

# Effects on the central and peripheral nervous activity in rats elicited by acute administration of lead, mercury and manganese, and their combinations

András Papp,\* László Pecze, Andrea Szabó and Tünde Vezér

Department of Public Health, University of Szeged, Hungary

Received 19 January 2006; Revised 27 March 2006; Accepted 3 April 2006

**ABSTRACT:** Adult male Wistar rats were treated with inorganic lead, mercury and manganese, and their double combinations, in acute application. The aim was to study the effects on spontaneous and stimulus-evoked cortical, and evoked peripheral, nervous activity, to detect any interaction of the metals and any correlation between the changes caused in the spontaneous and stimulus-evoked electrical activity of the primary somatosensory cortical area, and the compound action potential of the tail nerve. In the frequency distribution of the spontaneous cortical activity, a shift to lower frequencies was seen. The cortical responses evoked by whisker or tail stimulation showed an increase of the peak-to-peak amplitude and peak latency on administration of the metals and metal combinations. With the metal combinations, synergism was observed. Correlations found between alterations of the spontaneous and evoked, or between cortical and peripheral, activity were evaluated in terms of mechanism. According to the results, combined exposure to the three heavy metals studied might lead to synergistic action, indicating an increased health risk in settings with exposure to several heavy metals. Copyright © 2006 John Wiley & Sons, Ltd.

**KEY WORDS:** mercury; lead; manganese; cortical activity; peripheral activity; combination; rat

## Introduction

Heavy metals belong to the environmental and/or occupational toxicants with known neurotoxicity.

Mercury (chemical symbol: Hg) is present in occupational and environmental settings in various chemical forms. Bulk amounts of liquid Hg are used in chloralkali plants or in low-tech gold mining. For the general population, a frequent source of exposure is Hg released from dental amalgam fillings (WHO, 1991). Following occupational exposure to inorganic Hg, alterations in spontaneous (Piikivi and Tolonen, 1989) and stimulus-evoked (Lille *et al.*, 1988; Discalzi *et al.*, 1993) cortical electrical activity have been reported. Mercury in animal experiments affected a number of ion channels in the peripheral and central nervous system (Sirois and Atchison, 1996). Hg<sup>2+</sup> also interfered with calcium homeostasis, by disturbing Ca<sup>2+</sup> uptake to the endoplasmic reticulum (Freitas *et al.*, 1996). In rat brain slices treated with Hg<sup>2+</sup>, a higher than normal release of noradrenaline (Gasso *et al.*, 2000) was described. Increased dopamine release after intrastriatal application of Hg<sup>2+</sup> was also reported (Faro *et al.*, 2001). *In vitro* ligand binding of rat cortical muscarinic receptors also was

negatively affected by Hg<sup>2+</sup> (Castoldi *et al.*, 1996). Previous studies by our group evaluated the effects of Hg<sup>2+</sup> on cortical activity. In these studies rats, receiving subchronic oral HgCl<sub>2</sub> treatment at 0.4 and 1.6 mg kg<sup>-1</sup>, showed alterations in spontaneous (Dési *et al.*, 1996) and stimulus evoked (Schulz *et al.*, 1997) cortical activity.

Lead (Pb) exposure is seen in the primary production and reclamation of this metal and its processing to batteries, piping, paints, solders etc. The use of tetraethyl lead as a petrol additive is still common practice in certain countries, resulting in airborne population exposure. In lead-exposed humans, various forms of central and peripheral evoked activity, such as sensory evoked potentials and nerve conduction velocity, were affected (Lille *et al.*, 1988; Discalzi *et al.*, 1993; Araki *et al.*, 2000). Deposited in the central nervous system, first of all in the cortex and hippocampus (Grandjean, 1978), Pb<sup>2+</sup> disturbs the regulatory functions of Ca<sup>2+</sup> (Sandhir and Gill, 1993; Bettaiya *et al.*, 1996), and in this way affects several transmitter systems. In synaptosomes from brains of rats exposed to Pb<sup>2+</sup> (ca. 2 mg per os for 3 months; Jablonska *et al.*, 1994) GABA uptake was decreased and dopamine uptake increased. Alterations in the dopaminergic, cholinergic and glutamatergic control of behavior were observed in Pb<sup>2+</sup>-treated animals (Cory-Slechta, 1995). In our earlier studies (Nagymajtényi *et al.*, 1997) changes of cortical electrical activity and of open field behavior were seen in rats after 4 to 12 weeks oral exposure to 80 and 320 mg kg<sup>-1</sup> Pb<sup>2+</sup>.

\* Correspondence to: A. Papp, Department of Public Health, University of Szeged, H-6720 Szeged, Dóm tér 10, Hungary.  
E-mail: ppp@puhe.szote.u-szeged.hu

In contrast to lead and mercury, manganese (Mn) is essential for living organisms in small amounts and toxic only when overdosed. The presence of Mn in the general environment is partly due to human activity including the use of methylcyclopentadienyl manganese tricarbonyl (MMT) as an anti-knock petrol additive in certain countries (Lynam *et al.*, 1999). Other Mn-compounds have widespread agricultural application as fungicides (Ferraz *et al.*, 1988). Beyond these, spent dry cells contribute to the Mn content of solid household waste. Inorganic Mn, once absorbed into the bloodstream, can pass the blood-brain barrier in transferrin-bound form, and as free  $Mn^{2+}$  ion via a cation transporter (Aschner *et al.*, 1999) and can deposit in the brain. In chronic human disease resulting from long-term occupational exposure to Mn, functional (Shinotoh *et al.*, 1997) and structural (Yamada *et al.*, 1986) damage to the dopaminergic systems were described. In animals, Mn was found to interfere with CNS synaptic functions in several ways.  $Mn^{2+}$  is known to block voltage-dependent Ca-channels of neurons and presynaptic endings (Nelson, 1986). Calcium currents of cortical neurons, induced by local application of excitatory amino acids, were blocked by  $Mn^{2+}$  (Pumain *et al.*, 1987). The release of the excitatory transmitters themselves, and of GABA, was found to be reduced by moderate doses of  $Mn^{2+}$  (Takeda, 2003). Another effect of  $Mn^{2+}$ , inhibition of astrocytic glutamate uptake, can, on the contrary, enhance synaptic transmission in the cortex (demonstrated *in vitro* with 100  $\mu M$   $Mn^{2+}$ ; Hazell and Norenberg, 1997).

