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Experimental investigation of the neurotoxicity of environmental micropollutant heavy metals

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SUMMARY

Microelements may be essential for humans, toxic, or both depending on dose. In this paper, selected results of the neurotoxicological work done at the Department of Public Health at the University of Szeged, Faculty of Medicine, are presented. All experiments were done on Wistar rats. Acute application of Pb, Hg, Mn and their combinations induced a shift to lower frequencies of the spontaneous cortical electric activity. The evoked cortical responses showed an increase of the peak-to-peak amplitude and peak latency. When Pb or Hg was given, combined with 5% alcohol in the drinking water, for 12 weeks, evoked potential latency was significantly increased by both metals and by their combination in the water-drinking rats, and the effect of Pb, but not of Hg, seemed to be abolished by ethanol. When Hg was given during pre- and postnatal development, it was found that prenatal exposure increased the effect of postnatal Hg administration, and that the changes of peripheral nerve activity indicated the effect of Hg as sensitively as some traditional toxicological parameters. More practical biomarkers of the functional alterations caused by micropollutant metals would be advantageous. The results of research done at our Department may contribute to achieving that.

INTRODUCTION

Environment is the final source of all substances we need to consume to stay alive. Beyond water for drinking and bulk nutrients of plant and animal origin, the presence or absence of certain molecules and chemical elements may have decisive role in health. Some micro-elements are essential for humans (iron, copper, zinc etc.), others are toxic (mercury, lead, cadmium etc.), and still others can play both roles depending on the dose. An example for the latter is manganese, being essential in micro, but toxic in macro, amounts.

Environmental Mn originates from organo-Mn fungicides (*Ferraz et al.* 1988), a petrol additive (*Lynam et al.* 1999), and other sources. Its intestinal absorption is limited and is linked to Fe supply (*Davis et al.* 1992). Once absorbed, Mn passes the blood-brain barrier, partly in transferrin-bound form (*Aschner* and *Gannon* 1994) and deposits in the brain. In human chronic (occupational) exposure to Mn, the leading feature is functional brain damage (*Shinotoh et al.* 1997).

Lead has been used – and has caused occupational, foodborne and other exposures – for thousands of years. Pb is well absorbed after inhalation and ingestion, in a process linked to the absorption of Ca, and permeates readily the blood-brain barrier (*Grandjean* 1978). Intestinal absorption of Pb is increased in persons with Ca and/or Fe insufficiency (*Mahaffey* and *Annest* 1986, *Mahaffey et al.* 1986). The neurological consequences of Pb intake include impaired IQ and different behavioural difficulties (*Needleman* and *Gatsonis* 1990), and EEG and auditory evoked potential alterations (*Otto et al.* 1985), mainly in children.

In the mercury exposure of the population, the amount released into the environment and contained in dental amalgam are the major sources. Environmental Hg finally takes the form of methyl and dimethyl Hg, both being lipophilic, and hence accumulated and concentrated along the food chain. Both inorganic and organic Hg forms can pass the blood-brain barrier (*Aschner* and *Aschner* 1990) and the placental barrier (*Clarkson* 1989). Hg burden of the mothers' organism (food-borne methyl mercury, dental amalgam etc.) was shown to pass the placenta and appear in breast milk (*Plockinger et al.* 1993, *Sakamoto et al.* 2002) so that babies are exposed this way for several months (*Grandjean et al.* 2003, *Oskarsson et al.* 1996). Mercury, when present in the nervous system, affects the bioelectric processes of nerve cells by influencing the operation of voltage-activated Ca^{2+}-channels and the release or turnover of neurotransmitters. In persons occupationally exposed to inorganic mercury, various disorders of the EEG (*Piikivi* and *Tolonen* 1989) and evoked-responses (*Discalzi et al.* 1993) were seen.

The Department of Public Health at the University of Szeged, Faculty of Medicine, has been active in experimental investigations of the neurotoxicity of environmental chemicals for ca. 25 years. In this paper, selected results of our work are presented.

