International Journal of Environmental Health Research **8**, 121–135 (1998)

CARFAX

Consequences of lead exposure of rats during pregnancy, lactation, and postweaning. A combined behavioural and neurotoxicological study

LÁSZLÓ NAGYMAJTÉNYI, ILLÉS DÉSI, HORST SCHULZ and ANDRÁS PAPP

Department of Public Health, Albert Szent-Györgyi Medical University Dóm tér 10, 6720 Szeged and WHO Collaborating Centre for Chemical Safety, Szeged, Hungary

Behavioural and electrophysiological changes caused by inorganic lead treatment at different phases of the ontogenesis were investigated in Wistar rats. They were treated by gavage with 80.0, 160.0, and 320.0 mg/kg lead during the 5th–15th days of pregnancy, or the 5th–15th days of pregnancy + 4 weeks of lactation (females of P generation), or the 5th–15th days of pregnancy + 4 weeks of lactation (females of P generation) + 8 weeks after weaning (males of F1 generation). The behavioural (open field) and electrophysiological (electrocorticogram, cortical evoked potentials, etc.) parameters of F1 male rats in the above groups were investigated at the age of 12 weeks. Only rats exposed to lead during pregnancy exhibited a significant hyperactivity in the OF test expressed as a higher ambulation rate in the first minute in the OF and as longer running distances. Grooming was also increased. The spontaneous and evoked electrophysiological functions showed dose- and treatment-dependent changes (e.g. decreased mean amplitude and increased frequency of the electrocorticogram, lengthened latency and duration of the evoked potentials, etc.). The results indicate that the low-level pre- and postnatal inorganic lead exposure considerably affects both the behaviour and the function of the nervous system.

Keywords: conduction velocity; cortical evoked potential; electrocorticogram; lead; open field behaviour; pre- and postnatal exposure; rat; refractory period.

Introduction

Heavy metals including lead belong to the most harmful environmental pollutants. Beyond being present in different industrial workplaces lead contaminates our air, drinking water and food. As a consequence, the chronic low-level lead exposure, especially in the highly industrialized regions, represents an important risk for practically the whole population (WHO 1989, Trotter 1990).

Human studies and animal experiments have revealed that inorganic lead passes the placenta barrier and accumulates in foetal tissues showing correlation between the maternal exposure and lead concentrations in foetal organs (Schramel *et al.* 1988, Goyer 1990, Klein *et al.* 1994). Lead is also excreted into the human breast milk and animal milks (Ong *et al.* 1985, Sternovsky and Wessolowsky 1985, Schramel *et al.* 1988).

Correspondence to László Nagymajtényi.

0960-3123/98/020121-15 $7.00

Acute and chronic lead exposure affects the nervous system and causes different behavioural and neurological disorders, e.g. impairment in learning process, attention, response speed, manual dexterity, visual perception, nerve demyelination, encephalopathy, etc. (Cory-Slechta 1995, Tang *et al.* 1995). Functional alterations of the central and peripheral nervous system (e.g. changes of EEG, motor and sensory evoked potentials, nerve conduction velocity) were also found (Bordo *et al.* 1982, Seppalainen *et al.* 1983, Lille *et al.* 1988, 1994). Human epidemiological surveys showed a higher incidence of such disorders in lead-exposed populations, especially in children who have higher susceptibility to lead (Otto *et al.* 1985, Davies 1990, Needleman *et al.* 1990, Winneke *et al.* 1990, 1994, Winneke 1995).

In a previous experiment we have seen that the relatively low-level lead treatment of adult rats caused significant, dose- and time-dependent behavioural and functional changes (Nagymajtényi *et al.* 1997). Another experiment with three generations of rats also confirmed that administration of low-level lead had a dose- and generation-dependent influence on behavioural and electrophysiological processes (Dési *et al.* forthcoming).

In the present study, we undertook simultaneous behavioural and neurophysiological investigations concerning the effects in rats treated with relatively low lead doses in different schedules during pregnancy, lactation and after weaning.

Materials and methods

Animals and treatment

Wistar rats were obtained from the SPF breed of RILA (Research Institute of Laboratory Animals, Gödöllö, Hungary) and used as the parent generation. The animals (altogether 120 female and 60 male rats – P generation: 120 male rats – F1 generation) were kept at standard conditions (up to five rats/cage, 22 ± 2°C, 60 ± 10% humidity, 12 h light – dark cycle starting at 6.00 a.m. with light, rodent food and water were available *ad libitum*) for 3 weeks and were mated at the age of 11 weeks (two females and one male per cage).

Lead treatment was performed by gavage giving 80.0, 160.0 and 320.0 mg/kg b.w. lead in form of lead acetate ($C_4H_6O_4Pb.3H_2O$; mol.wt.: 379.33; purity: 99.5%, supported by REANAL, Hungary), dissolved in distilled water and administered in 1 ml/kg b.w. volume. The control rats received the same value of distilled water.

Treatment schedules. Pregnancy (P) variation: the females of the parent generation were daily treated from 5th to 15th day of pregnancy. Pregnancy + lactation (P + L) variation: the parent females were daily treated from 5th to 15th day of pregnancy and also during lactation, i.e. from the 2nd day after delivery until separation of the offspring at their age of 4 weeks. Pregnancy + lactation + post weaning (P + L + P) variation: the parent females were daily treated from 5th to 15th day of pregnancy and during lactation from the 2nd day after delivery until separation the offspring at their age of 4 weeks, and the weaned young male rats were further on treated for 8 weeks in a 5 days per week schedule. The number and body weight of the pups was determined within 12 h from birth. On the 4th day, the litter size was adjusted to eight (up to five males per litter).

