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Effect of acute administration of certain heavy metals and their combinations on the spontaneous and evoked cortical activity in rats

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Abstract

The aim of this study was to see the effect of acutely administered inorganic lead, mercury, manganese, and their combinations, on the electrical activity in the somatosensory system of rats. Male Wistar rats were anaesthetised with urethane, the head was fixed in a stereotaxic frame and the left hemisphere was exposed. Weak electric shocks to the whiskers and the tail served as stimuli. Spontaneous and stimulus-evoked activity was recorded from the primary projection area of the whiskers and the tail. After an hour of control recording, one of the following was given to the rat i.p.: 1000 mg/kg Pb^{2+}, 7 mg/kg Hg^{2+}, 50 mg/kg Mn^{2+}, 500 mg/kg Pb^{2+} + 25 mg/kg Mn^{2+}, or 500 mg/kg Pb^{2+} + 3.5 mg/kg Hg^{2+}. Lead caused a massive increase in the cortical response amplitude, starting immediately after administration and developing in the next 40–50 min. Latency showed a minimal increase. The spontaneous activity was moderately shifted to lower frequencies. The effect of Hg^{2+} on the response amplitude and on the ECoG was similar but stronger than that of Pb^{2+}. The effect of Mn^{2+} on the evoked activity was marked but less strong than with Pb^{2+}. The ECoG shift was moderate. With Hg^{2+} and Mn^{2+}, the response amplitude showed first a decrease than an increase. The effect of the Pb^{2+} + Mn^{2+} combination on the activities was not additive but the correlation between the alteration of the ECoG and the evoked potential was stronger than with any of the metals alone. With Pb^{2+} + Hg^{2+}, the effect of Pb^{2+} dominated on the evoked and that of Hg^{2+} on the spontaneous activity. In the peripheral nerve, action potential amplitude and conduction velocity were decreased.

These alterations of the spontaneous and stimulus-evoked cortical activity probably reflected a specific action of the heavy metals on the nervous activity.

Keywords: Neurotoxicity; Cortical activity; Lead; Mercury; Manganese; Combined exposure

1. Introduction

One of the major risks arising from environmental pollution by heavy metals is the damage to the nervous system of exposed individuals. Lead has been, and still is, used in large amounts for a number of purposes. Metal and inorganic lead occurs in batteries, piping, paints, solders etc. Alkylated lead was, and in a number of countries still is, used as a car fuel additive. Lead in any form is accumulated in the central nervous system, first of all in the cortex and hippocampus (Grandjean, 1978). Pb^{2+} interferes with Ca-dependent regulation of protein kinase C, calmodulin, ATPases, etc. due to the competition with Ca^{2+} ions (Sandhir and Gill, 1993; Bettaiya et al., 1996). Partly due to the interference with Ca^{2+}, lead also affects several transmitter systems. GABA uptake was decreased and dopamine uptake increased in synaptosomes from brains of rats exposed to daily oral lead doses of ca. 2 mg for 3 months (Jablonska et al., 1994). Alterations in the dopaminergic, cholinergic and glutamatergic control of behavior were observed in lead-treated animals (Cory-Schlechta, 1995). In humans, alterations of various forms of central and peripheral evoked activity, like sensory evoked potentials and nerve conduction velocity, were described in lead-exposed individuals having ca. 50 μg/dl blood lead levels (Araki et al., 2000; Lille et al., 1988). In our earlier studies, similar changes were found in rats after up to 12 weeks oral exposure by 80 and 320 mg/kg Pb^{2+} (Nagymajtényi et al., 1997).

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Mercury is another heavy metals known to be harmful for the nervous system. In occupational exposure to inorganic mercury, alterations of the spontaneous (Piikivi and Tolonen, 1989) and stimulus-evoked (Lille et al., 1988) 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^{2+} also interfered with calcium homeostasis, by disturbing Ca uptake to the endoplasmic reticulum (Freitas et al., 1996.). In rat brain slices treated with ionic mercury, higher than normal release of noradrenaline (Gasso et al., 2000) was described. Increased dopamine release after intrastriatal application of Hg^{2+} was also reported (Faro et al., 2001). In vitro ligand binding of rat cortical muscarinic receptors also was negatively affected by Hg^{2+} (Castoldi et al., 1996). Previous studies by our group evaluated the effects of mercury on cortical activity. In these studies rats, receiving subchronic $HgCl_2$ treatment of 0.4 and 1.6 mg/kg p.o., showed alterations in the spontaneous (Dési et al., 1996) and stimulus-evoked (Schulz et al., 1997) cortical activity.

In contrast to lead and mercury, manganese 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 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 the chronic human disease resulting from long-term occupational exposure to manganese, functional (Shinotoh et al., 1997) and structural (Yamada et al., 1986) damage to the dopaminergic systems were described.

In animals, manganese 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 μM Mn^{2+}; Hazell and Norenberg, 1997).

The aim of the present work was to see how the 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.