Materials and methods

All experiments were done on Wistar rats. In the subacute/subchronic scheme, 10 weeks old male rats were exposed by the investigated metal orally (by gavage) for 5 to 12 weeks, 5 times a week. In the developmental scheme, pregnant females were treated daily from the 5 to 15 day of pregnancy (P protocol); or were treated as above plus during lactation from the 2 day after delivery until weaning (pregnancy+lactation: P + L protocol); or the male offspring after the P + L protocol was treated for further 8 weeks in a 5 days per week schedule (P + L + P protocol).

For electrophysiological recording after the treatment period, the rats were anaesthetised with urethane, the skull was opened, and spontaneous electrical activity of the cortex (electro-

corticogram, ECoG), and sensory evoked potentials (EPs) were recorded from the primary sensory areas by silver electrodes. From the rats' tail, compound action potential of the tail nerve was taken. In case of the ECoG, the frequency spectrum was determined (delta, theta etc. waves) and/or ECoG index (obtained as the [delta + theta]/[beta1 + beta2] activity ratio) was calculated. The measured parameters of EPs were latency and duration. The calculated parameters of the tail nerve were conduction velocity and refractory period. Significance of the differences was tested by ANOVA. All investigations were performed in accordance with the principles of the Ethical Committee for the Protection of Animals in Research of our University. Some experiments were done under GLP-certified conditions (certification No: 3837/48/2007, issued by the Hungarian National Institute of Pharmacy).

Results and discussion

Acute nervous system effects of Pb, Hg, Mn and their combinations (*Papp et al.* 2006). Adult male rats were treated with inorganic lead (Pb-acetate, 1000 mg/kg b.w.; doses given for pure metal), mercury ($HgCl_2$, 7 mg/kg) and manganese ($MnCl_2$, 50 mg/kg), and their double combinations (Pb + Hg, 500 + 3.5 mg/kg; Pb + Mn, 500 + 25 mg/kg); in acute application, that is, during recording. The aim was to see the effects on the spontaneous and stimulus-evoked cortical, and the evoked peripheral nervous activity,

Table 1. Relative change (group mean ± SD, n = 8) of the ECoG index ([delta + theta]/[beta1 + beta2] activity ratio), evoked potential amplitude, and evoked potential latency; 60 and 160 min after ip. application of the metals and combinations indicated

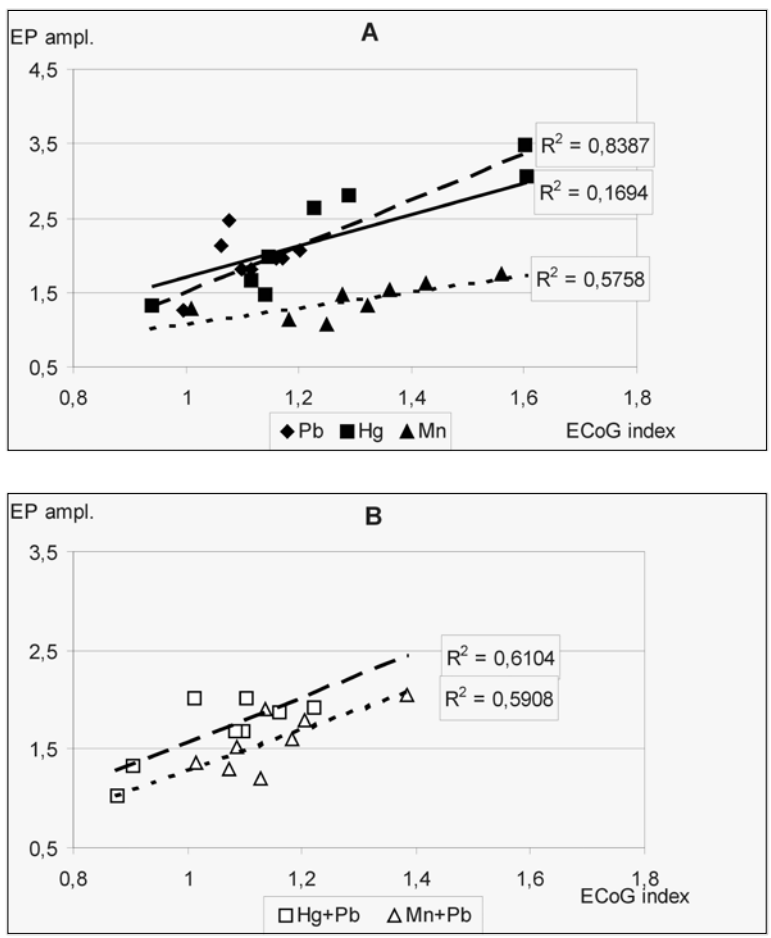
Treatment	ECoG index		EP peak-to-peak amplitude		EP 1st peak latency	
	60 min	160 min	60 min	160 min	60 min	160 min
Control	1.1539 ± 0,0867	1.1634 ± 0.0945	1.0611 ± 0.0799	1.0615 ± 0.0959	1.0065 ± 0.0292	1.0008 ± 0.0441
Pb^{2+}	1.1397 ± 0.0764	1.0871 ± 0.0467	1.8208*± 0.2339	2.4752* ± 0.3742	0.9774 ± 0.0426	1.0029 ± 0.0803
Hg^{2+}	1.1153 ± 0.0852	1.6038* ± 0.1215	1.6557 ± 0.2079	3.0613* ± 0.7013	1.0592* ± 0.0791	1.1673* ± 0.0781
Mn^{2+}	1.1832 ± 0.0708	1.5592* ± 0.0910	1.1423 ± 0.1289	1.7414* ± 01.939	1.0247 ± 0.05588	1.0299 ± 0.03833
Pb^{2+} + Hg^{2+}	1.1002 ± 0.0395	1.2192 ± 0.0566	1.6782 ± 0.1593	1.9203 ± 0.2448	1.0494 ± 0.0734	1.0910* ± 0.1031
Pb^{2+} + Mn^{2+}	1.0707 ± 0.0697	1.3785 ± 0.1725	1.3043 ± 0.1985	2.0489* ± 0.3717	1.0609* ± 0.0526	1.0905* ± 0.05853

