage with 80.0, 160.0 and 320.0 mg/kg lead (in form of lead acetate) for 4, 8 and 12 weeks. The changes of certain behavioural features (exploratory horizontal and vertical ambulation scores, grooming in an open field situation) as well as of some parameters of the spontaneous (electrocorticogram, ECoG) and evoked electrical activity in the somatosensory, visual and auditory cortical foci, and also the conduction velocity and refractory periods of peripheral nerve were analyzed. Treated animals showed a significant time-dependent reduction of horizontal exploratory activity and a dose- and time-dependent reduction of grooming behaviour. The electrophysiological data showed that each investigated parameter changed in a dose- and time-dependent manner: increased mean ECoG frequencies accompanied by decreased mean amplitudes, lengthened latencies and durations of evoked potentials, decreased conduction velocity and increased refractory periods were observed. By the end of the 12-week treatment period, changes were significant in the highest, or in the two higher dose groups. The results suggest that the subchronic, low-level exposure by lead has, after a 4 to 12-week treatment, clear effects on behaviour as well as on spontaneous and evoked electrical activity of the rats' nervous system.

KEY WORDS: Lead acetate, open field behaviour, electrocorticogram, cortical evoked potentials, conduction velocity, refractory period, rat

INTRODUCTION

Lead is one of the most important pollutants being present in the whole environment. Not only occupationally exposed persons, but practically the entire population can be continuously exposed to it, especially in highly industrialized areas (WHO, 1989, Trotter, 1990). Both acute and chronic lead exposure can cause behavioural and neurological disorders in animals as well as in humans. Impairment of learning processes with involvement of dopaminergic, cholinergic, and glutaminergic neurotransmitter systems, decreased attention, response speed, manual dexterity, perceptual-motor speed and visual perception have been reported (Cory-Slechta, 1995, Tang et al., 1995). Beside these effects high order central activity, related functional expressions like changes of EEG, of motor and sensory evoked potentials, and of nerve conduction velocity, etc. have also been found, together

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with structural disorders like encephalopathy, nerve demyelination, etc. (Bordo et al., 1982, Seppalainen, 1984, Lille et al., 1988, 1994). Human epidemiological investigations showed that these alterations can appear in larger populations, especially in children who have higher susceptibility to lead exposure (Otto et al., 1985, Needleman et al, 1990, Davis, 1990, Winneke et al. 1990, 1994, Winneke 1995).

The aim of this study was to investigate the simultaneously occurring behavioural and neurophysiological effects of a subchronic, relatively low-level lead exposure, and to define the measured functional changes as sensitive biomarkers of early alterations of the brain.

METHODS

In our experiments, 10-week old male Wistar rats (10 per group, altogether 120 rats) were used. The animals were kept under conventional standard housing conditions (temperature 20–22°C, humidity 60–70 %, 12-hours light-dark cycle) and fed with standard rodent chow (Research Institute of Laboratory Animals, Gödöllő, Hungary). Food and water were available *ad libitum*.

The rats were treated, in a 5 days per week schedule, through gavage with 80.0, 160.0, and 320.0 mg/kg b.w. lead as lead acetate ($C_4H_6O_4Pb \cdot 3H_2O$; mol.wt 379.33; purity: 99.5 %, supplied by REANAL, Hungary), dissolved in distilled water and administered in 1 ml/kg b.w. volume for 4, 8 or 12 weeks. Control rats received the same volume of distilled water. The animals were observed daily for symptoms of intoxication (e.g. salivation, shivering, muscle tonus, etc.), body weight was registered weekly.

BEHAVIOURAL INVESTIGATION

For the investigation of motility and exploration an automatized open field (OF) was used (ACTIFRAME, Gerb Electronic, Berlin, Germany). By means of infrared sensors (IR) located at two different levels, horizontal as well as vertical exploratory activity or motility was electronically registered. The size of the OF was 40×40×40 cm, the distance between the sensory elements was 1.11 cm. Sensors for vertical movements (rearing) were located 10 cm above the floor. The sampling rate of IR signals was organized by an intelligent interface and the signals were stored in digital form by a PC. Every intermission of an infrared sensor resulted in an electric impulse registered by the interface as a count used for further computational estimation of the behavioural changes exhibited by the individual in the open field box. Automatic data collection for every animal occurred during a 10-minute session between 8:00 a.m. and 2:00 p.m. in a sound proofed room following habituation of the animals to the laboratory situation. Illumination of the floor of the OF was about 10±2 lx, background white noise (about 40 dB) was provided by a cooler fan. Computing of the behavioural data was done by a special PC program (ARNO, Dr. J. Wolffgramm, Dept. Psychopharmacology, Free University Berlin, Germany).

