# CENTRAL NEUROPHYSIOLOGICAL CHANGES CAUSED BY COMBINED TREATMENT WITH ENVIRONMENTAL XENOBIOTICS IN RATS

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ABSTRACT: Nowadays, the population mostly undergo multiple exposition to various chemical compounds. In our previous experiments aimed at modelling human exposure to pesticides and heavy metals, it was found that combinations like pesticide+pesticide or metal+metal caused different functional alterations of the nervous system than the administration of single compounds did. The aim of this study was to analyse the changes in certain central neurofunctional parameters caused with combined treatment by low doses of three xenobiotics. The animals were given the substances (pesticide+pesticide+pesticide, or metal+metal+metal) in different stages of the intra- and extra-uterine development. The doses, given by gavage, were 7.0 or 28.0 mg/kg dimethoate, 4.3 or 17.0 mg/kg propoxur, 5.4 or 21.6 mg/kg cypermetrine and 80.0 or 320.0 mg/kg lead, 0.4 or 16 mg/kg mercury, 3.5 or 14.0 mg/kg of cadmium (in form of $C_4H_6O_4Pb$, $HgCl_2$, $CdCl_2$, respectively). The neurophysiological function investigated was the spontaneous activity of the brain (electrocorticogram) in the somatosensory, visual, and auditory areas. The results showed that, compared to the alterations caused by combinations of two pesticides or metals, the changes to three xenobiotics seemed to be more marked or even significant. The neurophysiological alterations found point to increased risk to simultaneous exposure to different environmental xenobiotics

KEY WORDS: Electrocorticogram, pesticide, heavy metal, rat

## INTRODUCTION

The population, especially in the developed countries, is continuously exposed to various neurotoxic environmental pollutants, heavy metals originating from combustion of fossil fuels and industry, pesticide residues in food, etc. (ATSDR, 1997, 1999a,b; WHO, 1986, 1989). These chemicals can cause considerable biochemical, functional and morphological alterations in the central and peripheral nervous system. As people are generally exposed to several xenobiotics simultaneously, it seems to be important to investigate the changes in the neurofunctional parameters caused by combined administration of relatively low doses of, e.g., heavy metals and pesticides.

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In our previous experiments, it was found that, compared to the effect of the administration of a single substance, simultaneous treatment of rats with low-level doses of dimethoate combined with lead, mercury, or cadmium in different stages of the intra- and extrauterine development caused more marked changes of certain spontaneous and evoked activity forms of the nervous system (Nagymajtényi et al., 1997, 1998).

In this study, we investigated the alterations of certain functional parameters of the spontaneous central nervous activity after combined treatment with relatively low doses of three heavy metals (lead+mercury+cadmium) or commonly used pesticides (dimethoate+propoxur+cypermetrine) given in different intra- and extrauterine developmental phases.

## MATERIALS AND METHODS

Female Wistar rats (10 animals/group) were orally treated by gavage with 80.0 (low dose: LD) and 320.0 (high dose: HD) mg/kg lead ($C_4H_6O_4Pb \cdot 3H_2O$), 0.4 (LD) and 1.6 (HD) mg/kg mercury ($HgCl_2$), 3.5 (LD) and 14.0 (HD) mg/kg cadmium ($CdCl_2$); or 7,0 (LD) and 28,0 (HD) mg/kg dimethoate, 4.3 (LD) and 17,0 (HD) mg/kg propoxur and 5.4 (LD) and 21.6 (HD) mg/kg cypermetrine. The treatments were done from day 5 to 15 during pregnancy (P variation), from day 5 to 15 of pregnancy + for 4 weeks of lactation (P+L variation), or from day 5 to 15 of pregnancy + for 4 weeks of lactation + male offspring (F1 generation) further treated for 8 postweaning weeks (P+L+P variation). The dose combinations were LD+LD+LD or HD+HD+HD. The metal and pesticide control rats were orally given saline or sunflower oil, respectively.

The neurophysiological investigation of the F1 male rats was performed at the age of 12 weeks. The rats anesthetized with 1000.0 mg/kg ip. of urethane were placed in a stereotaxic instrument. After opening the skull, silver electrodes were directly placed on the primary somatosensory, visual and auditory areas (Zilles, 1982). The electrocorticogram (ECoG) was simultaneously recorded from the three cortical centers for 15 minutes. The analyzed parameters were power spectrum of the frequency bands and ECoG index (the ratio of the slow (delta+theta) and the fast (beta1+beta2) frequencies; Dési, 1983).

The statistical analysis was performed by two-factorial ANOVA.

The study was approved by the Ethical Committee for the Protection of Animals in Research of the University.

## RESULTS

Litter size, average body weight of pups, the body weight gain of the treated rats did not considerably differ from those of the controls except in the groups receiving HD+HD+HD doses of metals or pesticides.

Compared to the control, pesticide administration altered the spectral distribution of the electrocorticogram. The differences in the individual frequency bands were small *(Fig. 1)* but the ECoG index of the spectrum was markedly different from the control (*Fig. 2*).

Corresponding to these trends in the frequency spectrum, the changes of the ECoG index in the somatosensory area were slight in the groups given the combination of small doses, and more marked in the P+L+P group given HD+HD+HD (*Fig. 2*). Compared to the control, the index value was lower in the P variation and higher in the P+L group, while in the P+L+P variation smaller again. The changes were not significant, but the decrease of the index of the P+L+P rats was on the borderline of significance.