During the whole treatment period, all animals were continuously observed for symptoms of intoxication (e.g. salivation, muscle tonus, etc.) which, together with the body weight, were recorded. Behavioural and neurotoxicological investigations of the male rats were done at the age of 12 weeks.

Behavioural investigations

Motility and spontaneous exploration was investigated in an automatic open field (OF: 40 × 40 × 40 cm; ACTIFRAME, Gerb Electronic, Berlin, Germany). Horizontal as well as vertical movements were electronically detected by means of infrared (IR) sensors located to 1.11 cm distance at two different levels in the OF. An intelligent interface was used to sample the IR signals and to store these in digital form by a mini computer. Every intermission of an infrared sensor resulted in an electric impulse registered by the computers interface as a count used for further computational estimation of the behavioural changes exhibited by the individual in the open field box. Automated data collection from each animal occurred during a 10 min session between 8:00 a.m. and 2:00 p.m. in a soundproof room following habituation of the rats to the laboratory situation. Illumination of the OF floor was ca. 10 ± 2 lux, background white noise provided by a computer cooler fan was of about 40 dB. Computing of the behavioural data was done by means of a special PC program (ARNO, Dr J. Wolffgramm, Department of psychopharmacology, Free University of Berlin, Germany).

Neurophysiological investigations

There was an interval of 1 to 2 days between the behavioural and electrophysiological investigations. The anaesthetized animals (urethane, 1000.0 mg/kg i.p.; Bowman and Rand 1980) were placed in a stereotaxic instrument and the left hemisphere was exposed. Silver electrodes were placed on the primary somatosensory (Par1), visual (Oc1M) and auditory centers (Te1) (Zilles 1982). Half an hour later, electrocorticogram (ECoG) of the rats was recorded from the three sensory areas simultaneously for 5 min. Parameters calculated from the ECoG were: mean amplitude, mean frequency, and the power spectrum. 'ECoG index', the ratio of the spectral power slow and fast bands (delta + theta/$beta_1$ + $beta_2$), was also calculated and was found to be more sensitive than conventional power spectrum analysis (Dési 1983). The recorded ECoGs were also analysed by a graphic analysis program (Waterfall, Cambridge Electronic Ltd, UK).

Cortical evoked potentials were recorded subsequently via the same electrodes. Somatosensory stimulation occurred via electrodes inserted into the nasal part of skin, in the area of the whiskers. 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) delivered by a flash-producing device via an optical fibre directly into the contralateral eye of the rat. The acoustic stimulation occurred by application of clicks (1 Hz, 40 dB), produced by a sound generator and applied by a small earphone put into the ear of the rat. Following application of 50 stimuli of each modality per rat, averaging of the evoked potentials was done by an appropriate computer program (Cambridge Electronics Ltd, UK). Latency and duration of the averaged somatosensory, visual and auditory evoked potentials were measured on the display by hand.

Conduction velocity of a peripheral nerve (the tail nerve) was studied by the modified Miyoshi method (at room temperature, 21–22°C, instead of 37°C; Miyoshi and Goto 1973). The relative and absolute relative periods were investigated according to Anda *et al.* (1984).

After finishing electrophysiological recording, the rats were killed by an overdose of urethane. The weight of the brain, liver, heart, lung, kidneys, thymus and adrenal glands was determined and the relative organ weights as related to that of the brain weight calculated.

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

Statistical evaluation

First, the data were checked for normal distribution using the Kolmogorov-Smirnov test. Effects of the treatment on behaviour was tested in a 3 × 4 (time × doses) designed experiment, following square-root transformation of the data and equal cell content, by means of multivariate ANOVA (normal distribution) or by Kruskal-Wallis ANOVA (non-normality). Electrophysiological data were analysed by univariate ANOVA. *Post hoc* analysis of group differences was performed by a subsequent LSD test setting the probability level at $p < 0.05$.

Results

General toxicology

None of the treatment schedules induced a significant alteration in the body weight gain of the rats (Table 1). The weight increase of rats in the P + L and P + L + P treatment variations was slightly slower compared to that in the P variation, and this was a bit slower than that of the control animals, but the differences among all variations (schedules, doses, control) were below significance ($p > 05$; NS in all cases).