ELECTROPHYSIOLOGICAL INVESTIGATION

The electrocorticograms (ECoGs) and sensory evoked potentials were recorded 1 to 2 days after the behavioural investigations. The rats were anaesthetized with urethane (1000.0 mg/kg i.p.; Bowman and Rand, 1980) and placed in a stereotaxic frame. The skull was opened and silver electrodes were placed onto the left primary somatosensory, visual and auditory cortical areas (Par1, Oc1B, Te1 areas, after Zilles, 1982). 30 minutes later ECoGs from each area were simultaneously recorded for 5 minutes. The analyzed ECoG parameters were: mean amplitude, mean frequency, activity of the frequency bands (power spectrum) and the "CoG index" the ratio of the slow (delta+theta) and fast ($beta_1$+$beta_2$) bands expressing the distribution of ECoG waves in a sensitive marker (Dési, 1983). The recorded ECoGs were also analyzed by a Waterfall program (Cambridge Electronic Ltd., UK).

The cortical evoked potentials were recorded by the same electrodes. Somatosensory (electric shock) stimulation was carried out by a pair of electrodes pricked into the whiskery part of the skin. The parameters of the rectangular stimuli were: 1 Hz, 3-4 V, 0.2 msec. Visual stimulation was performed by flashes (1 Hz, 60 lux) directed to the contralateral eye via an optical fiber. Acoustic stimulation was performed by clicks (1 Hz, 40 dB), produced by a small earphone put into the contralateral ear of the rat. The recorded evoked potentials (n=50) were averaged by a computer program (Cambridge Electronics Ltd., UK). Latency and duration of the averaged evoked potentials were measured off line, on the screen.

The conduction velocity of the peripheral nerve (tail nerve) was measured by the modified Miyoshi method; instead of 37 °C, recording was done at 21–22 °C (Miyoshi and Goto, 1973). The relative and absolute refractory periods were determined and studied according to Anda et al. (1984).

Following the recording, the rats were overdosed with urethane and certain internal organs (brain, liver, heart, lung, kidneys, thymus, and adrenal glands) were weighed; the relative organ weights were calculated as related to the brain weight.

The lead concentration in the different organs (brain, etc.) will be measured in the near future.

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

STATISTICS

The distribution of data was checked for normality by Kolmogorov-Smirnov test. Behavioural effects were analyzed by uni- and/or multivariate ANOVA following square root transformation of the data by a 3 x 4 design for equal cell content or by nonparametric Kruskal-Wallis ANOVA. Electrophysiological data were analyzed by a one-way ANOVA with subsequent post hoc analysis of group differences by the use of LSD test. A probability level of $p<0.05$ was accepted as significant.

RESULTS

General toxicology

A reduction of body weight was observed only in the 12-week group ($p<0.0284$; *Table 1*). The relative weight of the kidneys showed a dose-dependent increase ($p<0.0173$). The relative weight of the thymus decreased dose- ($p<0.0260$) and time-dependently ($p<0.001$). Weights of other investigated organs (liver, heart, lung, spleen, adrenals) in the treated animals did not significantly differ from that in the controls. No clinical signs of lead intoxication were observed during the treatment of up to 12 weeks in any group.