Relative change was calculated by normalizing all individual data to the average of the 5 pre-administration control records.

* $p < 0.05$ (LSD after ANOVA).

to detect any interaction of the metals and any correlation between the changes caused in the spontaneous and stimulus-evoked electrical activity, in the primary somatosensory cortical area, and on compound action potential of the tail nerve. On administration of the metals and metal combinations, a shift to lower frequencies was seen in the spectrum of the spontaneous cortical activity, (resulting in increasing ECoG index values, *Table 1.*). The cortical responses showed an increase of the peak-to-peak amplitude and peak latency. The correlation of ECoG index and parameters of the EPs was good for Hg, Mn, and the combinations, but poor for Pb *(Figure 1.).*

Figure 1. Correlation diagram of the ECoG index (abscissa) and cortical evoked potential amplitude (ordinate). Effects of Pb, Hg and Mn given alone (**A**), and effect of the Pb + Hg and Pb + Mn combination (**B**). Symbols for the treatments in the insert below the graph. Linear trend lines fitted by EXCEL. Inserts at the right-side end of the trend lines give the correlation coefficients.



This was interpreted by the effect of the metals on the ascending cholinergic cortical activation (choline acetyltransferase activity is reduced by Hg: *Dwivedi et al.* 1980, and Mn: *Martinez* and *Bonilla* 1981) and on glutamatergic thalamocortical input. Hg inhibits the glial uptake of Glu (*Brookes* 1992), and Mn inhibits its breakdown (*Normandin* and *Hazell* 2002), leading finally to increased cortical excitation. The results with the metal combinations (two halved, themselves ineffective, doses together) indicated synergism. Pb, for example, was alone weaker than the two other metals, but its low dose greatly enhanced the effect of dose of Hg and Mn on the EP, probably due to the breakdown of the blood brain barrier caused by Pb (*Bradbury* and *Deane* 1993).

Subchronic effects of Pb and Hg, interaction with alcohol (*Lukács et al.* 2007).

Absorption, accumulation and toxicity of heavy metals is influenced by a number of nutritional, physiological and environmental factors, alcohol consumption being one of these. In this experiment, 12 weeks oral administration (by gavage) of Pb and Hg to adult male rats was combined with 5% alcohol in the rats' drinking water as shown in *Table 2*.