The aim of the present work was to see how spontaneous and stimulus-evoked cortical and stimulus-evoked peripheral electrical activity of rats was altered by acute doses of lead, mercury and manganese and combinations of these.

## Materials and Methods

Adult male Wistar rats of ca. 350 g b.w. were used in the experiments. The rats were obtained from the Breeding

Center of the University and kept under standard conditions (up to five rats per cage, 12 h light–dark cycle,  $22 \pm 2$  °C,  $70 \pm 10\%$  humidity, standard rodent food and water *ad libitum*) until used. For preparation, the animals were anesthetized by i.p. urethane (1000 mg  $kg^{-1}$  b.w. i.p.; Bowman and Rand, 1980), the head was clamped in a frame and the left hemisphere was exposed. Wounds were sprayed with 10% lidocaine, the exposed cortex was covered with warm paraffin oil, and the animals (wrapped in a warm cloth) were put aside for a 30 to 40 min recovery. Then, the rats were moved to the stereotaxic apparatus, where the head was fixed and ball-tipped silver electrodes were positioned on the somatosensory projection area of the whiskers (barrel field; Tracey and Waite, 1995) and of the tail. The corresponding peripheral sites (whiskery skin and base of tail) were stimulated with weak electric pulses (ca. 4 V, 0.05 ms) at 1 Hz frequency. First, 5 min of spontaneous activity (electrocorticogram, ECoG) was taken from both sites simultaneously, then one train of 20 stimuli was applied to each of the peripheral sites and the cortical evoked potentials (EPs) were recorded. During stimulation of the tail, the compound action potential of the tail nerve was also recorded. This scheme was repeated every 20 min. Recording and analysis of the activities was PC-based using the NEUROSYS software (Experimetria Ltd, UK). The stereotaxic apparatus had a heated support plate (36.5 °C) to help maintain constant body temperature of the rats during the whole recording.

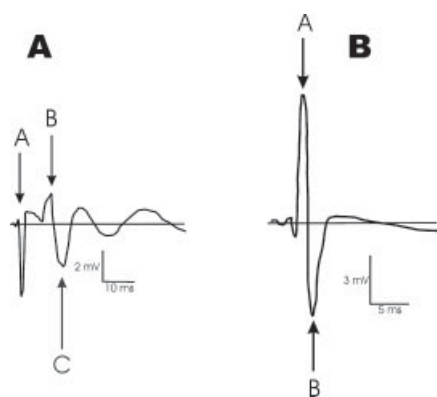
Five pre-treatment control records were taken, then one of the metals or metal combinations (Table 1) was administered via a peritoneal cannula, and a further ten records were made (after 5 h of deep anesthesia, the general state of the animal usually started to deteriorate and the experiment was finished by an overdose of urethane).

From the ECoGs, the software automatically determined the band activities (EEG standard, delta to gamma; Kandel and Schwarz, 1985). From that, the so-called ECoG index (relation of the low and high frequencies in the recorded ECoG;  $[\delta + \theta]/[\beta_1 + \beta_2]$ ) was calculated. Records of the cortical and peripheral evoked

**Table 1.** Doses of the metals and combinations used in the experiments

Group	Chemical	Dose (mg $kg^{-1}$ b.w.)	
Control	(none) distilled water		
$Pb^{2+}$	$Pb(CH_3COO)_2 \cdot 3 H_2O$	High	1000
		Low	500
$Hg^{2+}$	$HgCl_2$	High	7
		Low	3.5
$Mn^{2+}$	$MnCl_2 \cdot 4 H_2O$	High	50
		Low	25
$Pb^{2+} + Hg^{2+}$		Low + low	500 + 3.5
$Pb^{2+} + Mn^{2+}$		Low + low	500 + 25

Solutions were made up in distilled water to give an administration volume of 1 ml  $kg^{-1}$  b.w. The doses were chosen to cause a significant effect within the time limits of the acute preparation, as described in Pecze *et al.* (2003).



**Figure 1.** Measurements on the records of evoked activity. (A) Somatosensory cortical evoked potential. 1st peak latency was measured between A and B, and peak-to-peak amplitude, between B and C. (B) Tail nerve compound action potential. 1st peak latency was measured between time zero and A, and peak-to-peak amplitude, between A and B

activity were averaged, and the 1st peak latency and peak-to-peak amplitude of EP or nerve action potential was measured (Fig. 1). To make the results comparable from animal to animal and from group to group (consisting of eight animals each, including eight untreated parallel controls), the average of the four or five pre-administration control records was taken as a base and all individual values were normalized to that.

As the effect of the metals developed during the period following administration, the difference in time trend of the measured parameters (amplitude, latency, EEG index) of the control and treated animals was a suitable indicator of the effect. The significance of the differences was tested by means of the ANOVA-based 'General Linear Model' in the SPSS 9.0 software pack (SPSS Inc., Chicago, Ill. USA; see Pecze *et al.*, 2003 for details). The method of least square differences (LSD) was used for post-hoc testing. A possible correlation between the

different parameters, as an indicator of a mechanistic relationship, was tested by generating parameter to parameter plots in EXCEL and obtaining the regression coefficient ( $R^2$ ).