TABLE 1. Relative organ weights

Treatment	Dose (mg/kg)	Relative organ weights±						
		liver	lung	heart	kidney	spleen	thymus	adr.gland
4 weeks	Control	7.51	1.06	0.60	1.33	0.34	0.24	0.03
		±0.41	±0.04	±0.03	±0.05	±0.04	±0.03	±0.001
	80.0	7.45	1.04	0.62	1.33	0.33	0.22	0.03
		±0.35	±0.03	±0.04	±0.05	±0.03	±0.03	±0.001
	160.0	7.44	1.00	0.61	1.34	0.34	0.21	0.03
		±0.33	±0.05	±0.03	±0.06	±0.03	±0.03	±0.002
	320.0	7.44	1.04	0.60	1.37	0.31	0.22	0.02
		±0.26	±0.04	±0.03	±0.07	±0.03	±0.04	±0.002
8 weeks	Control	7.50	1.05	0.62	1.35	0.31	0.24	0.03
		±0.36	±0.04	±0.04	±0.07	±0.04	±0.03	±0.003
	80.0	7.42	1.03	0.63	1.37	0.32	0.23	0.03
		±0.33	±0.02	±0.03	±0.06	±0.03	±0.03	±0.002
	160.0	7.44	1.04	0.61	1.40	0.32	0.22	0.03
		±0.32	±0.05	±0.04	±0.04	±0.02	±0.03	±0.001
	320.0	7.41	1.04	0.61	1.43	0.32	0.22	0.03
		±0.26	±0.04	±0.03	±0.06	±0.04	±0.03	±0.003
12 weeks	Control	7.54	1.06	0.61	1.33	0.33	0.22	0.03
		±0.33	±0.07	±0.03	±0.05	±0.03	±0.03	±0.002
	80.0	7.45	1.04	0.60	1.40	0.32	0.21	0.03
		±0.31	±0.03	±0.04	±0.06	±0.03	±0.03	±0.002
	160.0	7.43	1.01	0.60	1.42*	0.31	0.20	0.03
		±0.43	±0.05	±0.03	±0.04	±0.04	±0.04	±0.001
	320.0	7.40	1.00	0.59	1.53**	0.34	0.18*	0.03
		±0.39	±0.05	±0.05	±0.06	±0.03	±0.02	±0.003

*p<0.05, **p<0.01 (compared to the 12-week control)

± Relative organ weight: absolute organ weight (g) / absolute brain weight (g), (Mean ± SD)