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

Agent	Chemical	Dose (mg/kg b.w.)	
Pb^{2+}	$Pb(CH_3COO)_2 \cdot 3H_2O$	High	1000
		Low	500
Hg^{2+}	$HgCl_2$	High	7
		Low	3.5
Mn^{2+}	$MnCl_2 \cdot 4H_2O$	High	50
		Low	25
$Pb^{2+} + Hg^{2+}$		Low + low	500 + 3.5
$Pb^{2+} + Mn^{2+}$		Low + low	500 + 25
$Hg^{2+} + Mn^{2+}$ [a]		Low + low	3.5 + 25
None (control)	Distilled water		

Solutions were made up in distilled water to give an administration volume of 1 ml/kg b.w.

[a] In this combination, the animals died in 10 min after administration of the mercury and manganese.

2. Materials and methods

Adult male Wistar rats of ca. 350 g b.w. were used in the experiments. After urethane anaesthesia (1000 mg/kg b.w. i.p.; Bowman and Rand, 1980), the animals' head was fixed in a stereotaxic frame and the left hemisphere was exposed. Wounds were sprayed with 10% lidocaine and the exposed cortex was covered with warm paraffin oil. After an hour of recovery, 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. Spontaneous and stimulus-evoked electrical activity of the cortical sites was recorded and analyzed using a PC with the NEUROSYS software (Experimetria Ltd., UK). Five minutes of electrocorticogram (ECoG) were simultaneously taken from both sites, then one train of 20 stimuli was applied to each of the peripheral sites and the cortical evoked potentials (EPs) recorded. During stimulation of the tail, compound action potential of the tail nerve was recorded also. This scheme was repeated every 20 min.

After at least 4 pre-treatment control records, one of the metals or metal combinations (Table 1) was administered via a peritoneal cannula and the recording was continued for at least 2.5 h. (After this time, the general state of the animal usually started to deteriorate and the experiment was finished by an overdose of urethane. The higher dose for each metal was determined in pre-experiments to give a firm effect within the above time limit, without signs of general acute toxicity like cramps, dyspnea, flattening ECoG etc. Hence, and because of the way of administration, the doses were not comparable to any human, e.g. occupational, exposure.)

Eight such recordings each were taken with each of the doses and another eight with distilled water as parallel controls. From the ECoGs, the software automatically determined the band activity (standard, delta to gamma; Kandel and Schwartz, 1985). From that, the so-called ECoG index

(relation of the low and high frequencies in the recorded ECoG; delta + theta/beta1 + beta2) was calculated. Records of cortical and peripheral evoked activity were averaged, and the latency and amplitude of EP or nerve action potential measured. (A typical evoked potential is given as insert in Fig. 2(A). Latency was measured between the stimulus artefact and the main waves A or B, and amplitude, between A and B.)

To make the results comparable animal-to-animal and group-to-group, the average of the four or five pre-administration control records was taken as a base and all individual values were normalised to that.

As the differences between parameters (amplitude, latency and EEG index) from the control and treated groups was seen in the respective time trends, the significance of these differences was tested by means of the "General Linear Model" (GLM) of the SPSS software (SPSS Inc., Chicago, Ill. USA). This model uses univariate ANOVA to test differences in trends. Group (control or treated) was taken as fixed variable, time as covariate, and the significance of group *and* time effect was checked (as applied in Pecze et al., 2004). L.S.D. was used to find data points responsible for significant ($p < 0.05$) group differences. Possible correlation between different parameters was tested by generating parameter-to-parameter plots in EXCEL and obtaining the regression coefficient (R^2).

3. Results

3.1. Effects on the spontaneous cortical activity

Among the metals, Hg had generally the strongest and Pb had the weakest effect on the frequency distribution of the spontaneous cortical activity. The direction of the change, a shift to lower frequencies, was the same with every metal. As shown in Fig. 1(A), the increase of the ECoG index (equivalent with the mentioned frequency shift, see Methods) in the barrel field started with some delay after the metal had been injected and kept on until the end of the recording. The delay was longer with Hg than with Mn. By the end of recording the effect of these two metals was significant ($F[3,28] = 9.41$, $p < 0.001$; L.S.D.: $p < 0.05$ versus control for both). In Fig. 1(B) and (C), the effect of the high and low doses of Hg and Mn are compared to that of the Hg + Pb ($F[4,35] = 8.87$, $p < 0.001$) and Mn + Pb ($F[4,35] = 3.56$, $p < 0.001$) combinations. The graphs show that Pb, beyond not having significant influence on the ECoG frequency distribution alone (L.S.D.: n.s. versus control), had no interaction with the two other metals in this parameter (L.S.D.: n.s. versus single low doses). In the projection area of the tail, the effects were highly similar.

