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CHANGES IN BRAIN BIOELECTRICAL ACTIVITY (EEG) AFTER REPETITIVE EXPOSURE TO AN ORGANOPHOSPHATE ANTICHOLINESTERASE. II. RAT

SŁAWOMIR GRALEWICZ, TADEUSZ TOMAS, ROMAN GÓRNY, WOJCIECH KOWALCZYK and RENATA SOĆKO

Laboratory of Neurotoxicology, Institute of Occupational Medicine, Lodz, Poland

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Abstract. Effects of repetitive exposure (ten times in a period of two weeks) to chlorphenvinphos (CVP), at daily doses of 0.5 and 1.0 mg/kg, i.p., were studied in adult male Wistar rats of imp-DaK stock. It was found that 3 hrs after the last exposure, the cholinesterase (ChE) activity in the blood and brain was close to 50% of the control value in the 0.5 mg/kg group and less than 50% in the 1.0 mg/kg group. In both groups, normalization of ChE activity in plasma took less time than in erythrocytes, and the normalization of ChE activity in erythrocytes proceeded faster than in the majority of the brain areas studied. Electrophysiological investigations revealed a retardation of age-related epileptic-like cortical activity. This effect, however, was present only in the 1.0 mg/kg group, and only in the period of decreased ChE activity in the brain. Spectral analysis revealed an increase in 1–4 Hz activity in the cortical EEG of the 0.5 mg/kg group and heightened theta activity (4–7 and 7–9 Hz bands) in the hippocampal EEG of the 1.0 mg/kg group. The later effects were detected after a time sufficient for full normalization of ChE activity and manifested themselves most clearly in the presence of an acoustic stimulus associated with pain. The above results are in agreement with earlier observations on rabbits exposed repetitively to CVP. Data from both species suggest that, in the case of repetitive exposure to CVP, neither plasma nor erythrocyte ChE activity is a reliable indicator of toxicity, and that such exposure to this OP may lead to changes in EEG outlasting the period of lowered ChE activity in the blood and brain.

Address reprint requests to S. Gralewicz, Laboratory of Neurotoxicology, Institute of Occupational Medicine, P.O. Box 199, 90-950, Lodz, Poland.

INTRODUCTION

Some clinical observations (6, 11) as well as experimental data obtained from laboratory animals (4) show that exposure to warfare organophosphate anticholinesterases may result in longlasting changes in EEG. It has been suggested that these changes represent a persistent effect of cholinergic hyperactivity in the past (6). One may ask whether exposure to less toxic organophosphorus compounds (OP), used widely in agriculture as pesticides, may lead to similar consequences. In our previous experiments (7) we studied hippocampal and neocortical EEG in rabbits exposed repetitively (ten times in a period of two weeks) to an OP pesticide, chlorphenvinphos (phosphoric acid, 2-chloro-1-/2,4- -dichlorophenyl/vinyl Diethyl Ester (CVP)), at the daily dose of 14 mg/kg (i.p.). Overt behavioral symptoms did not appear at this level of dosing and the ChE activity in blood at the end of the exposure period was close to 50% of the pre-exposure value. The analysis of EEG-s revealed no changes in the spontaneous hippocampal activity 24 hrs after the exposure. Eight weeks after the exposure, however, the hippocampal arousal response (theta rhythm or RSA) to strong, sporadic stimuli, as well as to stimuli associated with pain was heightened. Moreover, spectral analysis revealed a slowing of cortical activity in the exposed animals in comparison with the control ones.

The purpose of the present work was to find out what changes in brain electrical activity can result from repetitive exposure to CVP in the rat. The separation of the studies on rats from those on rabbits is justified by two reasons. First, the rat is exceptionally vulnerable to CVP (9). Second, the rat EEG differs with some respects from that of the rabbit; the main difference is the presence in the rat, but not in the rabbit, of an age-related epileptic activity, which takes the form of high voltage spindles (HVS), in the neocortex (2, 5, 12, 13). It offers some additional possibilities of testing the effects of the exposure.