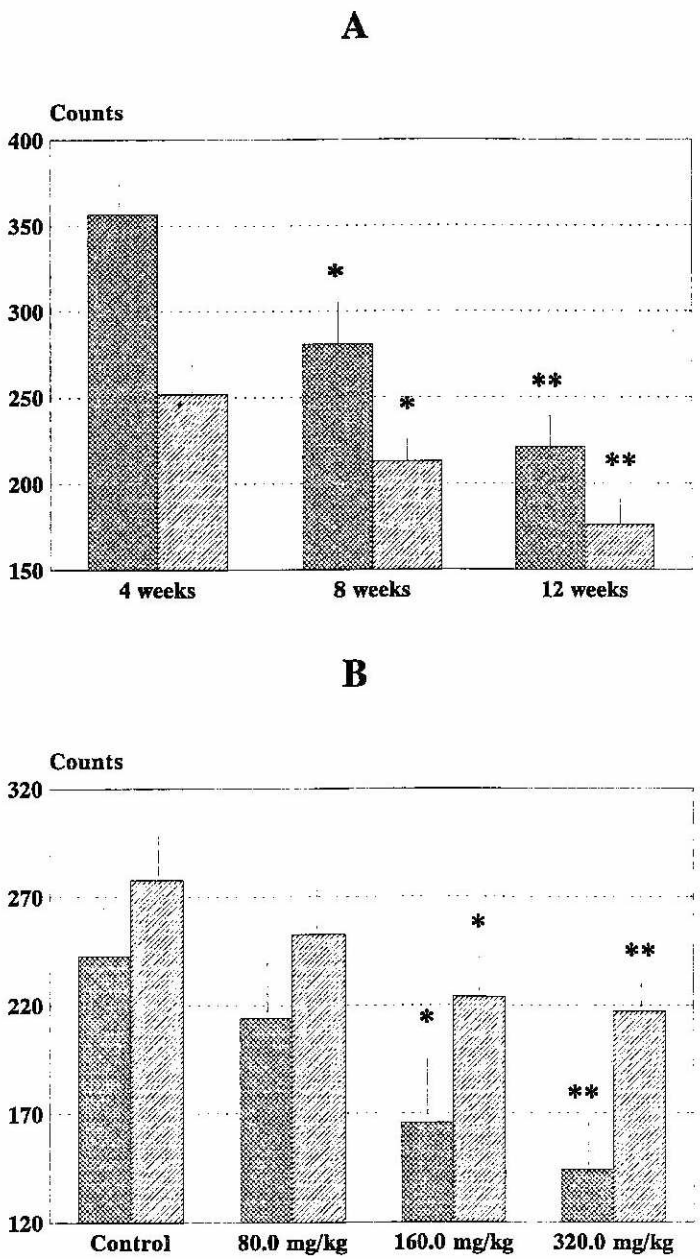

*Figure 1. Dose-dependent decrease at week 12 of total horizontal ambulation activity (squared columns) (A) and of grooming activity (dashed colunms) as well as treatment time dependent decrease of horizontal ambulation (squared colums) and of grooming activity (B); Ordinates: counts of fotobeams intermissions by the animal expressed as counts; abscissa – duration of treatment (A); doses of lead (B); error bar: SD; significance: *p<0.05, **p<0.01.*

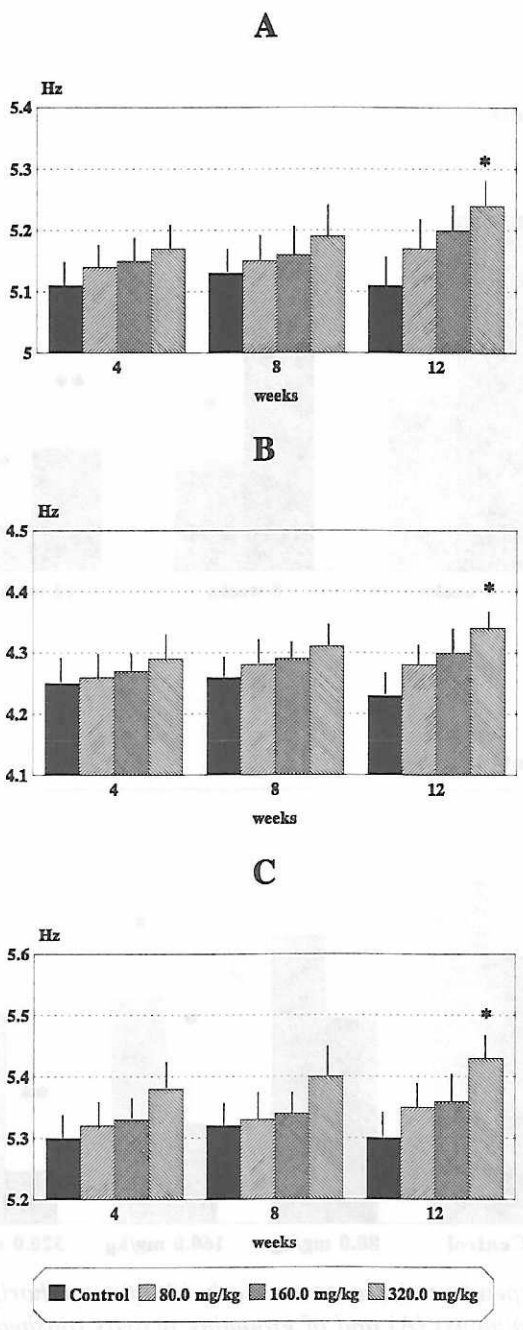

*Figure 2. Changes of the average ECoG frequency in the three cortical foci (A – somatosensory; B – visual; C – auditory). Ordinate: ECoG frequency; abscissa: weeks of lead treatment, error bar: SD; insert: control and treatment doses; significance: * $p<0.05$.*