3.2. Effects on the somatosensory evoked cortical activity

In the evoked activity, latency and amplitude were the main parameters analyzed. The effect of the metals investigated on the evoked response amplitude was considerable. Fig. 2(A) shows the amplitude change on administration of the high dose of the three metals. The general form of change was an increase, elicited most strongly by Hg, followed by Pb and Mn (Fig. 2(A)). The increase was significant versus control ($F[3,28] = 25.81$, $p < 0.001$) with all three metals (L.S.D.: $p < 0.05$). In the effect on the amplitude, interaction was much more marked than what was seen on the ECoG. Mn and Pb had a clear positive interaction, resulting in an amplitude increase ($F[4,35] = 10.87$, $p < 0.001$) which was significant (L.S.D.: $p < 0.05$) versus control and also versus the slight effect of the low dose Pb or Mn alone (L.S.D.: both n.s. versus control), and even stronger than that of the high dose Mn (Fig. 2(B)). Pb and Hg also had a positive interaction (Fig. 2(C)); the resulting effect was bigger than that of the low dose Pb or Hg alone but less than that of the high dose Hg ($F[4,35] = 11.69$, $p < 0.001$; but L.S.D.: n.s for the combination versus control).

Hg was the only metal which had a massive effect on the latency of the somatosensory evoked potential. Latency of the two main peaks (see insert in Fig. 2(A)) was separately measured but the effects were parallel so that only effects on the latency of the 1st peak will be detailed. The latency lengthening induced by high dose Hg evolved with a similar time course as the amplitude increase did (Fig. 2(D), cf. Fig. 2(A)) while in the Pb- and Mn-treated animals the latency remained at about control level ($F[3,28] = 61.24$, $p < 0.001$). The effect of the combinations was similar to that seen on the amplitude: Pb + Mn (Fig. 2(E)) gave a strong potentiation ($F[4,35] = 71.69$, $p < 0.001$; L.S.D.: $p < 0.05$ versus control) while the lengthening evoked by Pb + Hg (Fig. 2(F)) was greater than the effect of low dose Hg alone but less than the effect of high dose Hg ($F[4,35] = 11.43$, $p < 0.001$; L.S.D.: $p < 0.05$ versus control).

3.3. Effects on the peripheral nerve evoked activity

All three metals caused a gradually evolving, significant (L.S.D.: $p < 0.05$ versus control) reduction of the conduction velocity of the tail nerve (Fig. 3(A); $F[3,28] = 14.55$, $p < 0.001$). The quantitative differences between the effects of the metals were below significance. When applied in combination, both Pb + Hg (Fig. 3(B); $F[4,35] = 14.08$, $p < 0.001$) and Pb + Mn (Fig. 3(C); $F[4,35] = 49.91$, $p < 0.001$) gave an effect which was significant versus control (L.S.D.: $p < 0.05$) and was stronger than the effect of low dose Hg or Mn alone. The effect of Hg + Pb was ca. equal to that of high dose Hg alone; in case of Mn, the effect of the combination was greater. Low dose Hg alone (but not Pb or Mn) had here a slight but significant (L.S.D.: $p < 0.05$) effect (Fig. 3(B)).

Reduction of the tail nerve action potential amplitude was the strongest with high dose Hg. The two other metals had approximately the same effect (Fig. 3(D); $F[3,28] = 9.51$, $p < 0.001$; all significant versus control by L.S.D.). On this parameter, the peculiarity of the Hg effect – a transient, weak