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

## Results

### Effects on Spontaneous Cortical Activity

The effect of the metals and metal combinations on the spontaneous activity, if there was an effect at all, was a shift to lower frequencies. As shown by the ECoG index data in Table 2, the high dose  $Hg^{2+}$  generally had the strongest effect while the change caused by the high dose  $Pb^{2+}$  was insignificant. The frequency shift in the barrel field started with some delay (longer in the case of  $Hg^{2+}$  than with  $Mn^{2+}$ ) after the metal had been injected, and continued up to the end of recording (160 min after application). By then, the effect of the high dose  $Hg^{2+}$  and  $Mn^{2+}$  was significant ( $F[3,28] = 9.41$ ,  $P < 0.001$ ; Table 2). Comparison of the effect of the metals at the low dose alone, and in the combinations listed in Table 1, showed that  $Pb^{2+}$  at either dose had no noteworthy influence on the ECoG frequency distribution alone, and had no interaction with the two other metals.

### Effects on Somatosensory Evoked Cortical Activity

The investigated metals and combinations all caused an increase in the amplitude of EP obtained by whisker stimulation. Again,  $Hg^{2+}$  had the strongest effect, followed by  $Pb^{2+}$  and  $Mn^{2+}$  (Table 2). The increase was significant vs control ( $F[3,28] = 25.81$ ,  $P < 0.001$ ) with all

**Table 2.** Relative change (group mean  $\pm$  SD,  $n = 8$ ) of the ECoG index, evoked potential amplitude, and evoked potential latency (in the barrel field); 60 and 160 min after i.p. 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 $\pm$ 0.0867	1.1634 $\pm$ 0.0945	1.0611 $\pm$ 0.0799	1.0615 $\pm$ 0.0959	1.0065 $\pm$ 0.0292	1.0008 $\pm$ 0.0441
$Pb^{2+}$ High	1.1397 $\pm$ 0.0764	1.0871 $\pm$ 0.0467	1.8208 <sup>a</sup> $\pm$ 0.2339	2.4752 <sup>a</sup> $\pm$ 0.3742	0.9774 $\pm$ 0.0426	1.0029 $\pm$ 0.0803
$Pb^{2+}$ Low	1.1568 $\pm$ 0.1028	1.1089 $\pm$ 0.1460	0.9147 $\pm$ 0.0725	1.0673 $\pm$ 0.0995	0.9944 $\pm$ 0.01723	0.9986 $\pm$ 0.0307
$Hg^{2+}$ High	1.1153 $\pm$ 0.0852	1.6038 <sup>a</sup> $\pm$ 0.1215	1.6557 $\pm$ 0.2079	3.0613 <sup>a</sup> $\pm$ 0.7013	1.0592 <sup>a</sup> $\pm$ 0.0791	1.1673 <sup>a</sup> $\pm$ 0.0781
$Hg^{2+}$ Low	1.0254 $\pm$ 0.1053	1.2138 $\pm$ 0.1184	1.0611 $\pm$ 0.059	1.4579 $\pm$ 0.1057	0.9907 $\pm$ 0.0253	0.9978 $\pm$ 0.0441
$Mn^{2+}$ High	1.1832 $\pm$ 0.0708	1.5592 <sup>a</sup> $\pm$ 0.0910	1.1423 $\pm$ 0.1289	1.7414 <sup>a</sup> $\pm$ 0.1939	1.0247 $\pm$ 0.05588	1.0299 $\pm$ 0.03833
$Mn^{2+}$ Low	1.1451 $\pm$ 0.0406	1.3262 $\pm$ 0.1017	1.0232 $\pm$ 0.0514	1.2659 $\pm$ 0.0711	1.0100 $\pm$ 0.06489	0.9901 $\pm$ 0.05385
$Pb^{2+}$ + $Hg^{2+}$ Low + low	1.1002 $\pm$ 0.0395	1.2192 $\pm$ 0.0566	1.6782 $\pm$ 0.1593	1.9203 $\pm$ 0.2448	1.0494 $\pm$ 0.0734	1.0910 <sup>a</sup> $\pm$ 0.1031
$Pb^{2+}$ + $Mn^{2+}$ Low + low	1.0707 $\pm$ 0.0697	1.3785 $\pm$ 0.1725	1.3043 $\pm$ 0.1985	2.0489 <sup>a</sup> $\pm$ 0.3717	1.0609 <sup>a</sup> $\pm$ 0.0526	1.0905 <sup>a</sup> $\pm$ 0.05853

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

<sup>a</sup> $P < 0.05$  (LSD after ANOVA).

**Table 3.** Relative change (group mean  $\pm$  SD,  $n = 8$ ) of the conduction velocity and compound action potential amplitude in the tail nerve; 60 and 160 min after i.p. application of the metals and combinations indicated