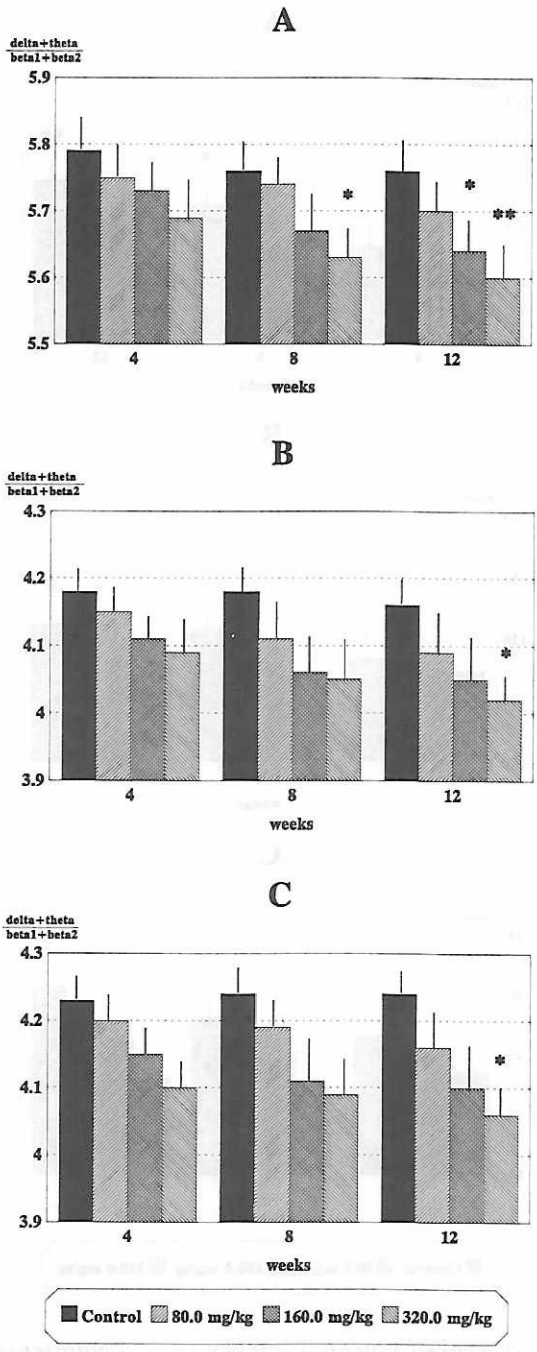

*Figure 3. Changes of the ECoG indices. (A – somatosensory; B – visual; C – auditory). Ordinate: index values; abscissa: weeks of lead treatment, error bar: SD; insert: control and treatment doses; significance: *p<0.05, **p<0.01.*