MATERIALS AND METHODS

Animals

The experiments were performed on male rats of the imp-DaK stock, outbreds, about 120 days old at the beginning of the experiments. A part of the animals served for biochemical studies (determination of ChE) only. The remaining ones were prepared for the electrophysiological experiments. The animals used for each type of experiment were assigned randomly into three groups: the High Dose Group (H group), the Low Dose Group (L group) and the Control Group (C group).

The pesticide and the exposure conditions

CVP (technical grade) was obtained from the manufacturer (Organika--Azot, Jaworzno, Poland). Before use, it was diluted in sterile, warm (40°C)

olive oil to the required concentration (0.5 mg/ml and 0.25 mg/ml). It was administered intraperitoneally ten times in a period of two weeks (one injection a day except Saturdays and Sundays), at the following doses: H group – 1.0 mg/kg, L group – 0.5 mg/kg. Based on previous (8) and some pilot observations, it was expected that in the L group the level of ChE activity in blood (in plasma and in erythrocytes) would not decrease below 50% of the control value, i.e. it would remain close to the safety limits accepted for workers in some countries (1). The rats of the C group were injected with 1.0 ml/kg pure olive oil.

ChE determinations

After the tenth injection, samples of animals (four to five animals per sample from the exposed groups and one to two from the controls) were killed by decapitation at predetermined intervals (3 hrs, 4, 7 and 14 days in case of the L group, and after 21, 35, 41 and 49 days in case of the H group). Samples of blood were collected from the cut neck vessels. Immediately after the decapitation the brains were removed from skulls and dissected on ice. Each part of the brain was carefully weighed and homogenized in a cold (4°C) Sorensen buffer. ChE was determined spectrophotometrically (Voss and Sachsee method, (14)) in plasma, erythrocytes and in the following parts of the brain: cerebellum, lower brain stem, diencephalon, hippocampus and in the anterior part of the cerebral hemispheres with the head of the caudate nucleus.

Electrophysiological techniques

Surgery. Each rat assigned for the electrophysiological studies was implanted with bipolar electrodes (into the anterior neocortex and into the dorsal hippocampus, bilaterally) under barbiturate anesthesia in the way described in our previous work (8).

Apparatus. Analog EEG records were made with the use of an 8-channel electroencephalograph at 1.0–35.0 Hz frequency band. In some cases (test 2) the EEG signals were digitalized and stored in the mass memory of a computer (IBM compatible). During the recording the animal remained in a plastic container ($25 \times 25 \times 40$ cm) or in a cage ($30 \times 60 \times 30$ cm) where it could move freely. The grid floor of the cage could be electrified. The container and the cage were located in a semi-sound-proof enclosure. A closed-circuit TV system made it possible to watch the animal's behaviour during the recording.

Procedure. Test 1. Testing effects of the exposure on HVS activity. Within about one month before the start of the exposure hippocampal and neocortical activity was recorded three times at 7 day intervals in each animal. Each session lasted at least two hours, during which the hippocampal and neocortical activity was recorded continuously (analog records). The animal

remained in the plastic container during the recording. After the third recording session, a preliminary analysis of the records was made in order to select animals with HVS in the cortical EEG (Fig. 1). The selected animals