Treatment	Conduction velocity		Action potential peak-to-peak amplitude	
	60 min	160 min	60 min	160 min
Control	1.0067 $\pm$ 0.0068	0.9851 $\pm$ 0.0141	0.9830 $\pm$ 0.0451	1.0167 $\pm$ 0.0359
Pb <sup>2+</sup>	0.9909 $\pm$ 0.0081	0.8891 <sup>a</sup> $\pm$ 0.0246	0.9324 $\pm$ 0.1189	0.6586 <sup>a</sup> $\pm$ 0.0489
Pb <sup>2+</sup>	0.9909 $\pm$ 0.0081	0.8891 <sup>a</sup> $\pm$ 0.0246	0.9324 $\pm$ 0.1189	0.6586 <sup>a</sup> $\pm$ 0.0489
Pb <sup>2+</sup>	0.9909 $\pm$ 0.0081	0.8891 <sup>a</sup> $\pm$ 0.0246	0.9324 $\pm$ 0.1189	0.6586 <sup>a</sup> $\pm$ 0.0489
Hg <sup>2+</sup>	0.9879 $\pm$ 0.0134	0.8607 <sup>a</sup> $\pm$ 0.0128	1.0535 $\pm$ 0.1033	0.4146 <sup>a</sup> $\pm$ 0.0765
Hg <sup>2+</sup>	0.9879 $\pm$ 0.0134	0.8607 <sup>a</sup> $\pm$ 0.0128	1.0535 $\pm$ 0.1033	0.4146 <sup>a</sup> $\pm$ 0.0765
Hg <sup>2+</sup>	0.9879 $\pm$ 0.0134	0.8607 <sup>a</sup> $\pm$ 0.0128	1.0535 $\pm$ 0.1033	0.4146 <sup>a</sup> $\pm$ 0.0765
Mn <sup>2+</sup>	0.9911 $\pm$ 0.0437	0.9187 <sup>a</sup> $\pm$ 0.0594	0.8517 $\pm$ 0.0326	0.6822 <sup>a</sup> $\pm$ 0.0626
Mn <sup>2+</sup>	0.9911 $\pm$ 0.0437	0.9187 <sup>a</sup> $\pm$ 0.0594	0.8517 $\pm$ 0.0326	0.6822 <sup>a</sup> $\pm$ 0.0626
Mn <sup>2+</sup>	0.9911 $\pm$ 0.0437	0.9187 <sup>a</sup> $\pm$ 0.0594	0.8517 $\pm$ 0.0326	0.6822 <sup>a</sup> $\pm$ 0.0626
Pb <sup>2+</sup> + Hg <sup>2+</sup>	0.9786 $\pm$ 0.0242	0.8603 <sup>a</sup> $\pm$ 0.0709	1.1087 $\pm$ 0.0415	0.5612 <sup>a</sup> $\pm$ 0.1394
Pb <sup>2+</sup> + Mn <sup>2+</sup>	0.9791 $\pm$ 0.0131	0.8689 <sup>a</sup> $\pm$ 0.1057	0.9592 $\pm$ 0.0742	0.4124 <sup>a</sup> $\pm$ 0.0828

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

<sup>a</sup>  $P < 0.05$  (LSD after ANOVA).

three metals given at the high dose. The interaction of the metals had a much more marked effect on the EP amplitude than on the ECoG. The low dose Mn<sup>2+</sup> and Pb<sup>2+</sup> had a slight effect; compared to which the effect of Mn<sup>2+</sup> + Pb<sup>2+</sup> was a significant increase in amplitude ( $F[4,35] = 10.87$ ,  $P < 0.001$ ). The increase was also stronger than that caused by the high dose Mn<sup>2+</sup>. Pb<sup>2+</sup> and Hg<sup>2+</sup> were in positive interaction, where the resulting effect was greater than that of the low dose Pb<sup>2+</sup> or Hg<sup>2+</sup> alone, but smaller than that of the high dose Hg<sup>2+</sup> ( $F[4,35] = 11.69$ ,  $P < 0.001$ ).

The 1st peak latency of the somatosensory EP was increased to a considerable extent only by the high dose Hg<sup>2+</sup> ( $F[3,28] = 61.24$ ,  $P < 0.001$ ). In the Pb<sup>2+</sup>- and Mn<sup>2+</sup>-treated animals, the latency remained at about the control level. The effect of the combinations was similar to that on the amplitude: Pb<sup>2+</sup> + Mn<sup>2+</sup> showed a strong positive interaction ( $F[4,35] = 71.69$ ,  $P < 0.001$ ) but the latency increase obtained by Pb<sup>2+</sup> + Hg<sup>2+</sup> was between that of the low dose Hg<sup>2+</sup> alone and the high dose Hg<sup>2+</sup> ( $F[4,35] = 11.43$ ,  $P < 0.001$ ).

### Effects on the Tail Nerve

The conduction velocity of the tail nerve was significantly reduced by the high dose of all three metals (Table 3;  $F[3,28] = 14.55$ ,  $P < 0.001$ ). The magnitude of change was very similar with each metal. With the low doses, only Hg<sup>2+</sup> had a moderate but significant effect. When applied in combination, the effect of both Pb<sup>2+</sup> + Hg<sup>2+</sup> ( $F[4,35] = 14.08$ ,  $P < 0.001$ ) and Pb<sup>2+</sup> + Mn<sup>2+</sup> ( $F[4,35] = 49.91$ ,  $P < 0.001$ ) was significant vs control and indicated a positive interaction. The low dose Hg, together with low dose Pb<sup>2+</sup>, caused about an equal change to the high dose Hg alone. In the case of Pb<sup>2+</sup> + Mn<sup>2+</sup>, the effect of the combination was greater than that of high dose Mn<sup>2+</sup>.