Table 2. Doses of lead, mercury and alcohol, with the corresponding group codes

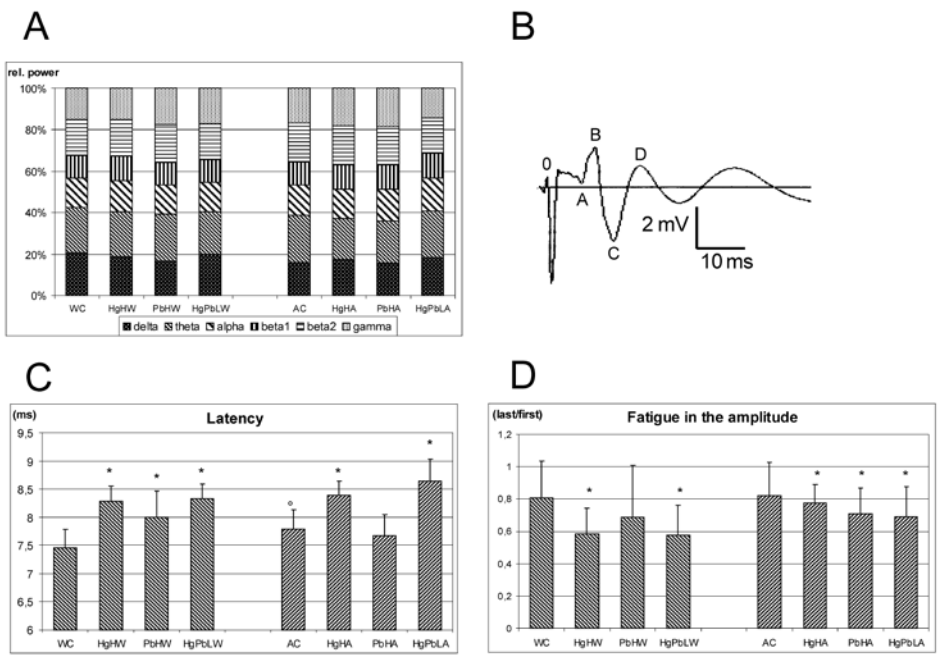
Group code	Treatment	Metal dose[a]	Alcohol
WC	water control	none	none
AC	alcohol control	none	5% in drinking water
PbLW	lead, low dose	80 mg/kg	none
PbHW	lead, high dose	320 mg/kg	none
PbLA	lead, low dose + alcohol	80 mg/kg	5% in drinking water
PbHA	lead, high dose + alcohol	320 mg/kg	5% in drinking water
HgLW	mercury, low dose	0.4 mg/kg	none
HgHW	mercury, high dose	1.6 mg/kg	none
HgLA	mercury, low dose + alcohol	0.4 mg/kg	5% in drinking water
HgHA	mercury, high dose + alcohol	1.6 mg/kg	5% in drinking water
HgPbLW	lead, low dose + mercury, low dose	80 mg/kg + 0.4 mg/kg	none
HgPbLA	lead, low dose + mercury, low dose + alcohol	80 mg/kg + 0.4 mg/kg	5% in drinking water

[a] Lead acetate and mercuric chloride was dissolved in distilled water to yield the given metal doses. Administration volume was 1 ml/kg b.w.

By the end of the 12 weeks, the higher doses of both metals induced some slight changes in the exposed rats' ECoG spectrum from the somatosensory cortex *(Figure 2. A).* The changes in the EP parameters (*Figure 2. B–D*) were more characteristic. Latency was significantly increased by both metals and by their combination in the water-drinking rats. In the alcohol-drinking groups the effect of Pb, but not of Hg, seemed to be abolished by ethanol, which alone caused some latency increase. The decrease of the EP amplitude during a stimulus series (a kind of fatigue, *Papp et al.* 2000) was moderate in the controls but was significantly increased in most metal-treated groups. Alcohol, a lifestyle factor