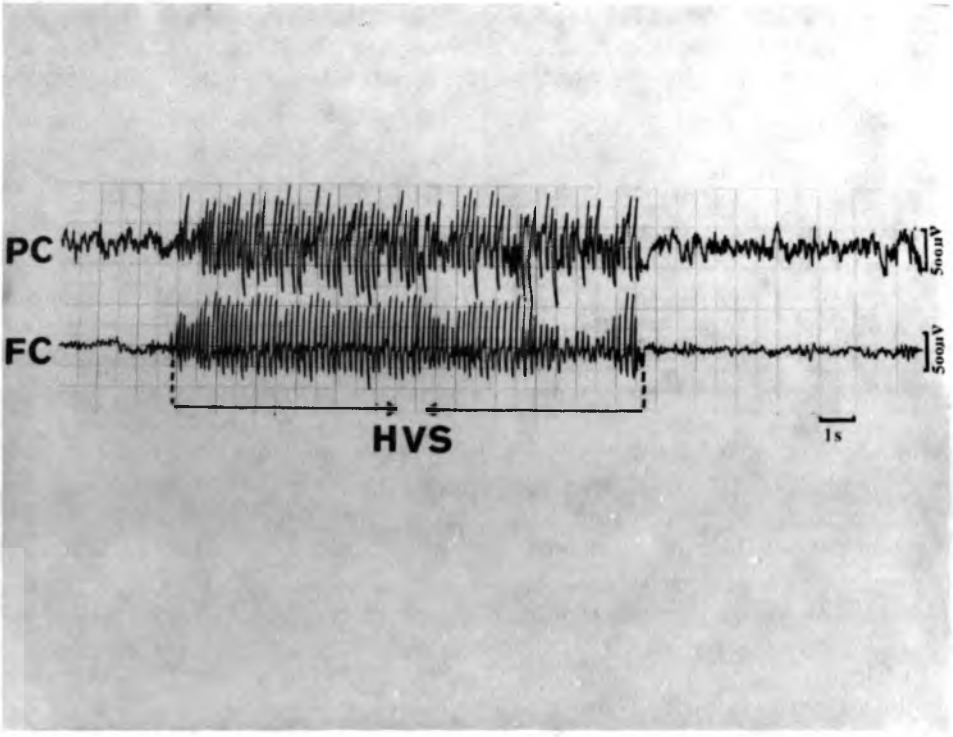


Fig. 1. An example of the spontaneous, age related, epileptic activity (high voltage spindle – HVS) in the rat electrocorticogram. Callibration: 1s, 200 μV.

were then separated into three groups, H group (n = 8), L group (n = 7) and C group (n = 8), as described above. Two additional recording sessions were performed two weeks and two months after the last injection of CVP.

Test. 2. Testing the hippocampal and neocortical EEG before and after a change of the biological significance of a sporadic stimulus.

This test was performed on the 39th – 41st day after the last injection of CVP. It consisted of three daily sessions. Session duration was 15 min. During the first 5 min the animal was not disturbed. During the next 10 min the rat was presented ten times with a 1000 Hz tone. The tone duration was 10 sec and the interstimulus interval was 50 sec. During the first session (Session I, habituation) the tone was presented alone. In the second session (Session II, conditioning) a series of electric footshocks (0.2 s bursts of AC at 1.5 mA applied at 1/sec frequency) accompanied the tone during the last 5 sec of its duration. In the third session (Session III, extinction) the tone was presented as

in the first one. EEG was recorded continuously (digital records) during the first and the third sessions only.

Data analysis. The analog, ink written EEG records (test 1) were analysed visually. The analysis consisted in establishing the number and the duration of HVS (Fig. 1) in the cortical EEG. HVS occur predominantly when the animal is aroused but immobile (5). Therefore, only this state was taken into account during calculation of the frequency of the HVS occurrence. The digital records (test 2) were subjected to spectral analysis using a computer programme which had been developed in our Laboratory. Averaged power spectra of the hippocampal and neocortical activity were made for selected EEG sections. Statistical comparisons were made with the use of parametric or nonparametric ANOVAs.