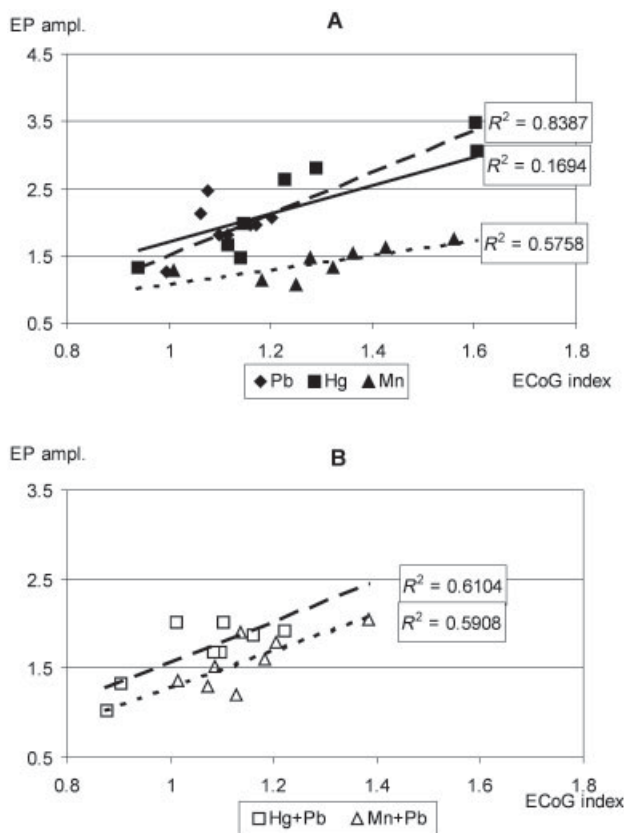
The tail nerve action potential amplitude was significantly diminished by the metals given in high dose (Table 3;  $F[3,28] = 9.51$ ,  $P < 0.001$ ). Again, the effect of Hg<sup>2+</sup> was the strongest (the effect of the low dose was significant, too), whereas that of Mn<sup>2+</sup> and Pb<sup>2+</sup> were quite similar. In the combinations, Pb<sup>2+</sup> + Hg<sup>2+</sup> had about the same effect as the high dose Hg alone ( $F[4,35] = 7.55$ ,  $P < 0.001$ ). The effect of low dose Mn<sup>2+</sup> was greatly potentiated by Pb<sup>2+</sup> also on this parameter ( $F[4,35] = 20.79$ ,  $P < 0.001$ ).

### Correlation between the Individual Effects

A correlation between the actual values of two measured parameters of the central or peripheral nervous system may indicate an underlying common mechanism. In the present experiment, a correlation of the ECoG index (representing spontaneous cortical activity) and the parameters of the cortical EP was obtained. It was supposed that the actual state of the cortex, reflected in the ECoG, was determined the amplitude and latency of the EPs. Figure 2A shows the correlation of ECoG index and EP amplitude in the case of the three metals given separately at the high dose. For Hg<sup>2+</sup> and Mn<sup>2+</sup>, the correlation was fair, but was rather poor for Pb<sup>2+</sup>. From the combinations (Fig. 2B), both Pb<sup>2+</sup> + Hg<sup>2+</sup> and Pb<sup>2+</sup> + Mn<sup>2+</sup> gave a rather good correlation.

The correlation of the ECoG index and EP latency is shown in Fig. 3. The relationship was solid in the case of Hg<sup>2+</sup>, both alone in high dose (Fig. 3A) and in combination with Pb<sup>2+</sup> (Fig. 3B), but the other two metals showed minimal correlation. The effect of Mn<sup>2+</sup> was stronger in combination with Pb<sup>2+</sup> than alone but, even in the former case,  $R^2$  was rather low.

As the actual shape of an EP can also be influenced by the peripheral input reaching the cortex, the correlation between the parameters of the tail nerve response and the

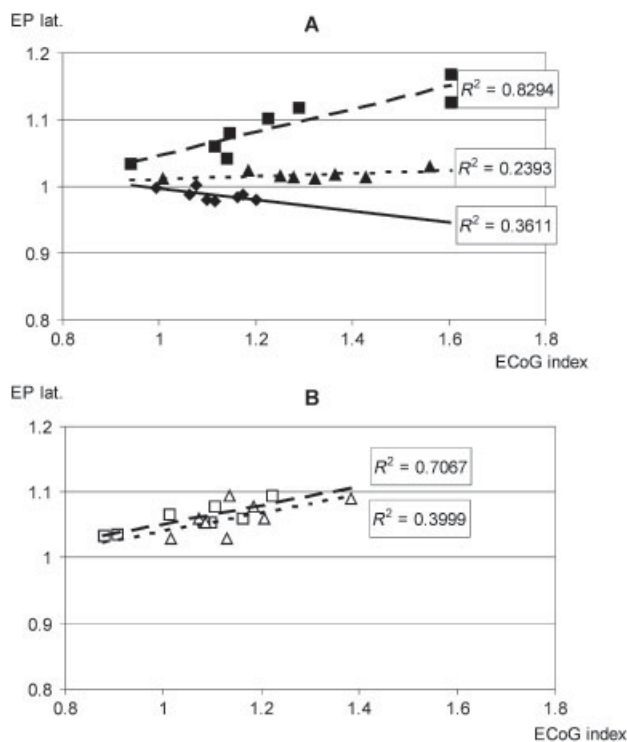


**Figure 2.** Correlation diagram of the ECoG index (abscissa) and cortical evoked potential amplitude (ordinate). Effects of high dose  $Pb^{2+}$ ,  $Hg^{2+}$  and  $Mn^{2+}$  given alone (A), and effect of the  $Pb^{2+} + Hg^{2+}$  and  $Pb^{2+} + Mn^{2+}$  combinations (B). Symbols for the treatments given in the insert below the graph. Linear trend lines fitted by EXCEL: (A) solid,  $Pb^{2+}$ ; long dashed,  $Hg^{2+}$ ; short dashed,  $Mn^{2+}$ ; (B) long dashed,  $Pb^{2+} + Hg^{2+}$ ; short dashed,  $Pb^{2+} + Mn^{2+}$ . Inserts at the right-side end of the trend lines give the correlation coefficients