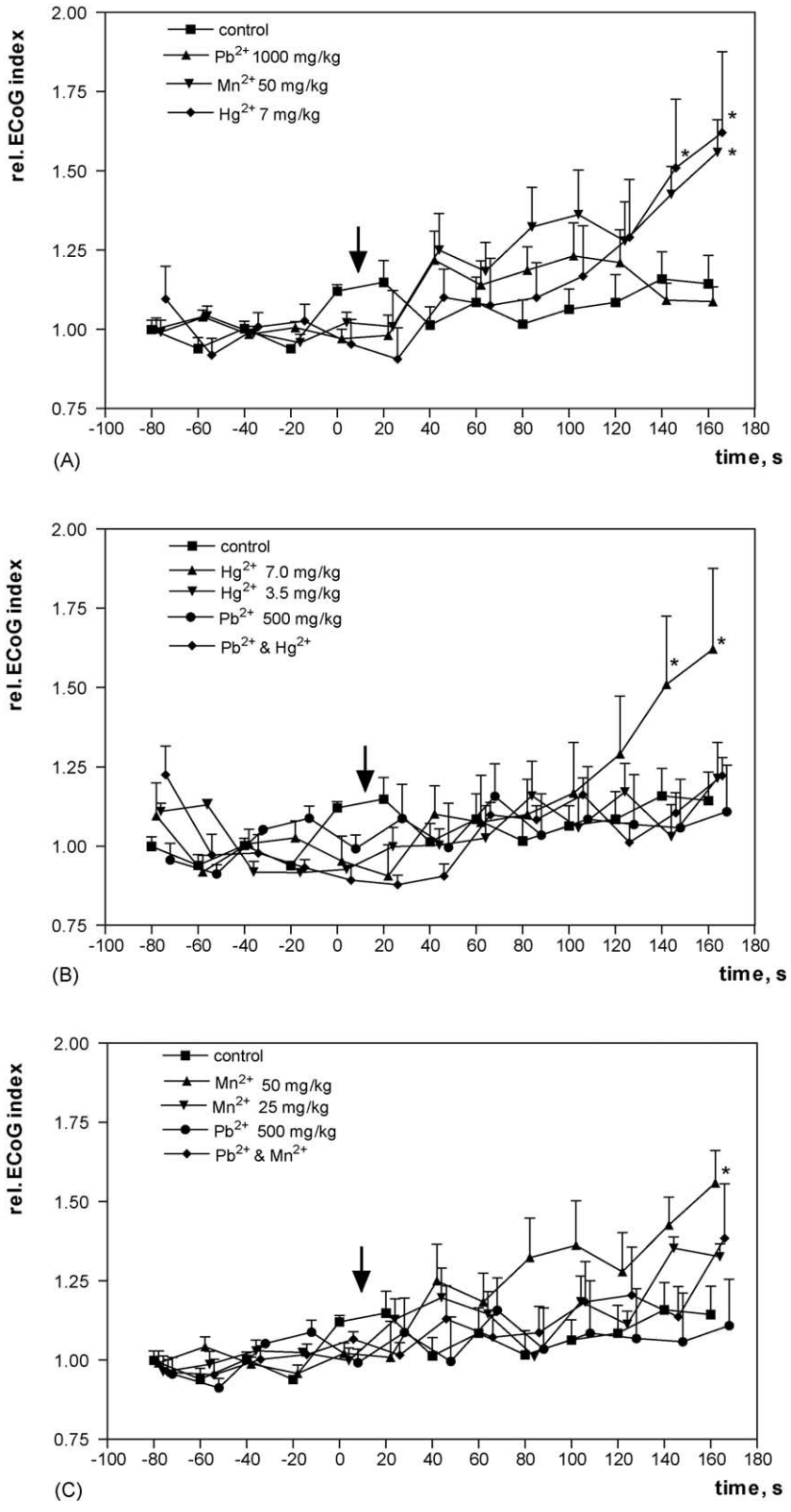


Fig. 1. Time course of the relative electrocorticogram index in control and treated rats. (For definition of the index and relative value calculation, see Methods). Abscissa: time in 20 min intervals, negative values are those before administration of the metal (arrow). Ordinate: relative ECoG index. The data points represent mean + S.E.M. ($n = 8$); $^{*}p < 0.05$ versus parallel control group. (A) High dose metals given alone. (B) High and low dose Hg and Hg + Pb combination. (C) High and low dose Mn and Mn + Pb combination. (For the doses, see Table 1).