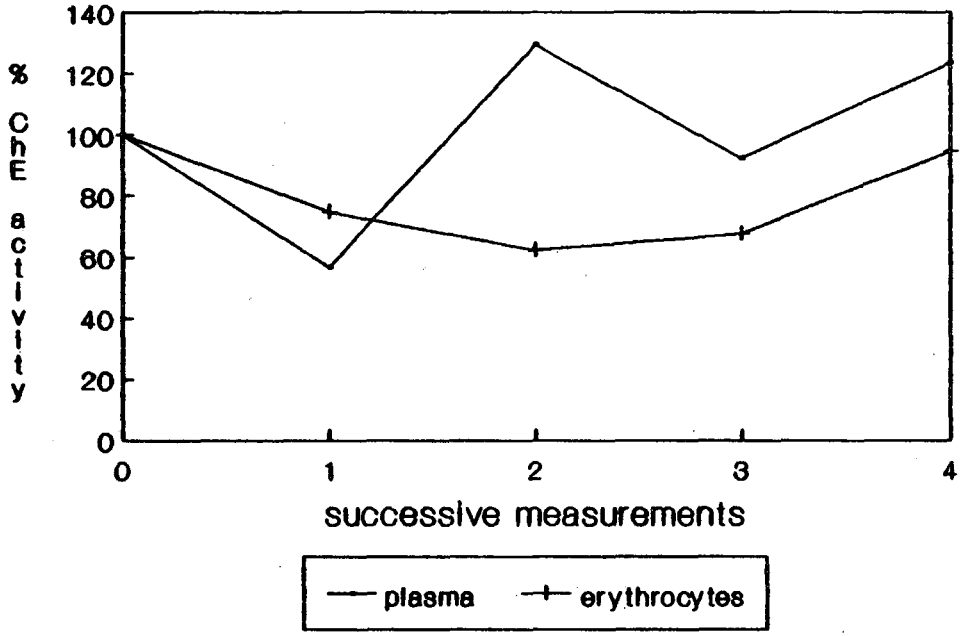
RESULTS

Cholinesterase activity after CVP exposure

Figs 2 and 3 illustrate the time course of changes in ChE activity in the blood and brain after repetitive CVP exposure. Kruskal-Wallis ANOVA and Conover's test were used for statistical comparisons (15). As expected on the basis of preliminary observations, in group L, 3 hrs after the last CVP injection, ChE activity in all the compartments studied was above 50% of the control value (i.e. the mean value obtained in control animals treated as one control group), and below 50% in the case of the H group.

In Group L, 3 hrs after the last CVP injection the depression of ChE activity in plasma, erythrocytes and in all parts of the brain was similar ($X^2 = 11.9$, df = 6, $p > 0.05$). However, the normalization proceeded faster in the case of plasma and cerebellum; in both cases the enzyme activity returned to a normal level within 4 days. Fourteen days after the exposure ChE activity in plasma, erythrocytes and cerebellum approached or exceeded control levels; but in the lower brain stem, diencephalon, hippocampus and the enterior part of the hemisphere, it was still slightly depressed ($p < 0.05$ in all cases).

In Group H, 3 hrs after the last CVP injection the level of ChE activity in all compartments, except plasma, was below 50% of the control values. There were also significant differences between compartments in the level of ChE inhibition at that time point ($X^2 = 12.9$, df = 6, $p < 0.05$). The erythrocyte ChE was affected significantly more than the plasma ChE ($p < 0.05$) and ChE of all parts of the brain except the enterior part of the hemisphere ($p < 0.05$ in all cases). In the brain, the degree of ChE inhibition was the lowest in the cerebellum (by 55%) and the highest in the anterior part of the hemisphere (by 71.9%). The normalization took place within 14 days in the case of plasma, and within 35 days in the case of erythrocytes. In the brain, 35 days sufficed for normalization of ChE in the cerebellum and in the anterior part of the hemisphere. On the 41st day the ChE activity in all compartments was normal.