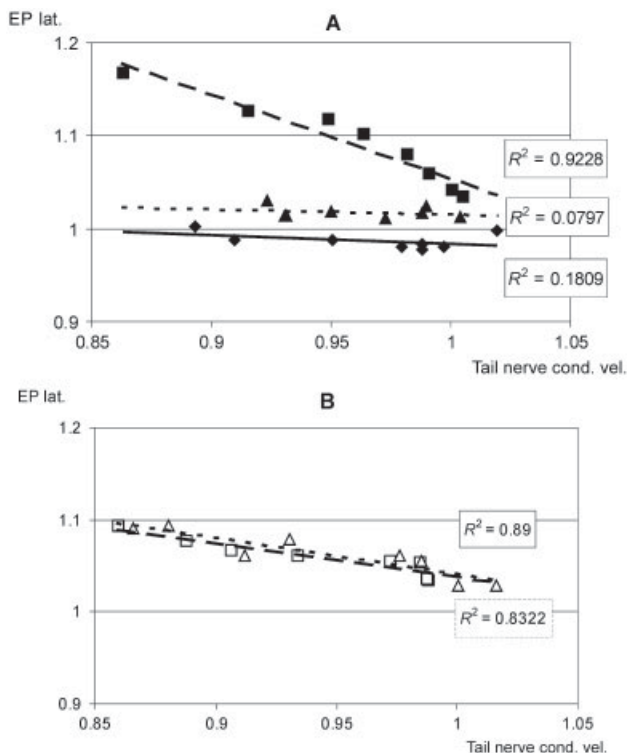
cortical EP was possibly also of interest. As seen in Fig. 4A,  $Hg^{2+}$  was the only metal for which there seemed to be a connection between the peripheral conduction velocity and the cortical response latency. The combinations (Fig. 4B) showed also a fair correlation.

**Discussion**

In the case of the acute effects of any external agent, the rate of absorption and transport to the site of action is crucial. The absorption of the three metals studied into the bloodstream, after i.p. injection, is good and fast (WHO, 1977, 1981, 1991). The blood–brain barrier can be passed by Mn in the free ionic ( $Mn^{2+}$ ) or transferrin-bound form (Aschner *et al.*, 1999). Both  $Hg^{2+}$  (Szumanska *et al.*, 1993) and  $Pb^{2+}$  (Bradbury and Deane, 1993) are known to damage the barrier in higher



**Figure 3.** Correlation diagram of the ECoG index and the cortical evoked potential 1st peak latency. Displayed as in Fig. 2



**Figure 4.** Correlation diagram of the tail nerve conduction velocity (abscissa) and the cortical evoked potential 1st peak latency (ordinate). Displayed as in Fig. 2, dashed insert frame indicates the  $R^2$  for  $Pb^{2+} + Mn^{2+}$



concentrations – which was most probably reached in this acute, high-dose administration. This then resulted in high levels in the brain, and was a possible explanation of the synergism observed in the  $\text{Hg}^{2+} + \text{Pb}^{2+}$  and  $\text{Mn}^{2+} + \text{Pb}^{2+}$  combinations.

The effect of  $\text{Hg}^{2+}$  and  $\text{Mn}^{2+}$  on the spontaneous cortical activity was similar. One of the major modulating factors of cortical activity is the ascending cholinergic activation (Metherate *et al.*, 1992) through the reticular formation.  $\text{Hg}^{2+}$  is known to decrease the activity of choline acetyltransferase (Dwivedi *et al.*, 1980) and the binding of acetylcholine to the muscarinic receptors (Rajanna *et al.*, 1997), resulting in reduced activation reaching the cortex. In the case of  $\text{Mn}^{2+}$  no effect on the muscarinic receptors has been described, but inhibition of choline acetyltransferase in different parts of the brain is known (Lai *et al.*, 1981; Martinez and Bonilla, 1981). Considering the rather good correlation of the ECoG index and the parameters of the cortical evoked potential in the case of exposure to high-dose  $\text{Hg}^{2+}$  and  $\text{Mn}^{2+}$ , as well as to  $\text{Hg}^{2+} + \text{Pb}^{2+}$  and  $\text{Mn}^{2+} + \text{Pb}^{2+}$  combinations, a possible explanation could be the known relationship of spontaneous and evoked cortical activity. Here, the preponderance of lower frequencies (indicated in our work by the higher ECoG index) is concomitant with more pronounced evoked responses (Herz *et al.*, 1967; Rémond and Lesévre, 1967).

Beyond that, effects on the specific ascending pathways may also have contributed to the effects of the metals on the cortical EPs. The excitatory thalamocortical input is glutamatergic.  $\text{Hg}^{2+}$  inhibits the glial uptake of Glu (Brookes, 1992), and  $\text{Mn}^{2+}$  inhibits its breakdown (Normandin and Hazell, 2002), leading finally to increased cortical excitation. The effect of  $\text{Pb}^{2+}$  in our study was weaker than that of the two other metals, but low-dose  $\text{Pb}^{2+}$  greatly enhanced the effects of the low dose (in itself ineffective) of  $\text{Hg}^{2+}$  and  $\text{Mn}^{2+}$  on the EPs. This synergism was probably due to the breakdown of the blood–brain barrier caused by  $\text{Pb}^{2+}$  (Bradbury and Deane, 1993).

In the case of cortical responses evoked by peripheral stimulation, any effect on the peripheral impulse conduction is probably reflected in some parameters of the cortical EP. The good correlation of the cortical EP latency and the peripheral conduction velocity with  $\text{Hg}^{2+}$  and with the metal combinations was in line with the above. Cationic channels of neurons and axons, first of all  $\text{Ca}^{2+}$  channels, are known target sites of heavy metals (Nelson, 1986; Büsselberg, 1995). Beyond this general mechanism, damage to (motor) axons by  $\text{Hg}^{2+}$  was described by Pamphlett and Coote (1998).  $\text{Pb}^{2+}$ , acting on voltage-dependent  $\text{Ca}^{2+}$  and  $\text{Ca}^{2+}$ -activated  $\text{K}^{+}$  channels, affects ion transport through the neuron membrane (Reuveny and Narahashi, 1991) and could slow down the conduction of action potentials. However,  $\text{Pb}^{2+}$ , alone, in our experiments had a minimal effect on the cortical EP latency,

and the correlation of the EP latency and the peripheral conduction velocity was very poor. Thus the action of  $\text{Pb}^{2+}$  alone was probably negligible, and the synergism, reflected in the good correlation of the EP latency and the peripheral conduction velocity in the  $\text{Hg}^{2+} + \text{Pb}^{2+}$  and  $\text{Mn}^{2+} + \text{Pb}^{2+}$  combinations, was instead due to the blood–brain barrier effect mentioned above.