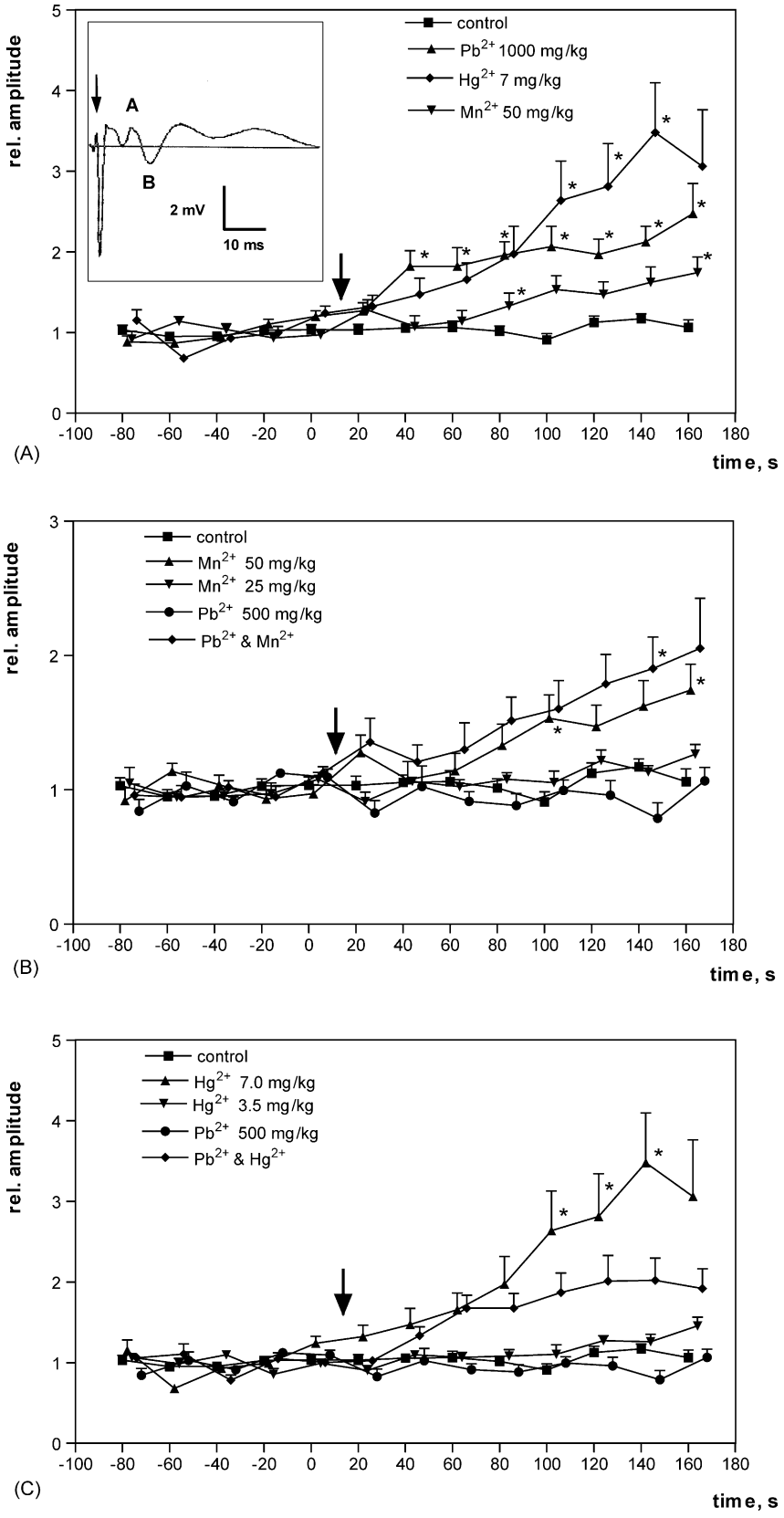
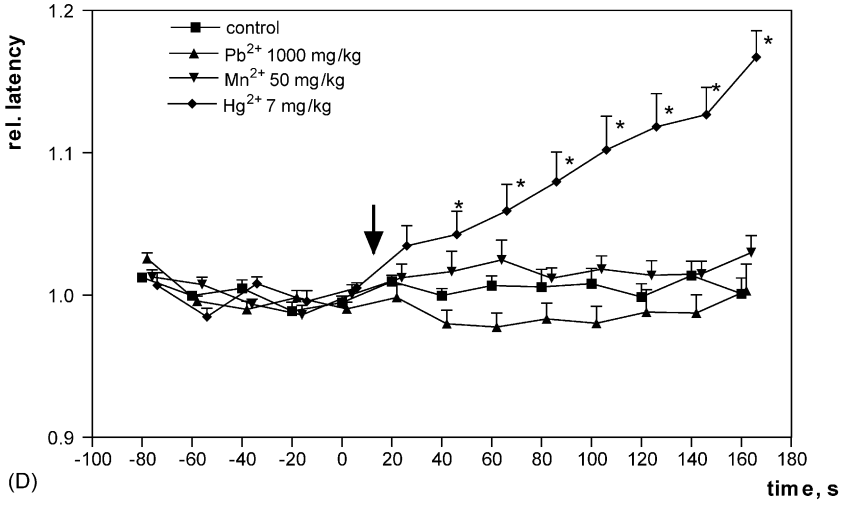
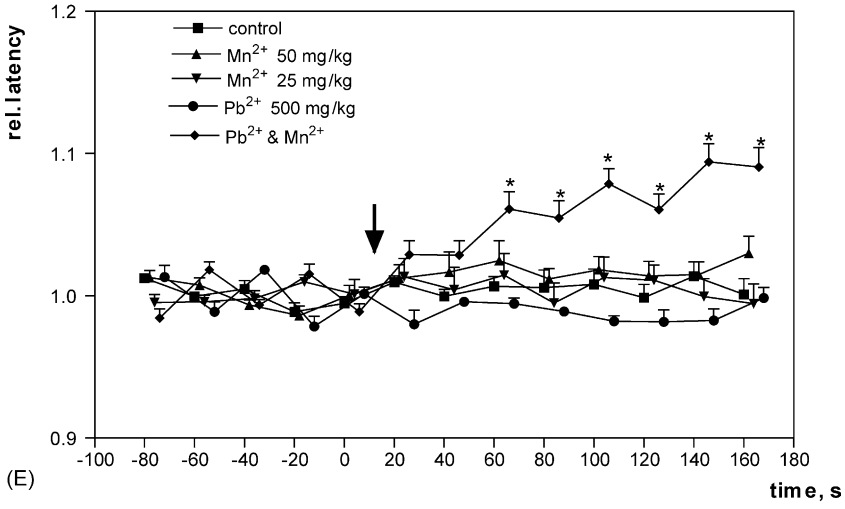


Fig. 2. Time course of the cortical evoked potential amplitude (A, B, C) and first main wave latency (D, E, F). Displayed as in Fig. 1. Ordinate: relative amplitude (A, B, C), relative latency (D, E, F).