Table 1. Body and relative organ weights of F1 male rats

		Body weight			*Relative organ weight*						
Treatment	*Dose (mg/kg)*	*Birth*	*4 weeks*	*12 weeks*	*Liver*	*Lung*	*Heart*	*Kidney*	*Spleen*	*Thymus*	*Adrenol gland*
P	Control	5.61 ± 0.42	98.3 ± 6.7	327.5 ± 22.7	7.48 ± 0.46	1.01 ± 0.04	0.62 ± 0.02	1.44 ± 0.05	0.35 ± 0.05	0.24 ± 0.03	0.03 ± 0.002
	80.0	5.62 ± 0.29	97.5 ± 4.2	326.4 ± 21.7	7.44 ± 0.31	1.01 ± 0.03	0.60 ± 0.03	1.41 ± 0.07	0.34 ± 0.04	0.21 ± 0.06	0.03 ± 0.001
	160.0	5.56 ± 0.36	97.4 ± 4.3	321.8 ± 19.1	7.41 ± 0.35	1.03 ± 0.05	0.61 ± 0.03	1.39 ± 0.09	0.30 ± 0.06	0.24 ± 0.03	0.03 ± 0.003
	320.0	5.43 ± 0.34	97.1 ± 5.1	318.1 ± 17.6	7.41 ± 0.42	1.02 ± 0.05	0.63 ± 0.02	1.40 ± 0.08	0.32 ± 0.04	0.22 ± 0.03	0.03 ± 0.002
P + L	Control	5.58 ± 0.42	97.1 ± 5.3	324.6 ± 19.8	7.42 ± 0.39	1.03 ± 0.05	0.62 ± 0.05	1.41 ± 0.06	0.32 ± 0.03	0.23 ± 0.04	0.03 ± 0.002
	80.0	5.52 ± 0.35	95.8 ± 5.6	319.1 ± 17.4	7.39 ± 0.37	1.04 ± 0.05	0.63 ± 0.03	1.39 ± 0.08	0.30 ± 0.04	0.24 ± 0.04	0.03 ± 0.04
	160.0	5.48 ± 0.31	95.3 ± 4.8	318.2 ± 16.8	7.38 ± 0.33	1.04 ± 0.05	0.62 ± 0.02	1.38 ± 0.07	0.33 ± 0.05	0.24 ± 0.03	0.03 ± 0.002
	320.0	5.44 ± 0.35	95.1 ± 5.2	317.2 ± 16.3	7.38 ± 0.36	1.02 ± 0.05	0.63 ± 0.04	1.38 ± 0.11	0.32 ± 0.04	0.24 ± 0.04	0.03 ± 0.003
P + L + P	Control	5.63 ± 0.36	97.3 ± 5.1	322.7 ± 16.2	7.39 ± 0.37	1.03 ± 0.05	0.62 ± 0.05	1.40 ± 0.05	0.32 ± 0.05	0.25 ± 0.05	0.03 ± 0.002
	80.0	5.55 ± 0.36	95.4 ± 8.6	315.8 ± 22.3	7.32 ± 0.25	1.02 ± 0.04	0.62 ± 0.04	1.36 ± 0.07	0.29 ± 0.05	0.23 ± 0.03	0.03 ± 0.003
	160.0	5.51 ± 0.34	94.7 ± 5.3	314.1 ± 20.1	7.30 ± 0.32	1.03 ± 0.06	0.62 ± 0.05	1.32 ± 0.09	0.30 ± 0.03	0.24 ± 0.05	0.03 ± 0.002
	320.0	5.47 ± 0.37	94.4 ± 6.4	313.0 ± 27.1	7.28 ± 0.44	1.06 ± 0.04	0.62 ± 0.05	1.32 ± 0.12	0.31 ± 0.03	0.25 ± 0.03	0.02 ± 0.003

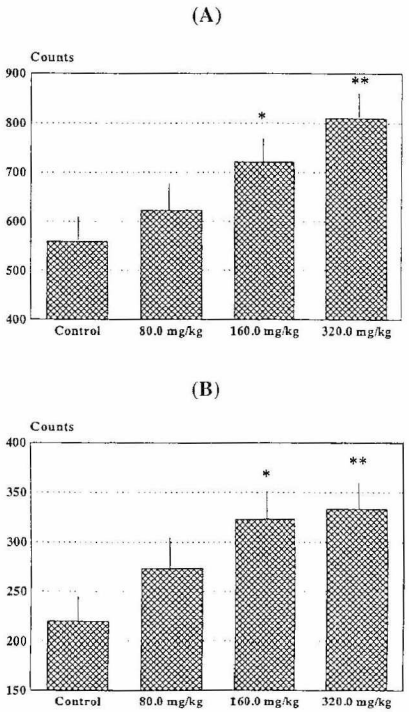

Fig. 1. Dose-dependent increase of horizontal ambulation of the first minute (A) and of grooming activity (B). Ordinates: counts of photobeam intermissions by the animal expressed as counts; abscissa: doses of lead; mean: ± SEM; significance: $*p < 0.05$, $**p < 0.01$.

Although the averaged number of newborn rats/litter was lower, especially in the higher dose groups of each variation, the litter sizes did not differ significantly from those in the control ($p > 05$; NS in all cases). The average body weight of the pups from treated females in all variations were hardly lower compared to the control ($p > 05$; NS in all cases). Neither macroscopic malformations nor visible clinical signs of chronic lead intoxication were observed, even in the groups receiving the highest dose.

Behavioural findings

It was the treatment during pregnancy that caused a significant hyperactivity in the offspring ($F(2, 27) = 5.55$; $p < 0.0096$) which was expressed in an increased ambulation rate in the first minute and in longer distances run in the OF. Grooming, too, was significantly increased ($F(2,27) = 3.64$; $p < 0.0398$). Treatment during pregnancy and including suckling period or during pregnancy and the subsequent 12 weeks did not result in significant changes of the adult rats' behaviour (Fig. 1).

Electrophysiological findings

The amplitude of the ECoGs from the somatosensory, visual and auditory cortical foci showed a dose- and variation-dependent, but not significant decrement. The decrease compared to the control was more pronounced in the P + L and P + L + P variation groups than in the P ones, but the variation-dependent differences between groups treated with the same dose were not significant either ($p > 0.05$; NS in all cases).

The mean frequency of the somatosensory ECoG increased in a dose- and variation-dependent manner with significant differences, compared to the control, with the two higher doses in the P + L + P variation ($F(3,28) = 2.64$; $p < 0.0162$, and $F(3,28) = 2.64$; $p < 0.0333$; Fig. 2A). Although the mean frequency of the visual and auditory ECoGs showed the same tendency, significant increases were achieved only in the highest dose group of P + L + P variation (visual: $F(3,28) = 1.77$; $p < 0.035$; auditory: $F(3,28) = 2.11$, $p < 0.0209$; Fig. 2B, C).

In the P + L + P variation, treatment with the two higher lead doses resulted in significantly lower somatosensory ECoG index ($F(3,28) = 2.8$; $p < 0.0133$, and $F(3,28) = 2.8$; $p < 0.0273$; Fig. 3A). In the group with P + L variation receiving the highest dose the decrease of the index was also considerable but not significant. The visual and auditory ECoG index was significantly decreased in the highest dose group with P + L + P variation (visual: $F(3,28) = 1.82$; $p < 0.0315$, and auditory: $F(3,28) = 3.17$; $p < 0.0104$), in all other variations the decreases were below significance (Fig. 3B, C). Between the ECoG indexes from the three sensory centres, no significant differences were observed ($p > 0.05$, NS in all cases).