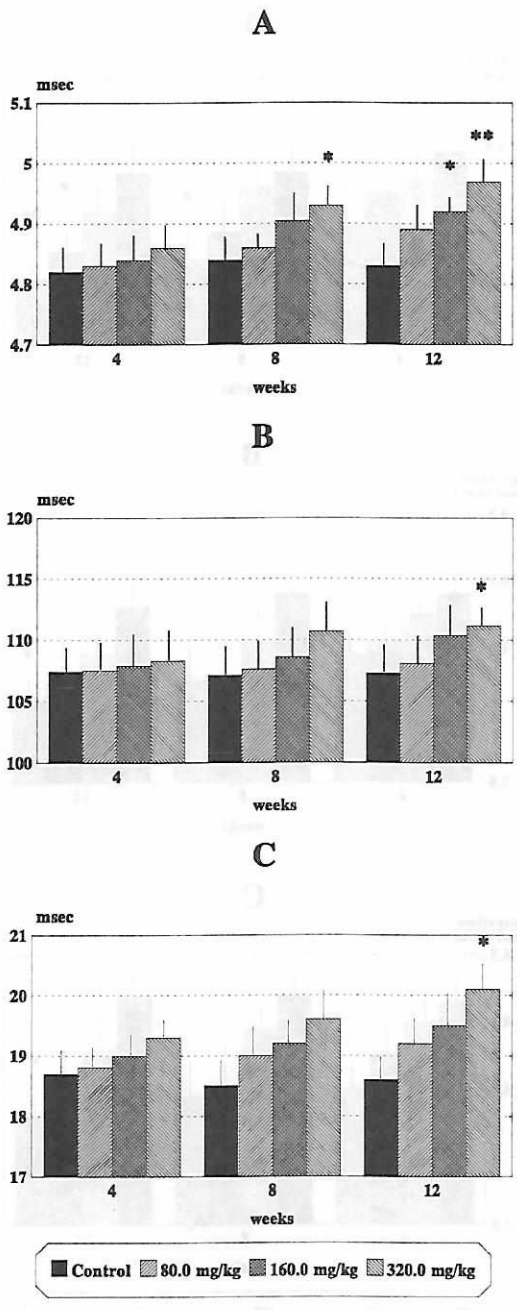

*Figure 4. Changes of evoked potential latency (A – somatosensory N1 wave; B – visual N2 wave; C – auditory N1 wave). Ordinate: latency; abscissa: weeks of lead treatment, error bar: SD; insert: control and treatment doses; significance: *p<0.05, **p<0.01.*

Behavioural findings

There was a clear time-dependent decrease of horizontal ambulation (i.e. a hypoactivity) ($p<0.0002$) and a dose- ($p<0.0002$) as well as time-dependent ($p<0.0001$;) decrease of grooming activity (*Fig. 1A, B*). Resting in the corner of the OF decreased with the treatment time ($p<0.0063$) and with the dose ($p<0.0382$). Interestingly, vertical exploratory activity (rearing), running speed or visits to the centre of the OF did not show any significant change on lead exposure.

Electrophysiological findings

The average amplitude of the somatosensory, visual and auditory ECoGs underwent a dose- and time-dependent decrease but this was not significant even in the group treated with the highest dose ($p>0.05$, *NS in all cases*).

The mean frequency of the somatosensory ECoG also increased in a dose- and time-dependent manner, with significant differences after a 12-week treatment with the highest dose ($p<0.0298$; *Fig. 2A*). In the visual and auditory ECoGs the average frequency did not differ significantly from the controls either except for the highest dose group after 12-weeks of treatment (visual – $p<0.0455$; auditory – $p<0.0476$; *Fig. 2B, C*).

Considering the so-called ECoG index, treatment with the highest dose of lead resulted in a significantly lower somatosensory ECoG index after 8 weeks ($p<0.0291$) and after 12-week ($p<0.0074$); the medium dose resulted in similar decrease only after a 12-week treatment ($p<0.026$; *Fig. 3A*). Index of the visual ECoG decreased significantly in the group which received the highest dose for 12-week ($p<0.0256$; *Fig. 3B*). In the auditory focus, the index was also lowered by all three doses but the change was significant only with the highest dose by the end of the 12 weeks treatment ($p<0.0388$; *Fig. 3C*).

The alterations of cortical electrical activity demonstrated by the ECoG indices were also seen in the simple power spectra of the recordings from the three cortical sensory centres but this kind of analysis did not show marked changes: the slow wave part (delta and theta) was slightly decreased and the fast wave part ($beta_1$ and $beta_2$) increased a bit in all dose groups at all treatment lengths ($p>0.05$, *NS in all cases*).

Comparing the identical parameters of the ECoGs recorded from the three cortical areas on the basis of dose or of treatment duration, no significant differences were found ($p>0.05$, *NS in all cases*).

The durations and latencies of the cortical evoked potentials following lead treatment increased in a dose- and time-dependent manner.

In the somatosensory evoked potentials the latency of the waves increased in all dose and treatment duration groups. The differences of wave N1 became significant at the two higher doses by the end of the 12-week period ($p<0.0203$) and, in case of the highest dose, also after 8 weeks ($p<0.0219$; *Fig. 4A*). The intervals between individual peaks were also lengthened but these differences were not significant ($p>0.05$, *NS in all cases*).