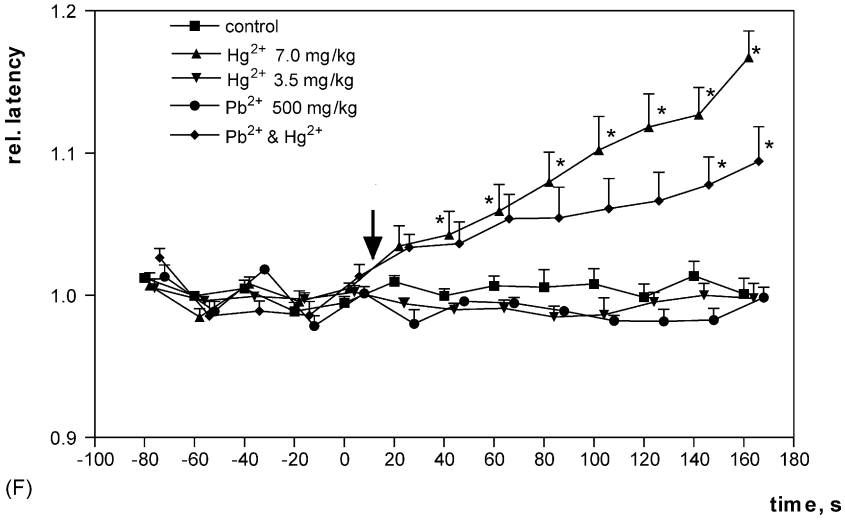


Fig. 2. (*Continued*.)

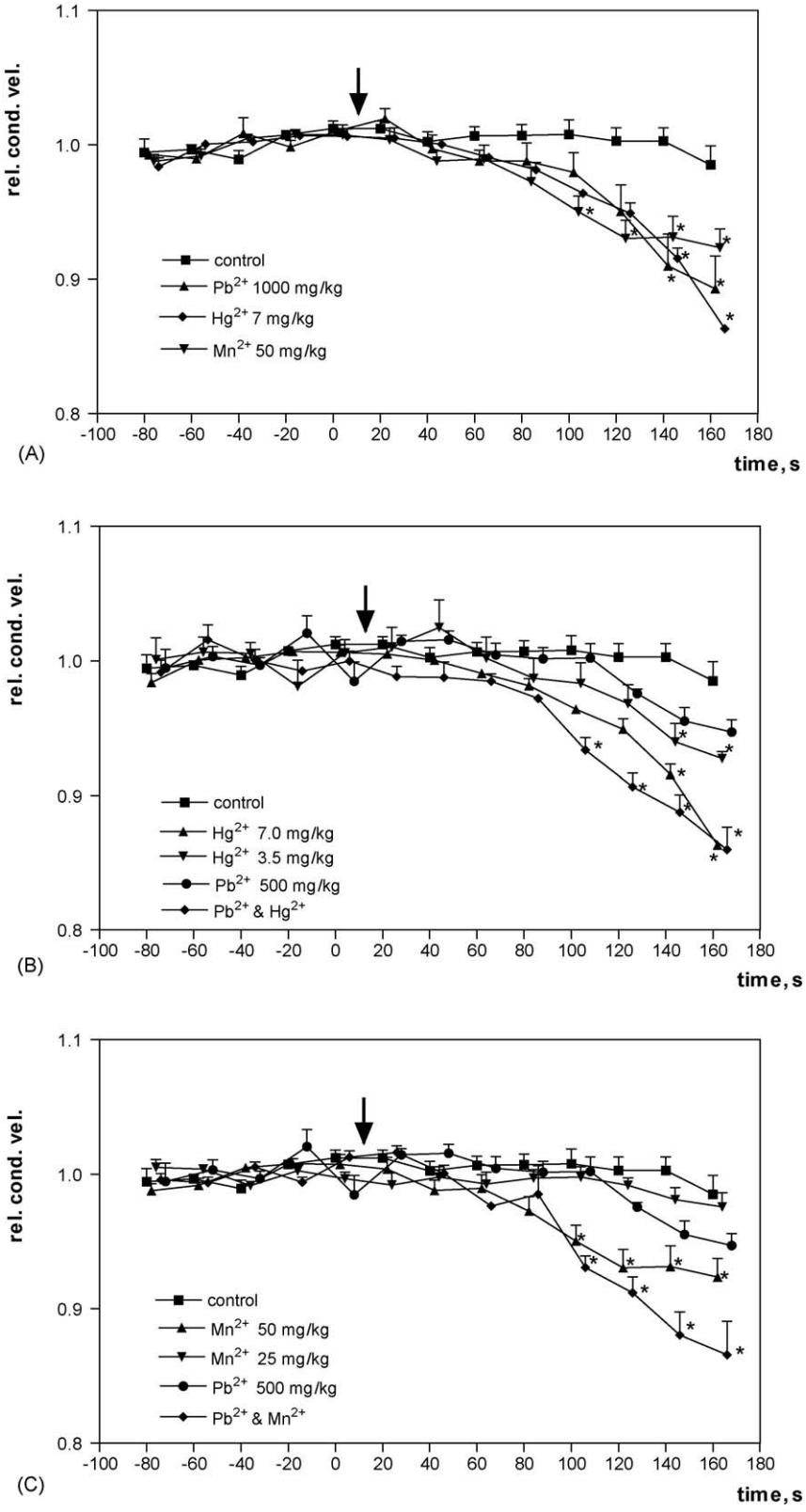


Fig. 3. Relative values of the peripheral nerve conduction velocity (A, B, C) and action potential amplitude (D, E, F). Displayed as in Fig. 1. Ordinate: relative conduction velocity (A, B, C), relative amplitude (D, E, F).