Such an approach to the effects of heavy metals on the nervous system could provide a better understanding of the mechanisms involved. In further experiments, including different exposure schemes (subacute and subchronic), certain alterations in the activity forms, reacting on exposure to Hg, Pb or Mn in a sensitive and specific way, could possibly be identified. These, in turn, have the potential to be developed into practical biomarkers of human heavy metal exposure, as has been suggested in the case of various other neurotoxic xenobiotics (Dési and Nagymajtényi, 1999; Papp *et al.*, 2000, 2001).

## References

- Araki S, Sato H, Yokoyama K, Murata K. 2000. Subclinical neurophysiological effects of lead: A review on peripheral, central, and autonomic nervous system effects in lead workers. *Am. J. Ind. Med.* **37**: 193–204.
- Aschner M, Vrana KE, Zheng W. 1999. Manganese uptake and distribution in the central nervous system (CNS). *Neurotoxicology* **20**: 173–180.
- Bettaiya R, Yallapragada PR, Hall E, Rajana S. 1996. *In vitro* effect of lead on  $\text{Ca}^{2+}$ -ATPase in synaptic plasma membranes and microsomes of rat cerebellar cortex and cerebellum. *Ecotoxicol. Environ. Saf.* **33**: 157–162.
- Bowman WC, Rand MJ. 1980. *Textbook of Pharmacology*. Blackwell Scientific Publications: Oxford; 7.15.
- Bradbury MW, Deane R. 1993. Permeability of the blood–brain barrier to lead. *Neurotoxicology* **14**: 131–136.
- Brookes N. 1992. *In vivo* evidence for the role of glutamate in the CNS toxicity of mercury. *Toxicology* **76**: 245–256.
- Büsselberg D. 1995. Calcium channels as target sites of heavy metals. *Toxicol. Lett.* **82**: 255–261.
- Castoldi AF, Candura SM, Costa F, Manzo L, Costa LG. 1996. Interaction of mercury compounds with muscarinic receptor subtypes in the rat brain. *Neurotoxicology* **17**: 735–741.
- Cory-Slechta DA. 1995. Relationships between lead-induced learning impairments and changes in dopaminergic, cholinergic and glutamatergic neurotransmitter system functions. *Annu. Rev. Pharmacol. Toxicol.* **35**: 391–415.
- Dési I, Nagymajtényi L. 1999. Electrophysiological biomarkers of an organophosphorus pesticide, dichlorvos. *Toxicol. Lett.* **107**: 55–64.
- Dési I, Nagymajtényi L, Schulz H. 1996. Effect of subchronic mercury exposure on electrocorticogram of rats. *Neurotoxicology* **17**: 719–724.
- Discalzi G, Fabbro D, Meliga F, Mocellini A, Capellaro F. 1993. Effects of occupational exposure to mercury and lead on brainstem auditory evoked potentials. *J. Psychophysiol.* **14**: 21–25.
- Dwivedi C, Raghunathan, R, Joshi BC, Foster HW. 1980. Effect of mercury compounds on acetylcholine transferase. *Res. Commun. Chem. Pathol. Pharmacol.* **30**: 381–384.
- Faro LR, do Nascimento JL, Alfonso M, Duran R. 2001. *In vivo* effects of inorganic mercury ( $\text{HgCl}_2$ ) on striatal dopaminergic system. *Ecotoxicol. Environ. Saf.* **48**: 263–267.
- Ferraz HB, Bertolucci PH, Pereira JS, Lima JG, Andrade LA. 1988. Chronic exposure to the fungicide maneb may produce symptoms and signs of CNS manganese intoxication. *Neurology* **38**: 550–553.