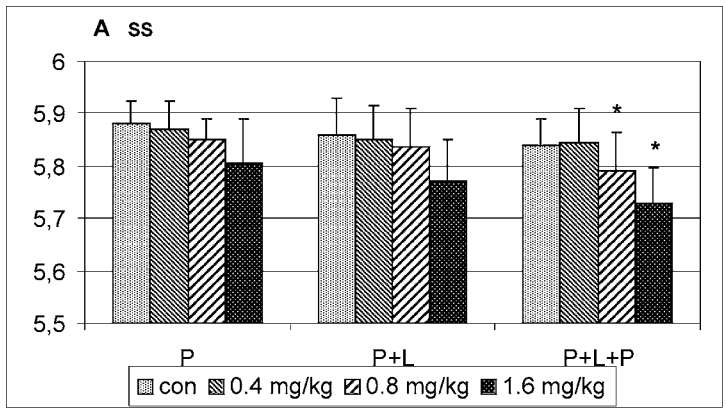
interacting with environmental toxicants (*Maranelli et al.* 1990) is known to enhance the toxicity of numerous chemicals in humans. One mechanism most likely involved in this effect is the increased permeability of the blood-brain-barrier (*Gulati et al.* 1985). Subchronic mercury treatment of rats in different phases of ontogenesis: functional effects on the central and peripheral nervous system (*Papp et al.* 2005).

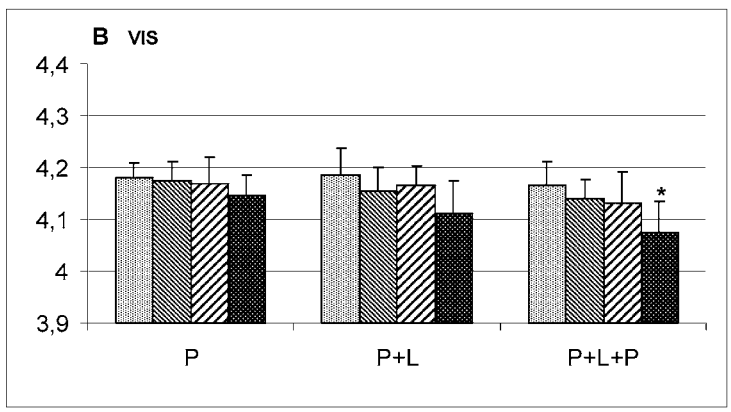
Figure 2. **A:** Power spectrum of the somatosensory cortex ECoG in the control and treated rats. Insert: bar pattern for the frequency bands. For group codes, see *Table 1.*
B: Measurements of the cortical evoked potentials (latency, between 0 and A; duration, between A and D; amplitude, between B and C).
C: Latency of the EP. Mean + SD, n = 10. * $p < 0.05$ vs. control; °$p < 0.05$ alcohol control vs. water control.
D: Fatigue, calculated from the amplitude of the EPs (see text for details).



In this study, the electrophysiological consequences of pre- and/or postnatal oral exposure to Hg were studied. Pregnant female rats were treated, by gavage, with 0.4, 0.8, or 1.6 mg/kg mercury ($HgCl_2$ diluted in distilled water) according to the P, P + L and P + L + P protocols described in Material and methods. From the male offspring, cortical spontaneous and evoked activity, and tail nerve action potential, was recorded at the age of 12 weeks. On the ECoG, a dose- and treatment protocol-dependent shift to lower frequencies was seen *(Figure 3.),* significant with the high dose and in P + L + P. In the high and middle dose P + L + P groups, EP latency was significantly lengthened in all sensory areas recoded *(Figure 4.).*

Figure 3. Changes of the ECoG index in the three cortical centers (**A** somatosensory; **B** visual; **C** auditory). Ordinate, index value (mean + SD), n = 8. Abscissa, treatment protocol. Bar pattern (insert): groups and doses. * $p < 0.05$ vs. control in the same protocol.