There were similar trends of changes of the ECoG power spectra. The slow wave part (delta and theta) was slightly decreased and the fast wave part ($beta_1$ and $beta_2$) increased to a small extent in all dose groups and variations ($p > 0.05$, NS in all cases).

Intervariational comparison of identical parameters (amplitude, frequency, index) of ECoGs revealed no significant differences ($p > 0.05$; NS in all cases).

The changes of latency and duration of the evoked potentials were also dose- and variation-dependent. In case of the somatosensory evoked potentials the latency of waves increased in all dose groups and variations. Lengthening of the latency of N1 wave (Fig. 4A) was significant in the high dose groups with P + L and P + L + P variation, and also in the middle dose groups with P + L + P variation (high dose – P + L: $F(3,28) = 2.15$; $p < 0.0189$, and P + L + P: $F(3,28) = 2.93$; $p < 0.0127$; middle dose – $F(3,28) = 2.93$; $p < 0.0205$). The change of the other waves' latency showed a similar trend. The interpeak durations also became longer, but the differences were not significant even in the high dose group with P + L + P variation ($p > 0.05$, NS in all cases). The intervariation analysis of the identical parameters did not show significant differences ($p > 0.05$; NS in all cases).

The latency of the waves in the visual evoked potential increased dose-dependence in all variations. The change of N2 wave was significant in the highest dose groups with P + L and P + L + P variations (P + L: $F(3,28) = 1.96$; $p < 0.0245$ and P + L + P: $F(3,28) = 3.88$; $p < 0.0045$), and also in the P + L + P middle dose group ($F(3,28) = 2.37$; $p < 0.0362$; Fig. 4B). The intervariation differences were insignificant ($p > 0.05$; NS in all cases). Longer interpeak durations were found in all treated groups with any variation, but the changes were not significant either ($p > 0.05$; NS in all cases).

The latency of the auditory evoked potential waves were also dose- and variation-dependently longer compared to the control. Latency increase of N1 waves (Fig. 4C) was significant only in the highest dose groups with P + L + P variation ($F(3,28) = 4.38$; $p < 0.0027$). Significant

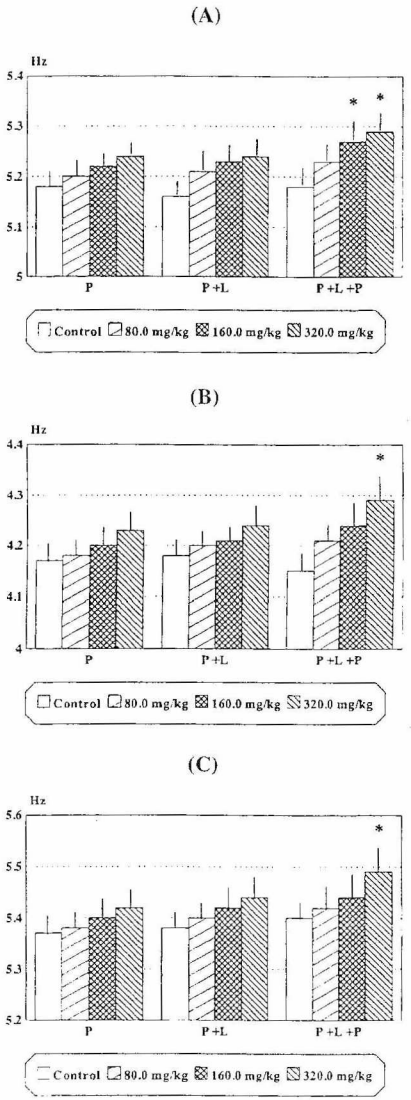

Fig. 2. Changes of the ECoG mean frequency in the somatosensory (A), visual (B) and auditory (C) centres. Ordinate: mean frequency; abscissa: treatment schedule; insert: control and treatment doses; significance: $^{*}p < 0.05$.

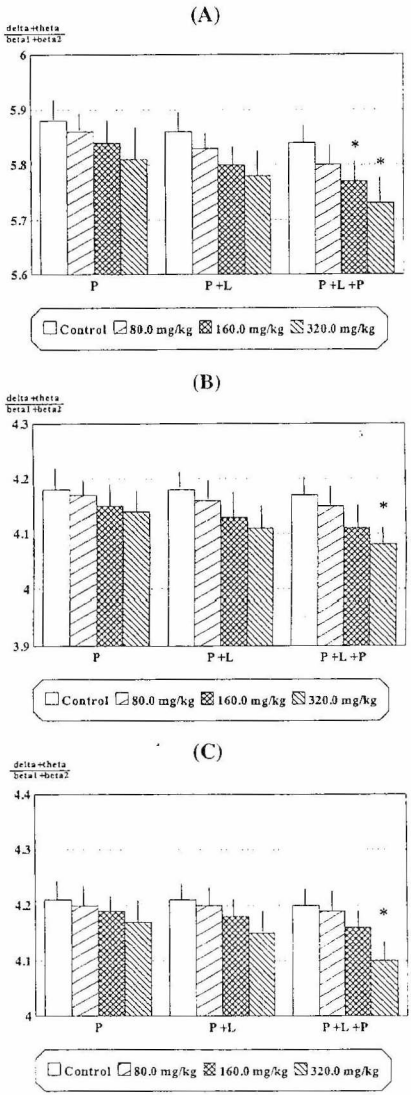

Fig. 3. Changes of the ECoG index in the three cortical centres (A – somatosensory; B – visual; C – auditory). Ordinate: index value; abscissa: treatment schedule; insert: control and treatment doses; significance: $^*p < 0.05$.