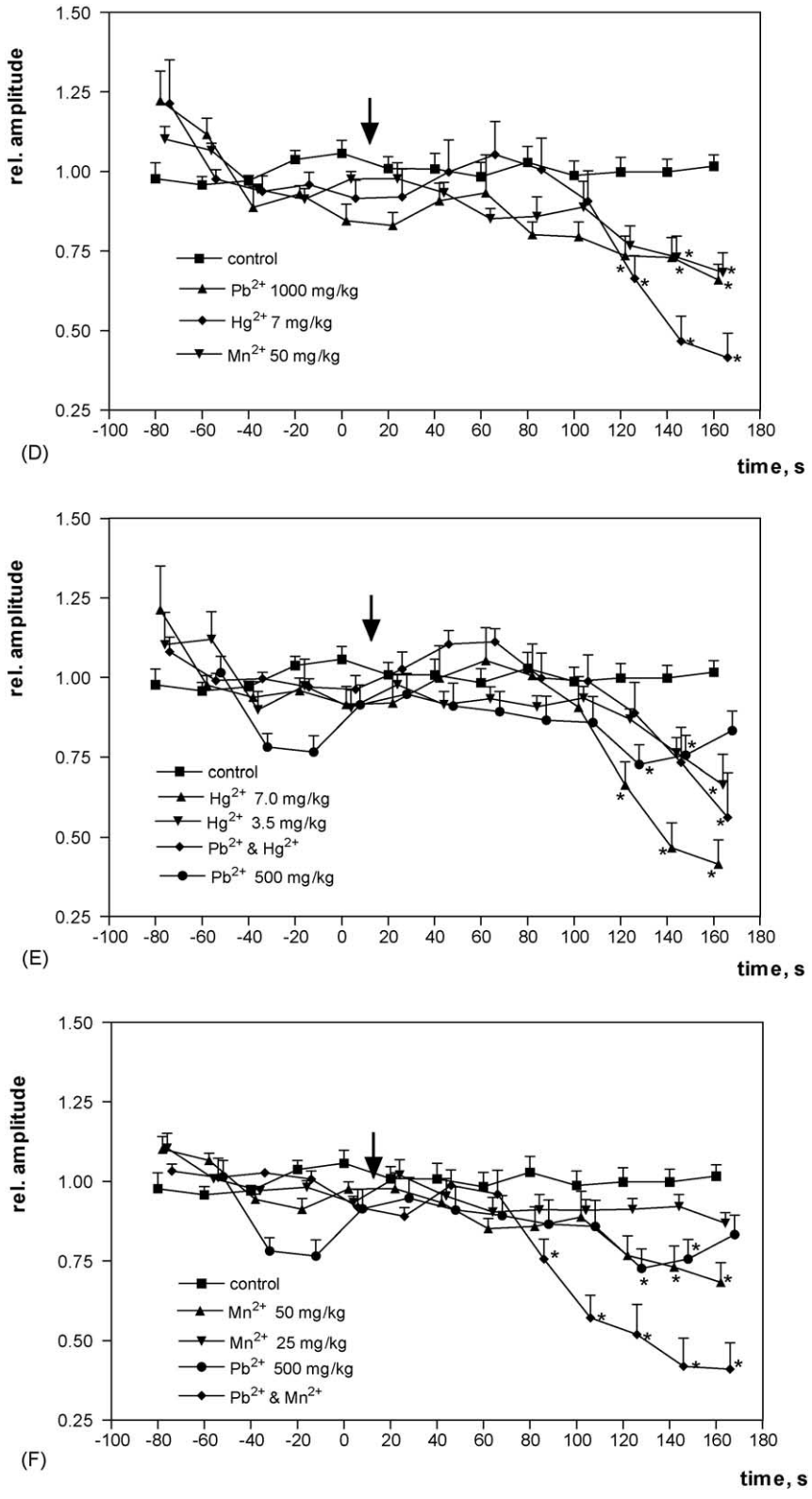


Fig. 3. (*Continued*.)

change in opposite direction to that of the final effect – was a bit more expressed than in the others mentioned above. In the combinations, Pb + Hg had about the same effect as low dose Hg alone (Fig. 3(E); $F[4,35] = 7.55$, $p < 0.001$; L.S.D.: $p < 0.05$ for the combination versus control). The effect of low dose Mn was, however, greatly potentiated by Pb (Fig. 3(F); $F[4,35] = 20.79$, $p < 0.001$; L.S.D.: $p < 0.05$ versus control and low dose Mn).