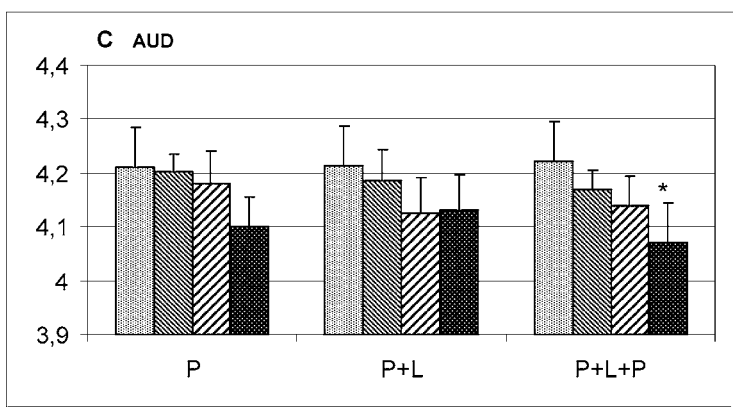
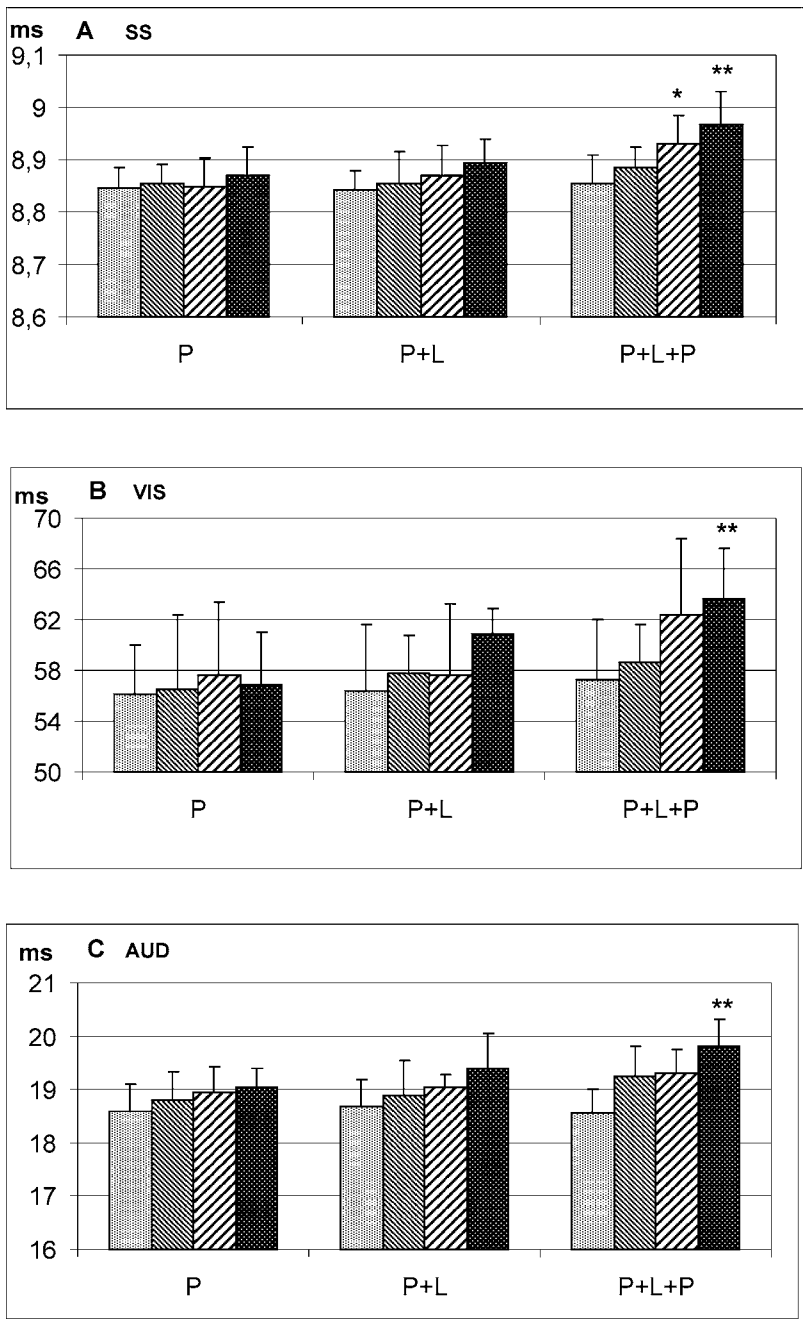
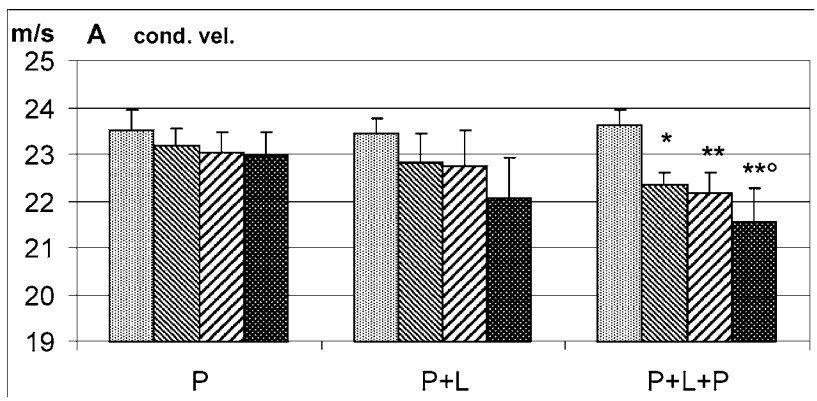