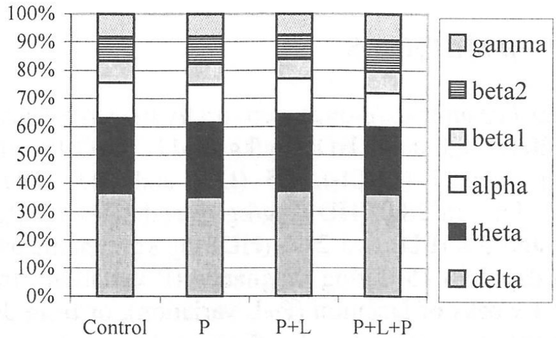


*Fig. 1. Spectral distribution of the somatosensory ECoG of rats treated with high dose of pesticides*

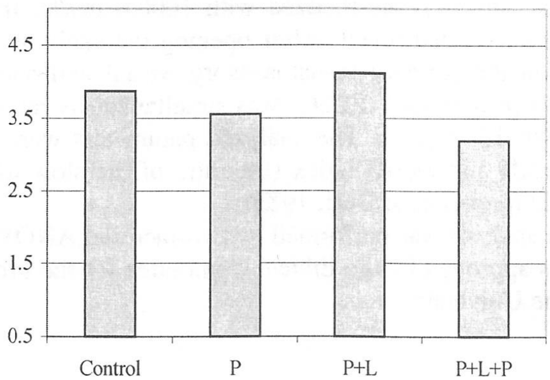


*Fig. 2. Changes of the somatosensory ECoG index of rats treated with high dose of pesticides*

Similar changes were observed in the visual and auditory ECoGs: higher activity of the faster frequency waves with parallel decrease of that of the slow ones, and more strongly reduced index in the P+L+P groups. In the auditory field ECoG, the activity of the delta end theta waves decreased, causing a lower ECoG index in all of the treated groups.

The effect of heavy metal administration on the spontaneous cortical activity of the treated animals was also dose- and treatment variation-dependent.

Compared to the control, the alteration of the spectral distribution of the ECoGs was slight in the LD+LD+LD groups but more marked in all HD+HD+HD groups. Especially in the P+L+P treatment variation, the activity of the slow-wave part of the somatosensory field ECoG was higher together with depressed fast wave activity (*Fig. 3*). Because of this tendency, the ECoG index was higher in all variations of treatment; compared to the control, the difference in the P+L+P variation was significant (*Fig. 4*).

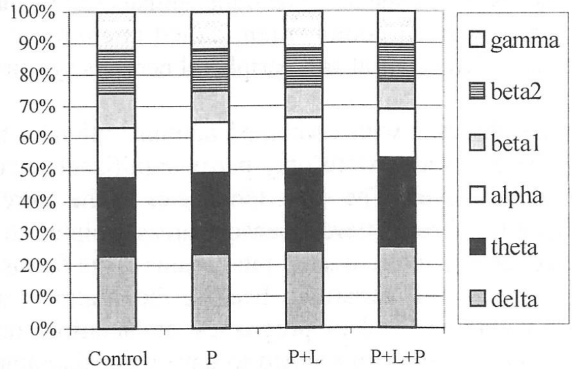


*Fig. 3. Spectral distribution of the somatosensory ECoG of rats treated with high dose of metals*

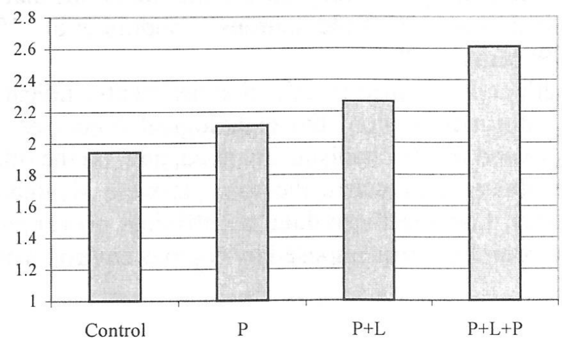


*Fig. 4. Changes of the somatosensory ECoG index of rats treated with high doses of metals (*p<0.05)*

Similar changes of the ECoG were seen in the visual and auditory foci: increased activity of the slower frequency waves together with simultaneous decrease of the fast ones, and, as a consequence, higher ECoG index in the treated groups. The activity of the delta and theta waves in the auditory focus decreased, causing a lower ECoG index in all the treated groups.

## CONCLUSIONS

Due to the increasing use of chemicals, people in developed countries are continuously exposed to various xenobiotics, among them to known toxicants. These substances are hazardous especially for the more susceptible part of the population, e.g., pregnant women, suckling babies, etc. (WHO 1976, 1992, 1995). Health effects can be more severe if they are simultaneously exposed to a number of compounds.

The results of our previous studies, where the animals were simultaneously treated with a pesticide+heavy metal combination, called attention to the more distinct functional alterations of the central and peripheral nervous system (Nagymajtényi et al., 1997, 1998).

The present data obtained with combined administration of three pesticides or metals, although the alterations were only partly significant, revealed the toxic effects of the compounds given. The same tendencies in the corresponding parameters, registered from the three different sensory areas, pointed to general alteration of the spontaneous activity in the treated rats' brains. This fact is especially important in case of the P and P+L variations, because the treatment of the rats in these groups was ceased with the end of pregnancy or lactation, respectively, which means that the combined exposure seemed to have a considerable hazardous effect during the early pre- and postnatal development of the brain.

The mechanism of the changes found seems to be rather complex. They can be caused by alterations of the neurotransmitter systems (cholinergic, adrenergic, GABAergic, etc.) and/or by affecting ion channel functions that results in changing transmembrane ionic currents of the neurons (Candura et al., 1997; Antonio 1999; Mejia et al., 1999; Minami, 2001).

Our results, although obtained in animal experiments, indicate the importance of completing the information about the pathological processes caused by multiple chemical exposure and the mechanisms involved, and, on the other hand, of collecting human data about cases where the subjects were simultaneously exposed to several xenobiotics. Lacking these data, no efficient preventive measures can be taken against the complex stress imposed by copious environmental xenobiotics.

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