- Freitas AJ, Rocha JBT, Wolosker H, Souza DOG. 1996. Effects of  $Hg^{2+}$  and  $CH_3Hg^+$  on  $Ca^{2+}$  fluxes in rat brain synaptosomes. *Brain Res.* **738**: 257–264.
- Gasso S, Sunol C, Sanfeliu C, Rodríguez-Farre E, Cristofol RM. 2000. Pharmacological characterization of the effects of methylmercury and mercuric chloride on spontaneous noradrenaline release from rat hippocampal slices. *Life Sci.* **67**: 1219–1231.
- Grandjean P. 1978. Regional distribution of lead in human brains. *Toxicology* **2**: 65–69.
- Hazell AS, Norenberg MD. 1997. Manganese decreases glutamate uptake in cultured astrocytes. *Neurochem. Res.* **22**: 1443–1447.
- Herz A, Fraling F, Niedner I, Färber G. 1967. Pharmacologically induced alterations of cortical and subcortical evoked potentials compared with physiological changes during the awake-sleep cycle in cats. In *The Evoked Potentials*, Cobb W, Morocutti C (eds). Elsevier: Amsterdam; 165–176.
- Jablonska L, Walski M, Rafalowska U. 1994. Lead as an inductor of some morphological and functional changes in synaptosomes from rat brain. *Cell. Mol. Neurobiol.* **14**: 701–707.
- Kandel ER, Schwartz JH. 1985. *Principles of Neural Science*. Elsevier: New York; 643–644.
- Lai JC, Leung TK, Kim L. 1981. Brain regional distribution of glutamic acid decarboxylase, choline acetyltransferase, and acetylcholinesterase in the rat. Effects of chronic manganese chloride administration after two years. *J. Neurochem.* **36**: 1143–1148.
- Lille F, Hazemann P, Garnier R, Dally S. 1988. Effects of lead and mercury intoxications on evoked potentials. *J. Toxicol. Clin. Toxicol.* **26**: 103–116.
- Lynam DR, Roos JW, Pfeifer GD, Fort BF, Pullin TG. 1999. Environmental effects and exposures to manganese from use of methylcyclopentadienyl manganese tricarbonyl (MMT) in gasoline. *Neurotoxicology* **20**: 145–150.
- Martinez H, Bonilla E. 1981. Water intake and brain choline acetyltransferase and acetylcholinesterase activities in manganese treated rats. *Neurobehav. Toxicol. Teratol.* **3**: 277–280.
- Metherate R, Cox CL, Ashe JH. 1992. Cellular bases of neocortical activation: modulation of neural oscillations by the nucleus basalis and endogenous acetylcholine. *J. Neurosci.* **12**: 4701–4711.
- Nagymajtényi L, Schulz H, Papp A, Dési I. 1997. Behavioural and electrophysiological changes caused by subchronic lead exposure in rats. *Centr. Eur. J. Occup. Environ. Med.* **3**: 195–209.
- Nelson MT. 1986. Interactions of divalent cations with single calcium channels from rat brain synaptosomes. *J. Gen. Physiol.* **87**: 201–222.
- Normandin L, Hazell AS. 2002. Manganese neurotoxicity: an update of pathophysiological mechanisms. *Metab. Brain Dis.* **17**: 375–387.
- Pamphlett R, Coote P. 1998. Entry of low doses of mercury vapour into the nervous system. *Neurotoxicology* **19**: 39–48.
- Papp A, Vezér T, Institoris L. 2001. An attempt to interpret the fatigue of the somatosensory cortical evoked potential during a stimulus train as a possible biomarker of neurotoxic exposure. *Centr. Eur. J. Occup. Environ. Med.* **7**: 276–281.
- Papp A, Vezér T, Nagymajtényi L. 2000. Possible functional biomarkers among the properties of cortical sensory evoked potentials of rats treated with xenobiotics. *J. Physiol.* **526**: 161P.
- Pecze L, Papp A, Nagymajtényi L. 2003. Simultaneous changes of the spontaneous and stimulus-evoked cortical activity in rats acutely treated with mercuric chloride. *Neurotoxicol. Teratol.* **26**: 131–137.
- Piikivi L, Tolonen U. 1989. EEG findings in chloralkali workers subjected to low long term exposure to mercury vapour. *Br. J. Ind. Med.* **46**: 370–375.
- Pumain R, Kurcewicz I, Louvel J. 1987. Ionic changes induced by excitatory amino acids in the rat cerebral cortex. *Can. J. Physiol. Pharmacol.* **65**: 1065–1077.
- Rajanna B, Chetty CS, Rajanna S, Hall E, Fail S, Yallapragada PR. 1997. Interaction of metals with muscarinic cholinergic and adrenoceptor binding, and agonist-stimulated inositol phospholipid hydrolysis in rat brain. *Comp. Biochem. Physiol.* **116C**: 111–116.
- Rémond N, Lesèvre V. 1967. Variations in average visual evoked potential as a function of the alpha rhythm phase (autostimulation). In *The Evoked Potentials*, Cobb W, Morocutti C (eds). Elsevier: Amsterdam; 42–52.
- Reuveny E, Narahashi T. 1991. Potent blocking action of lead on voltage activated calcium channels in human neuroblastoma cells SH-SY5Y. *Brain Res.* **545**: 312–314.
- Sandhir R, Gill KD. 1993. Alterations in calcium homeostasis on lead exposure in rat synaptosomes. *Mol. Cell. Biochem.* **131**: 25–33.
- Schulz H, Nagymajtényi L, Papp A, Dési I. 1997. Behavioural and neurophysiological consequences of subchronic mercury exposure in rats. *Centr. Eur. J. Occup. Environ. Med.* **3**: 210–223.
- Shinotoh H, Snow BJ, Chu NS, Huang CC, Lu CS, Lee C, Takahashi H, Calne DB. 1997. Presynaptic and postsynaptic striatal dopaminergic function in patients with manganese intoxication: a positron emission tomography study. *Neurology* **48**: 1053–1056.
- Sirois YE, Atchison WD. 1996. Effects of mercurials on ligand- and voltage-gated ion channels: A review. *Neurotoxicology* **17**: 63–84.
- Szumanska G, Gadamski R, Albrecht J. 1993. Changes of the Na/K ATPase activity in the cerebral cortical microvessels of rat after single intraperitoneal administration of mercuric chloride: histochemical demonstration with light and electron microscopy. *Acta Neuropathol. (Berlin)* **86**: 65–70.
- Takeda A. 2003. Manganese action in brain function. *Brain Res. Rev.* **41**: 79–89.
- Tracey DJ, Waite PM. 1995. Somatosensory system. In *The Rat Nervous System*, Paxinos G (ed.). Vol. 2. Academic Press: San Diego; 689–704.
- WHO. 1977. Lead. *Environmental Health Criteria* 3. WHO: Geneva.
- WHO. 1981. Manganese. *Environmental Health Criteria* 17. WHO: Geneva.
- WHO. 1991. Inorganic mercury. *Environmental Health Criteria* 118. WHO: Geneva.
- Yamada M, Ohno S, Okayasu I, Okeda R, Hatakeyama S, Watanabe H, Ushio K, Tsukagoshi H. 1986. Chronic manganese poisoning: a neuropathological study with determination of manganese distribution in the brain. *Acta Neuropathol. (Berlin)* **70**: 273–278.