Figure 4. Changes of the latency of evoked potentials. Displayed as in *Figure 1.*
* $p < 0.05$, ** $p < 0.01$ vs. control in the same protocol



The tail nerve conduction velocity was significantly decreased also in the low dose P + L + P group so that this parameter proved to be the most sensitive indicator *(Figure 5.).* In fact, it was found as sensitive as renal pathology (*Dieter et al.* 1992) but can, theoretically, be obtained non-invasively. It was also of interest that 8 weeks oral exposure of rats after intrauterine and milkborne exposure (P + L + P) had stronger effects than the same treatment of intact young rats in an earlier study (*Schulz et al.* 1997). The results emphasize the functional neurotoxic risk arising from the continuous presence of inorganic Hg in the human environment, and point to possible use of early functional changes in monitoring the effects of Hg.

Figure 5. Changes in the conduction velocity of the tail nerve
Displayed as in *Figure 2.*, ° $p < 0.05$ vs. lower doses within the same protocol



Conclusions

Due to the changes in the chemical composition of environmental media caused by human activity, environmental and occupational exposure to toxic heavy metals remains a problem. For a better protection of the population, more has to be learned about the mechanism of toxic actions. Not independently of that, better biomarkers of the resulting functional alteration would also be advantageous, compared to the ones used today which are based mostly on chemical detection of the micropollutants in biological samples or on peripheral biochemical alterations, and may be in loose relationship to damages in the central nervous system (*Manzo et al.* 1996). The results of research done at our Department may contribute to achieving these goals.

Környezeti mikroszennyező nehézfémek idegrendszeri toxicitásának kísérletes vizsgálata

PAPP ANDRÁS – SZABÓ ANDREA – LUKÁCS ANITA – VEZÉR TÜNDE

Szegedi Tudományegyetem
Általános Orvostudományi Kar
Népegészségtani Intézet
Szeged

Összefoglalás

A mikroelemek az ember számára lehetnek esszenciálisak, toxikusak, vagy dózistól függően mindkettők. Jelen közlemény a Szegedi Tudományegyetem Orvostudományi Karának Népegészségtani Intézetében folyó neurotoxikológiai munkából mutat be. A kísérleteket Wistar patkányon végeztük. Pb, Hg, Mn és kombinációik akut beadására a spontán agykérgi tevékenység lassabbá vált, a kiváltott kérgi válaszok amplitúdója és latenciája megnőtt. Ha Pb-t vagy Hg-t, az ivóvízbe adagolt alkohollal kombinálva, 12 hétig adtunk, a kiváltott potenciálok latenciája szignifikánsan nőtt mindkét fémtől és kombinációjuktól, és a Pb, de nem a Hg hatását az alkohol látszólag semlegesítette. Hg-t az egyedfejlődés születés előtti és utáni időszakában adva azt láttuk, hogy a prenatális Hg expozíció erősítette a születés után adott fém hatását, valamint, hogy a perifériás idegi aktivitás változása a Hg hatását ugyanolyan érzékenyen jelezte, mint egyes klasszikus toxikológiai jellemzők. Szükség lenne a fém mikroszennyezők funkcionális hatásaira irányuló, a jelenlegieknél alkalmasabb biomarkerekre. Az intézetben végzett kutatások hozzájárulhatnak ennek eléréséhez.

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