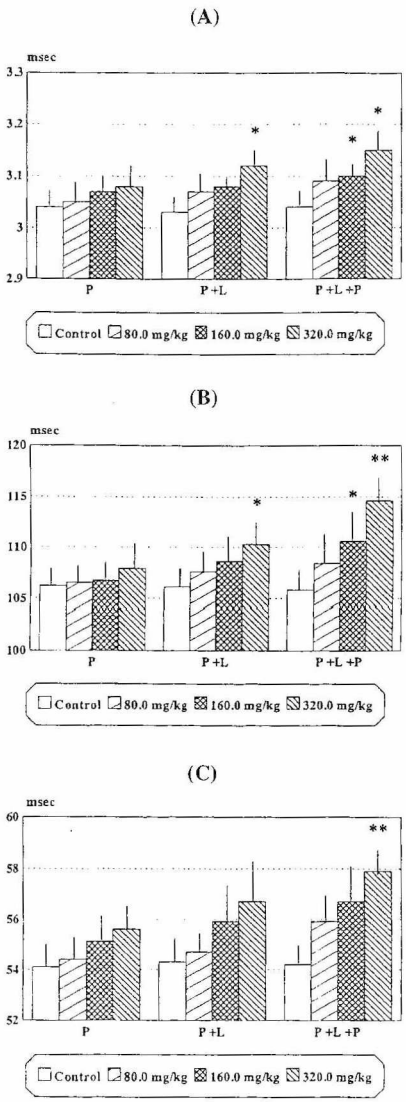

Fig. 4. Changes of the latency of certain evoked potential waves (A – somatosensory N1 wave; B – visual N2 wave; C – auditory N1 wave). Ordinate: latency; abscissa: treatment schedule; insert: control and treatment doses; significance: $^{*}p < 0.05$, $^{**}p < 0.01$.

intervariation differences between the identical parameters were not seen ($p > 0.05$; NS in all cases). In the treated groups, insignificantly prolonged interpeak durations were also observed ($p > 0.05$; NS in all cases).

The conduction velocity of the tail nerve was decreased in all treated groups, but the difference was significant only in the P + L + P groups in case of any dose (high dose: $F(3,28) = 5.25$; $p < 0.0011$, middle dose: $F(3,28) = 5.25$; $p < 0.0051$, and low dose: $F(3,28) = 5.24$; $p < 0.0145$; Fig. 5A). The relative and absolute refractory periods (Fig. 5B, C) were also significantly longer (relative: $F(3,28) = 4.89$; $p < 0.00061$, $F(3,28) = 4.89$; $p < 0.0132$, and $F(3,28) = 4.89$; $p < 0.0173$; absolute: $F(3,28) = 4.21$; $p < 0.0022$, $F(3,28) = 4.21$; $p < 0.0131$, and $F(3,28) = 4.21$; $p < 0.0294$). No significant intervariation differences of corresponding parameters from different variations were found ($p > 0.05$; NS in all cases).

Discussion

Pre- and postnatal inorganic lead exposure can cause morphological and functional disorders of the central and peripheral nervous system. Both human and animal investigations showed that inorganic lead can pass the placental barrier and affect the foetal organs including brain (Korpela *et al.* 1986, Mushak *et al.* 1989, Goyer 1990, Klein *et al.* 1994). Other human studies revealed that the functional state of the nervous system can be compromised even by a low level lead exposure of the foetus and newborn (Hammond and Dietrich 1990), and also that newborn babies can be more or less continuously exposed for several months by the lead content of breast milk (Schramel *et al.* 1988). As lead is continuously present in the environment and is regularly detected in food, the combined pre- and postnatal lead exposure seems to be a real risk to the population.

Results of animal experiments emphasize the above. When female rats were exposed to lead in drinking water altered synaptic processes were found in the pups as a consequence of inhibited synaptogenesis (McCauley *et al.* 1982). Others observed significantly lower GABA levels (Bailey and Kitchen 1986) or inhibited catecholamine synthesis (McIntosh *et al.* 1988). Vulnerability of neurotransmitter systems, that is, changed noradrenaline, dopamine, GABA and serotonin levels in different regions of the brain (hippocampus, cerebellum, hypothalamus, brainstem, etc.) was found in rats orally treated with lead acetate (Shailesh-Kumar and Desiraju 1990). Pre- and postnatal low-level lead exposure caused significant inhibition of the cholinergic marker enzyme choline acetyltransferase and altered the development of cholinergic muscarinic receptors in rats indicating that the cholinergic neurons, too, can be affected by lead (Widmar *et al.* 1992, Bielarczyk *et al.* 1994). The effects of lead on the neurotransmitter (e.g. cholinergic, dopaminergic, GABAergic) systems has also been described by Bressler and Goldstein (1991). Those changes in the neurotransmitter system will lead to changed behavioural outcomes, e.g. excessive hyperactivity as earlier also reported by changes of the dopaminergic system (Wirtshafter *et al.* 1988).