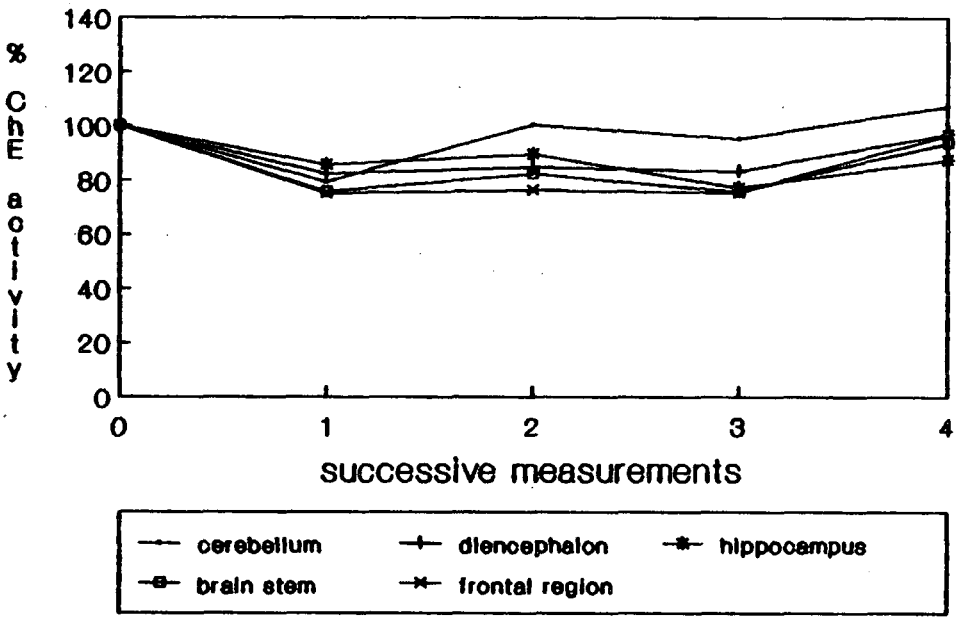


Fig. 2. Changes in ChE activity in blood (upper graph) and the brain (lower graph) after repetitive exposure to CVP in the dose of 0.5 mg/kg i.p. Successive measurements were made 3 hrs (1), 4 days (2), 7 days (3) and 14 days (4) after the last injection.