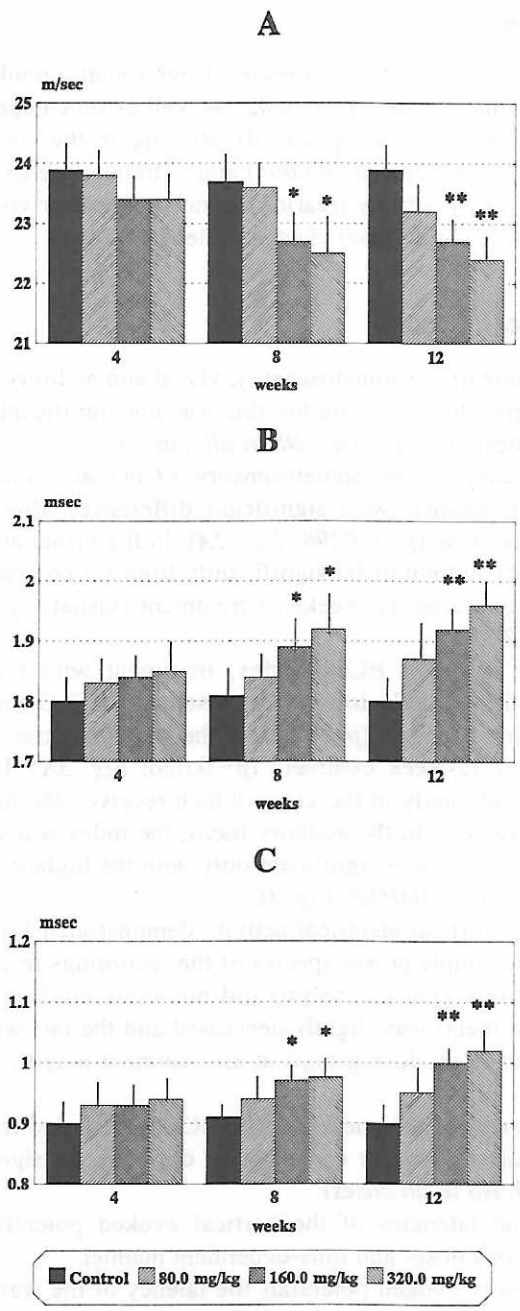

*Figure 5. Changes of the impulse conduction in the peripheral nerve. Ordinate: A – conduction velocity; B – relative refractory period; C – absolute refractory period; abscissa: weeks of lead treatment, error bar: SD; insert: control and treatment doses; significance: *p<0.05, **p<0.01*

The latencies of the waves of the visual evoked potentials all increased. In case of N2 wave the changes became more and more expressed in each treated group by the end of the 12-week treatment, but a significant prolongation was seen only at the top dose ($p<0.0243$; *Fig. 3B*). Like in case of somatosensory evoked potentials, longer durations between the peaks were also observed but were not significant ($p>0.05$, *NS in all cases*).

The latencies of the waves in the auditory evoked potential were all longer than the control values. In case of the highest dose, the difference of N1 wave was significant after 12 weeks ($p<0.039$; *Fig. 4C*). Lengthened, but not significantly longer, interpeak durations were also observed ($p>0.05$, *NS for all doses and treatment intervals*).

The conduction velocity of the peripheral nerve (*Fig. 5A*) was significantly lowered by the two higher lead doses when administered for 8 or 12 weeks (8 weeks – $p<0.0321$; and $p<0.0276$; 12 weeks – $p<0.0067$; and $p<0.00436$). The relative and absolute refractory periods in these groups became also significantly longer (relative: 8 weeks: $p<0.0372$, and $p<0.0291$; 12 weeks: $p<0.0072$, and $p<0.0192$; absolute: 8 weeks: $p<0.0321$, and $p<0.0259$; 12 weeks: $p<0.0023$, and $p<0.0198$; *Fig. 5B, C*)

DISCUSSION

The experimental results of the neurobehavioural testing demonstrate several clear measurable changes of behavioural outcomes of the subchronic lead exposure. The main result was the demonstration of hypoactivity considering horizontal exploration in the OF and grooming activity. Lead at low doses affect the neurotransmitter system of rats with significant changes mainly on dopaminergic and serotonergic levels. The most sensitive part of the brain (beside any others) is the nucleus accumbens (Kala and Jadha, 1995). Especially monkeys exposed to lead during pregnancy showed a negative shifting of the acquisition process in learning experiments in their adult lives (Newland et al., 1994). Similar behavioural deficits are also seen in children when exposed to lead exhibiting later in their life a slower sensorimotor and visual-motor development and having long-lasting deficits in cognitive performance (Goyer, 1996). The hypoactivity seen in our experiments may result at large extent to changes in the dopamine neurotransmitter system (Svensson et al., 1994).