Alterations at the level of synapses and transmitters can result in altered bioelectric activity. Low dose of lead increases the release of acetylcholine from presynaptic nerve endings (Suszkiv *et al.* 1984, Shao and Suszkiw 1991) and, as a consequence, causes excitation of the spontaneous activity of the brain expressed as higher EEG frequencies (Bringman 1995). Likewise, lead induced changes in dopamine metabolism and in dopaminergic receptor regulation altered brain functional processes (Lasley, 1992, Struzynska and Rafalowska 1994). Other sites of action of lead may also play a role in the altered function of the sensory pathways. Lead acts on the voltage-dependent Ca^{++}, and Ca^{++}-activated K^{+} channels

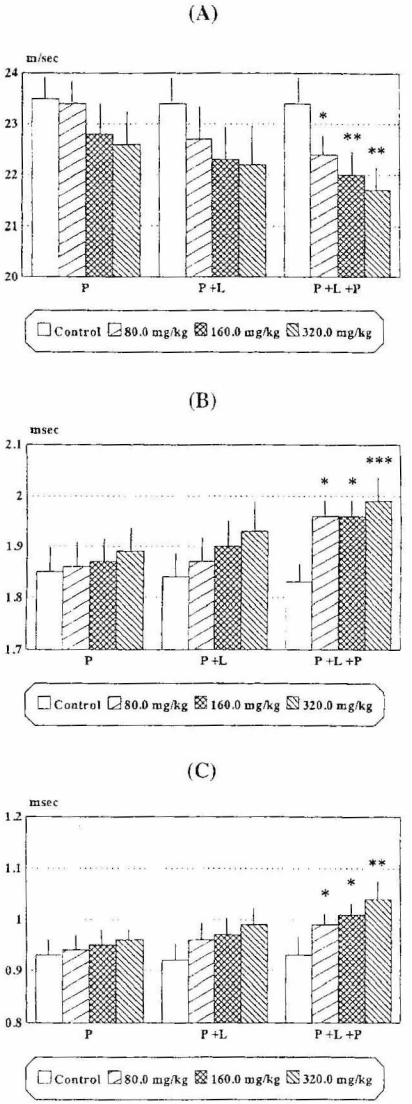

Fig. 5. Changes in the function of the tail nerve. A ordinate: conduction velocity; B ordinate: relative refractory period; C ordinate: absolute refractory period; abscissa: treatment schedule; insert: control and treatment doses; significance: $*p < 0.05$, $**p < 0.01$, $***p < 0.001$.

affecting the transmembrane ionic currents of the neurons resulting in slowed down conduction velocity and lengthened latencies (Audesirk and Audesirk 1991, Reuveny and Narahashi 1991, Leinders and Vijenberg 1992).

Our previous data showed that oral lead treatment of adult rats (with the same doses as in the present study) for 4, 8, or 12 weeks caused dose- and time-dependent signs of ECoG excitation: increased frequency and decreased amplitude, as well as lengthened latencies and duration of sensory evoked potentials (Nagymajtenyi *et al.* 1997). In a three-generation experiment, where lead treatment lasted from the conception to the end of the 12th week of age, alterations of the spontaneous and evoked activity of the brain were similar (Desi *et al.* forthcoming).

In our present study, the results pointed at the different sensitivity of central and peripheral functional processes to inorganic lead depending on the dose and timing of administration. Although the changes of the spontaneous and evoked cortical activity were not significant in the P treatment variation, the trends of the data unambiguously showed the affectedness of all investigated parameters. Continued administration of lead during lactation (P + L variation) considerably increased the functional alterations caused by the prenatal treatment. These pathologic trends seem very important if one takes into consideration that the electrophysiological recordings were made at the end of the 12th postnatal week when, in the P and P + L variations, the rats were no more lead-treated for 12 or 8 weeks, respectively. This means that the low-level inorganic lead exposure during the pre- and postnatal development of the central and peripheral nervous system can definitely alter certain bioelectric functions. In our previous three-generation experiment, the results seen in generation F1 were very similar to those obtained now with the P + L + P treatment variation. It is not surprising that some of the changes in this group were significant since the postweaning lead treatment could increase the altering effect of pre- and postnatal lead administration.

In a comparable experiment, Burdette and Goldstein (1986) found depression of the 6–7 Hz band of the hippocampal EEG, but no considerable change of the cortical EEG, in 8-week-old rats treated with 0.3% lead acetate in drinking water during 16–23 days of gestation, 1–8 and 9–16 days of nursing.

There is a clear correlation between some pathological changes and definite levels of the accumulated lead in brain (Kostial *et al.* 1986, Cory-Slechta *et al.* 1985, Cory-Slechta and Pokora 1991, Collins *et al.* 1992). In our investigation, a connection of the tendency of changes of the investigated (spontaneous and evoked) functional parameters to the different lead doses and treatment variation was unequivocal. Inorganic lead can, exposure-dependently, cross the placental and blood–brain barriers and accumulate in the nervous system. Considering the well-known high susceptibility of the pre- and postnatal developing brain to lead, the more expressed functional changes in the P + L and P + L + P generations can be explained by the continuous pre- and postnatal lead intake. And, as there were practically no qualitative differences in the trends of behavioural and/or neurophysiological changes among the treatment variations, it can be concluded that lead affects practically most functions of the whole nervous system.

Although it is difficult to relate or transfer the results of animal experiments directly to man, one can, relying on our data, on those of other animal experiments, and also human epidemiological investigations (Sborgia and Assenato 1983, Araki *et al.* 1987, Murata *et al.* 1987, Lilienthal *et al.* 1990), state that the chronic lead exposure of the population, originating from the vehicle emission and the industry, should be regarded as a real behavioural and functional neurotoxic risk, especially in case of pregnant and nursing women, more exactly, their foetuses and suckling babies. Based on the data of epidemiological studies made in children, our opinion is that the functional and behavioural effect of inorganic lead without other signs of

intoxication is more harmful than has been supposed. Thus, it seems to be necessary, beside the widely used biochemical monitoring (e.g. blood lead level, DALA in urine), to follow also the changes of certain behavioural and physiological biomarkers in the population using sensitive, non-invasive screening methods (e.g. EEG recording). Their use can give more informative data about early neurotoxic effect of low-level lead exposure which, in turn, would enable us to prevent its harmful effects on population level.