Table 2
Coefficients (R^2) of the correlation between the group average values in two combinations of parameters from groups of animals with various treatments

Parameters	Treatment	R^2
ECoG index vs. cortical evoked potential amplitude	Pb high	0.54
	Hg high	0.79*
	Mn high	0.79*
	Hg low	0.39
	Pb low + Hg low	0.31
	Mn low	0.30
	Pb low + Mn low	0.78*
Tail nerve conduction velocity vs. cortical evoked potential 1st peak latency	Pb high	0.18
	Hg high	0.92*
	Mn high	0.41
	Hg low	0.92*
	Pb low + Hg low	0.73*
	Mn low	0.01
	Pb low + Mn low	0.71*

* Significant correlation ($p < 0.05$).

3.4. Correlation of the individual effects

If the alteration in more than one of the studied parameters is based on the same effect, it is possible that their actual values show a correlation. To test this, two sets of parameters were plotted against each other by EXCEL and the regression coefficient was obtained by fitting a straight line on the resulting data points (Table 2). Between the ECoG index and the evoked potential amplitude, a fair regression coefficient was obtained for high dose Mn and Hg but a quite poor one for high dose Pb (for interpretation, see Discussion). In case of the low doses and combinations, the correlation was poor except for Mn + Pb.

4. Discussion

The effects obtained by the three heavy metals and combinations on the central and peripheral nervous activity in acutely treated rats were generally similar but of different intensity. In acute administration and effect, absorption of the agent and its access to the site of action is of great importance.

Although Pb^{2+} forms low solubility salts with physiological anions like Cl^-, lead is readily absorbed by various routes of exposure (WHO, 1977). Entering the blood stream, Pb^{2+} passes the blood–brain barrier above a concentration threshold (Bradbury and Deane, 1993). In case of high blood lead level, breakdown of the barrier was observed (Goldstein et al., 1974). Inorganic salts of Hg are generally supposed to have low penetration across the blood–brain barrier (Aschner and Aschner, 1990) but it is known that the barrier itself is damaged (Szumanska et al., 1993) shortly after administration of mercury. Mn^{2+}, once in the bloodstream, can pass the blood–brain barrier either bound to transferrin or as free Mn^{2+} (Aschner et al., 1999; Rabin et al., 1993). The rapid onset of the effects on cortical parameters indicates that transport was probably not a rate-limiting factor.

On the spontaneous cortical activity, the effect of Hg^{2+} and Mn^{2+} was comparable both in time course and in final strength. In case of Hg^{2+}, an effect on the ascending cholinergic activation is likely. Inorganic Hg is known to inhibit choline acetyltransferase (Dwivedi et al., 1980). It also decreases the binding of ACh on the muscarinic receptors (Rajanna et al., 1997) so that the activation to the cortex will be diminished. For Mn^{2+}, no such mechanism is known, at least the muscarinic receptors were found not to be influenced by manganese (Villalobos et al., 1994).

Inorganic lead could possibly interfere with the ascending cholinergic activation of the cortex (Metherate et al., 1992) by increasing the spontaneous and decreasing the stimulus-evoked synaptic release of ACh (Suszkiw et al., 1984) but in our experiments no significant effect of Pb^{2+} on the ECoG was seen.

There are several possible explanations of the increase in the cortical evoked potential amplitude. The excitatory thalamocortical input is glutamatergic. Hg^{2+} inhibits the glial uptake of Glu (Brookes, 1992) and Mn^{2+} inhibits its breakdown (Normandin and Hazell, 2002) both of which can result in increased cortical excitation. On the amplitude, the effect of the low dose of Hg^{2+} and Mn^{2+} was greatly enhanced by the low dose of Pb^{2+}. The low metal doses were, in respect to effects on the evoked potential, NOEL doses (caused no noteworthy change). So, their interaction was clearly synergistic, possibly due to the lead-induced increased permeability of the blood–brain barrier (Goldstein et al., 1974) and increased brain levels of the metals. It remains an open question, however, why no similar effect was seen on the spontaneous activity. An alternative explanation is provided by the known effect of Pb^{2+} on the hippocampal spontaneous and stimulus-evoked release of Glu and GABA (Braga et al., 1999a,b), partly similar to what was found by Suszkiw et al. (1984) on rat brain synaptosomes. Depending on the actual metal levels and IC_{50}s, the outcome may be more activation and less inhibition of the cortical neurons responsible for the generation of evoked potentials.

In case of Hg^{2+} and Mn^{2+} treatment, a fair correlation was found in our experiments between the alteration of the spontaneous and evoked cortical activity (Table 2). For Hg^{2+}, decreased cortical activation may be the primary event, and for Mn^{2+}, increased thalamocortical input. In case of $Mn^{2+} + Pb^{2+}$ (the only combination with a good correlation between spontaneous and evoked cortical activity) the common point may be glutamatergic transmission and/or Ca^{2+} channels.

The correlation coefficient of cortical response latency versus peripheral conduction velocity (Table 2) was good only for high dose Hg^{2+}. In case of the other metals, the latency of the cortical response is probably determined more by the central synaptic delay.

Although the kind and mechanism of interaction between the neurotoxic heavy metals lead, mercury and manganese, given acutely to rats in our experiments, is not yet clear, the above results indicate that combined exposure of humans to these metals may have unexpectedly severe consequences.

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