Electrophysiological observations show that all of the analyzed ECoG and evoked potential parameters underwent dose- and time-dependent changes. Alterations of the spontaneous electrical activity caused by lead have already been published in the literature. When rat dams were treated with 0.3 % lead acetate in drinking water during the 16^{th}–23^{rd} days of gestation or 1^{st}–8^{th} and 9^{th}–16^{th} days of nursing, depression of the 6–7 Hz part of the hippocampal EEG, but no considerable change in the cortical EEG was found in the offspring at the age of 42–45 days (Burdette and Goldstein, 1986). Significant reduction of the spectral power was observed during slow wave sleep on the motor cortex and hippocampal EEG of the rats treated with 400 μg/kg lead acetate through gavage for 60 days (Kumar and

Desiraju, 1992). In wakeful state, only activity of the theta and alpha bands was diminished.

It has been reported that several pathological changes are associated to a certain concentration of the accumulated lead in the brain (Kostial et al., 1978, Cory-Slechta et al., 1985, Cory-Slechta and Pokora, 1991, Collins et al., 1992); in our experiments the changes of the spontaneous activity were clearly proportional to the internal lead doses, as the alterations of ECoGs recorded from different cortical areas were dependent on treatment dose and time.

Similar to our findings, both occupational lead exposure of workers (Sborgia et al., 1983, Araki et al., 1987, Murata et al., 1987) and environmental exposure of children (Lilienthal et al., 1990) resulted in changes of the cortical evoked potentials (increased latencies, etc.). Likewise in monkeys, lead exposure manifested in a lengthening of auditory evoked potentials (Lilienthal and Winneke, 1996). In this context, results of the animal experiments show similar tendencies as human epidemiological data do.

As all latencies and durations of the sensory evoked potentials changed in our experiments, it seems obvious that lead affected each of the investigated sensory pathways. The dose- and time-dependency unequivocally indicates that these effects are to be ascribed to lead exposure. The significant changes of the peripheral function studied showed the neurotoxic effect on the peripheral nerves.

The above mentioned changes of behaviour and neurophysiology might be explained by the effect of lead on certain neurotransmitter (e.g. cholinergic, dopaminergic, GABAergic) systems (Bressler and Goldstein, 1991). Low doses of lead increase the release of acetylcholine from presynaptic nerve endings (Suszkiv et al., 1984, Shao and Suszkiv, 1991) and, as a consequence, cause excitation of the spontaneous activity of the brain being expressed in a higher average EEG frequency (Bringman, 1995) and possibly accompanied by disturbances of orientation thereby leading to hypoactivity in the OF. Several studies have proven that lead induces changes in the dopamine metabolism and in dopaminergic receptor sensitivity thus altering brain functional processes (Lasley, 1992, Struzynska and Rafalowska, 1994). This process may have its final expression in the decreased grooming activity, too. The ECoG changes in our experiments (increased mean frequency, decreased index) being signs of excitation of the gross electrophysiological activity, could also correlate with these neurotransmitter mechanisms. Beside those, however, other processes may also play a role in the altered function of the sensory pathways. It has been described that lead acts on the voltage-dependent Ca^{2+} and Ca^{2+}-activated K^{+} channels thereby affecting the ion transport through the neuron membrane (Audesirk and Audesirk, 1991, Reuveney and Narahashi, 1991, Leinders and Vijenberg, 1992) slowing down the conduction of the action potential and, consequently, increasing the latency of the evoked potentials as well as the relative and absolute refractory periods.

It is well known that the results of animal experiments cannot in general be directly transferred to man. In the Central and Eastern European countries, the level of chronic lead exposure, originating from vehicle emission and industry, is relatively high thus the risk of behavioural and functional neurotoxic effects cannot

be neglected. Monitoring only the biochemical changes in body fluids caused by low level lead exposure (blood lead level, DALA in urine, etc.) is, however, not appropriate if one has to detect the subchronic effect of such lead exposures which may cause only minimal observable behavioural and/or neurophysiological changes.

The implementation of sensitive but at the same time non-invasive functional screening methods, based on functional biomarkers such as behavioural investigations, EEG monitoring and measurements of cortical evoked potentials, and the decision making considering risk assessment are very important requirements in order to detect this type of early intoxication with lead and/or other heavy metals at population level.

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