Acknowledgement

The study was supported by the Hungarian OTKA grant No. T016735.

References

Anda, E., Dura, G. and Lörinczy, I. (1984) Effects of carbon monoxide on the peripheral nerves. *Egészségtudomány* **28**, 270–7 [in Hungarian].

Araki, S., Murata, K. and Aono, H. (1987) Central and peripheral nervous system dysfunction in workers exposed to lead, zinc and copper: a followup study of visual and somatosensory evoked potentials. *Int. Arch. Occup. Environ. Health* **59**, 177–87.

Audesirk, G. and Audesirk, T. (1991) Effects of inorganic lead on voltage-sensitive calcium channels in NIE-115 neuroblastoma cells. *Neurotoxicology* **12**, 519–528.

Bailey, C.C. and Kitchen, I. (1986) Ontogeny of catecholamine and GABA levels in rat brain: lack of effect of perinatal lead. *Toxicol. Lett.* **30**, 97–102.

Bielarczyk, H., Tomsig, J.L. and Suszkiw, J.B. (1994) Perinatal low-level lead exposure and the septo-hippocampal cholinergic system: selective reduction of muscarinic receptors and cholineacetyl-transferase in the rat septum. *Brain Res.* **18**, 211–7.

Bordo, B., Masetto, N., Musicco, I.M., Filippini, G. and Boeri, R. (1982) Electrophysiologic changes in workers with 'low' blood lead levels. *Am. J. Ind. Med.* **3**, 23–32.

Bowman, W.C. and Rand, M.J. (eds) (1980) *Textbook of Pharmacology*, Oxford: Blackwell Scientific.

Bressler, J.P. and Goldstein, G.W. (1991) Mechanism of lead neurotoxicity. *Biochem. Pharmacol.* **41**, 479–84.

Bringman, A. (1995) Topographic mapping of the cortical EEG power in the unrestrained rat: peripheral effects of neuroactive drugs. *Arch. Ital. Biol.* **133**, 1–16.

Burdette, L.J. and Goldstein, R. (1986) Long-term behavioral and electrophysiological changes associated with lead exposure at different stages of brain development in the rat. *Dev. Brain Res.* **29**, 101–10.

Collins, M.F., Hrdina, P.D. and Whittle, E. (1992) Lead in blood and brain regions of rats chronically exposed to low doses of the metal. *Toxicol. Appl. Pharmacol.* **65**, 314–22.

Cory-Slechta, D.A. (1995) Relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic, and glutaminergic neurotransmitter system functions. *Ann. Rev. Pharmacol. Toxicol.* **35**, 391–415.

Cory-Slechta, D.A. and Pokora, M.J. (1991) Behavioral manifestations of prolonged lead exposure initiated at different stages of the life cycle. I. Schedule-controlled responding. *Neurotoxicology* **12**, 745–60.

Cory-Slechta, D.A., Weiss, B. and Cox, C. (1985) Performance and exposure indicis of rats exposed to low concentrations of lead. *Toxicol. Appl. Pharmacol.* **78**, 291–9.

Davies, J.M. (1990) Risk assesment of the developmental neurotoxicity of lead. *Neurotoxicology* **11**, 285–91.

Dési, I. (1983) Neurological investigation of pesticides in animal experiments. *Neurobehav. Toxicol. Teratol.* **5**, 503–17.

Dési, I. Nagymajtényi, L. and Schulz, H. (Forthcoming) Behavioural and neurotoxicological changes caused by lead in three generations of rats. *Neurotox. Teratol.*

Goyer, R.A. (1990) Transplacental transport of lead. *Environ. Health Perspect.* **89**, 101–5.

Hammond, P.B. and Dietrich, K.N. (1990) Lead exposure in early life: health consequences. *Rev. Environ. Contam. Tox.* **115**, 91–124.

Klein, M., Kaminsky, P., Barbe, F. and Duc, M. (1994) Lead poisoning in pregnancy *Presse Med.* **23**, 576–80.

Korpela, H., Loueniva, R., Yrjanheikki, E. and Kauppila, A. (1986) Lead and cadmium concentrations in maternal and umbilical cord blood, amniotic fluid, placenta, and amniotic membranes. *Am. J. Obstet. Gynecol.* **155**, 1086–9.

Kostial, K., Kello, D. and Jugo, S. (1986) Influence of age on metal metabolism and toxicity. *Environ. Health Perspect.* **2**, 81–6.

Lasley, S.M. (1992) Regulation of dopaminergic activity, but not tyrosine hydroxylase, is diminished after chronic inorganic lead exposure. *Neurotoxicology* **13**, 625–35.

Leinders, T. and Vijenberg, H.P.M. (1992) Ca^{++} dependence of small Ca^{++}-activated K+ channels in cultured N1E-115 mouse neuroblastoma cells. *Pflügers Arch.* **422**, 223–32.

Lilienthal, H., Winneke, G. and Evert, T. (1990) Effects of lead on neurophysiological and performance measures. *Environ. Health Perspect.* **89**, 21–5.

Lille, F., Hazemann, P., Garnier, R. and Dally, S. (1988) Effect of lead and mercury intoxication on evoked potentials. *Clin. Toxicol.* **26**, 103–16.

Lille, F., Margules, S., Fournier, E., Dally, S. and Garnier, R. (1994) Efect of occupational lead exposure on motor and somatosensory evoked potentials. *Neurotoxicology* **15**, 679–83.

McCauley, P.T., Bull, R.J., Tonti, A.P., Lutkenhoff, S.D., Meister, M.V. and Doerger, J.U. (1982) The effect of prenatal and postnatal lead exposure on neonatal synaptogenesis in rat cerebral cortex. *J. Toxicol. Environ. Health* **10**, 639–51.

McIntosh, M.J., Meredith, P.A., Petty, M.A. and Reid, J.L. (1988) Influence of lead exposure on catecholamine metabolism in discrete brain nuclei, *Comp. Biochem. Physiol. C.* **89**, 211–3.

Miyoshi, T. and Goto, I. (1973) Serial *in vivo* determinations of nerve conduction velocity in rat tails. Physiological and pathological changes. *Electroenceph. Clin. Neurophysiol.* **35**, 125–31.

Murata, K., Araki, S. and Aono, H. (1987) Visual and brainstem auditory evoked potentials in lead-exposed workers. *Jpn. J. EEG EMG* **15**, 16–21.

Mushak, P., Davis, J.M., Crocetti, A.F. and Grant, L.D. (1989) Prenatal and postnatal effects of low-level lead exposure: integrated summary of a report to the US Congress on childhood lead poisoning. *Environ. Res.* **50**, 11–36.

Nagymajtényi, L., Schulz, H. and Dési, I. (1997) Behavioural and electrophysiological changes caused by subchronic lead exposure in rats. *Centr. Eur. J. Environ. Health* **3**, 195–209.

Needleman, H.L., Schell, A., Bellinger, D., Leviton, A. and Allred, E.N. (1990) The long-term effects of exposure to low doses of lead in childhood. *New Eng. J. Med.* **322**, 83–8.

Ong, C.N., Phoon, W.O., Law, H.Y., Tye, C.Y. and Lim, H.H. (1985) Concentrations of lead in maternal blood, cord blood, and breast milk. *Arch. Dis. Child.* **60**, 756–9.

Otto, D., Robinson, G., Baumann, S., Schroeder, S., Mushak, P., Kleinbaum, D. and Boone, L. (1985) 5-year follow-up study of children with low-to-moderate lead absorption: electrophysiological evaluation. *Environ. Res.* **38**, 168–86.

Reuveny, E. and Narahashi T. (1991) Potent blocking action of lead on voltage-activated calcium channels in human neuroblastoma cells SH-SY5Y. *Brain Res.* **545**, 312–14.

Sborgia, G. and Assenato, G. (1983) Comprehensive neurophysiological evaluation of lead-exposed workers. In *Neurobehavioral Methods in Occupational Health* (M. Gilioli, M. Cassito and M. Foa eds), pp. 283–294. London: Pergamon.

Schramel, P., Hasse, S. and Ovcar-Pavlu, J. (1988) Selenium, cadmium, lead, and mercury concentrations in human breast milk, in placenta, maternal blood, and the blood of the newborn. *Biol. Trace Elem. Res.* **15**, 111–24.

Shailesh-Kumar, M.V. and Desiraju, T. (1990) Regional alterations of brain biogenic amines and GABA/glutamate levels in rats following chronic lead exposure during neonatal development. *Arch. Toxicol.* **64**, 305–14.

Shao, Z. and Suszkiw, J.B. (1991) Ca2(+)-surrogate action of Pb2+ on acetylcholine release from rat brain synaptosomes. *J. Neurochem.* **56**, 548–57.

Sternovsky, H.J. and Wessolowsky R. (1985) Lead and cadmium in breast milk. Higher levels in urban vs rural mothers during the first 3 months of lactation. *Arch. Toxicol.* **57**, 41–5.

Struzynska, L. and Rafalowska, U. (1994) The effect of lead on dopamine, GABA and histidine spontaneous and KCl-dependent releases from rat brain synaptosomes. *Acta Neurobiol. Exp. Warsz.* **54**, 201–7.

Suszkiv, J., Toth, G., Murawsky, M. and Cooper, G.P. (1984) Effects of Pb and Cd on acethylcholine release and Ca movements in synaptosomes and subcellular fractions from rat brain and Torpedo electric organ. *Brain Res.* **323**, 31–46.

Tang, H.W., Liang, Y.X., Hu, X.H. and Yang, H.G. (1995) Alterations of monoamine metabolites and neurobehavioural function in lead-exposed workers. *Biomed. Environ. Sci.* **8**, 23–9.

Trotter, R.T. (1990) The cultural parameters of lead poisoning: a medical anthropologist's view of intervention in environmental lead exposure. *Environ. Health Perspect.* **89**, 79–84.

World Health Organization (1989) *Lead – Environmental Aspects*. Environmental Health Criteria, No. 85, Geneva: WHO.

Widmar, H.R., Vedder, H., Schlumpf, M. and Lichtensteiger, W. (1992) Concurrent changes in regional cholinergic parameters and nest odor preference in the early postnatal rat after lead exposure. *Neurotoxicology* **13**, 615–24.

Winneke, G. (1995) Endpoints of developmental neurotoxicity in environmentally exposed children. *Toxicol. Lett.* **77**, 127–36.

Winneke, G., Brockhaus, A., Ewers, U., Kramer, U. and Neuf, M. (1990) Results from the European multicenter study on lead neurotoxicity in children: implications for risk assessment. *Neurotoxicol. Teratol.* **12**, 553–9.

Winneke, G., Altmann, U., Kramer, U., Turfeld, M., Behler, R., Guthsmuths, F.J. and Mangold, M. (1994) Neurobehavioral and neurophysiological observations in six-year-old children with low lead levels in East and West Germany. *Neurotoxicology* **15**, 705–14.

Wirtshafter, D., Klitenick, M.A. and Asin, K.E. (1988) Is dopamine involved in the hyperactivity produced by injections of muscimol into the median raphe nucleus? *Pharmacol. Biochem. Behav.* **30**, 577–83.

Zilles, K. (1982) *The Cortex of the Rat. A Stereotaxic Atlas*. Berlin: Springer Verlag.