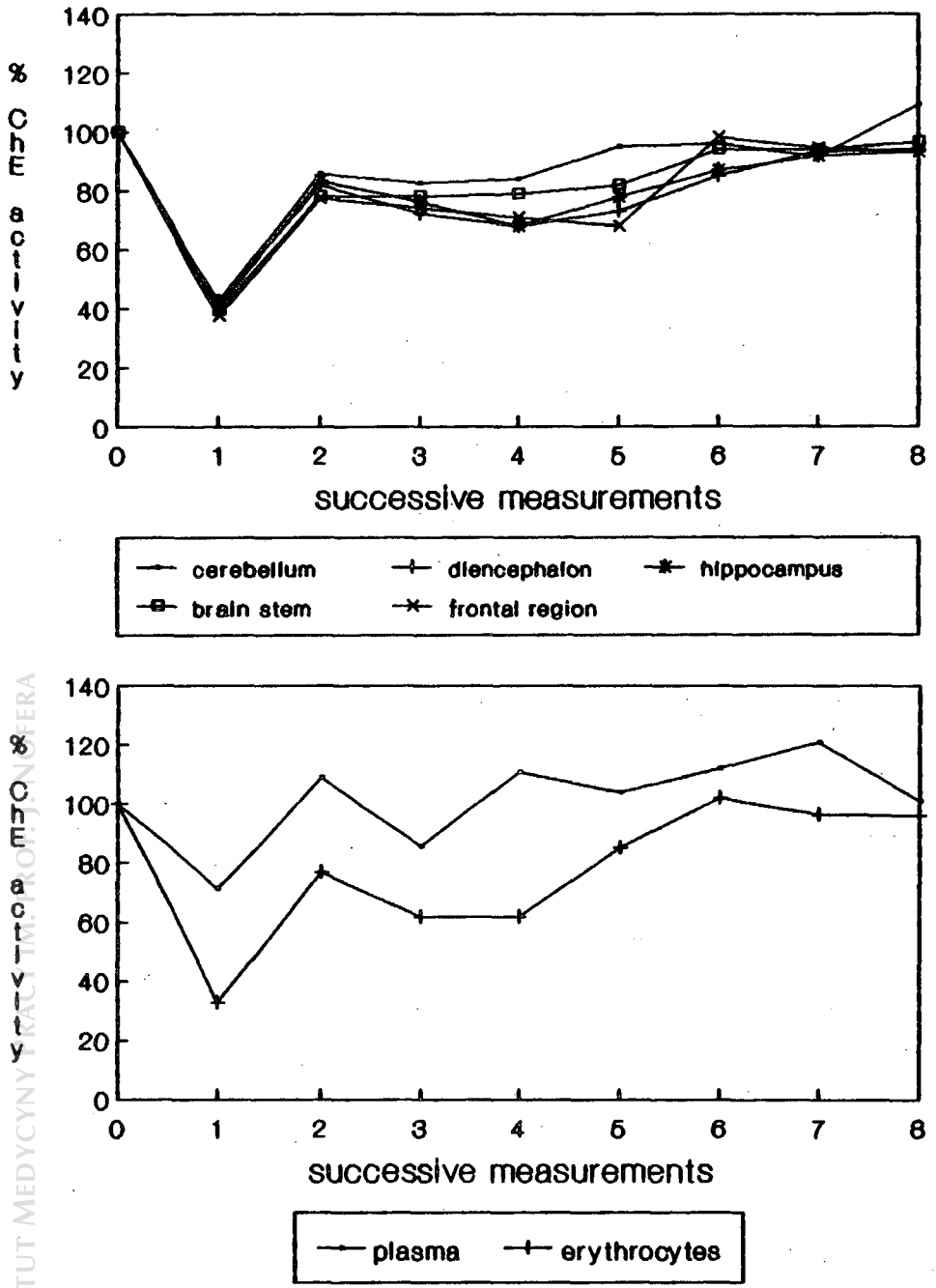


Fig. 3. Changes in ChE activity in blood (upper graph) and the brain (lower graph) after repetitive exposure to CVP in the dose of 1.0 mg/kg i.p. Successive measurements were made 3 hrs (1), 4 days (2), 7 days (3), 14 days (4), 21 days (5), 35 days (6), 42 days (7) and 49 days (8) after the last injection.

Effect of exposure to CVP on HVS activity

There were large individual differences within each group with respect to the HVS activity; during the first control session the number of HVS episodes per hour varied from 5 to 70 in different subjects. For statistical comparisons the direct HVS values were transformed into relative ones, i.e. for each rat the HVS numbers from successive recording sessions were expressed as a percentage of the value from the first session (Fig. 4). Two-way ANOVA

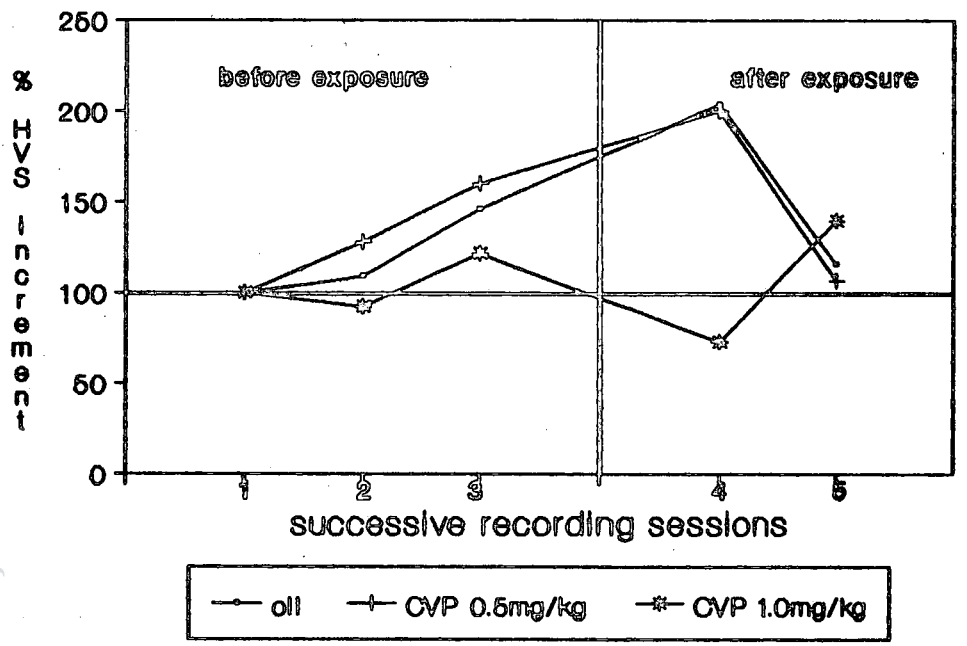


Fig. 4. Distribution of power within spectrum of the hippocampal EEG of control and CVP exposed rats before (habituation) and after (extinction) association of a 1000 Hz tone with unavoidable footshock. Each point represents an average from a 5 min EEG record. No stimulus was applied during the first 5 min of recording (sections 1 and 1'). During the last 10 min of each session (sections 2, 3, 2' and 3') the tone was applied once per min. The tone duration was 10 s. See text for further explanations.

(Groups × Sessions) revealed no Session or Group effect, but Groups × Sessions interaction was significant ($F_{2,20} = 8.61$, $p < 0.003$). Detailed comparisons showed that two weeks after the exposure the occurence of HVS in group H was significantly less frequent than in groups L and C ($p < 0.05$ in two latter cases). Comparisons between recording sessions, however, revealed no significant differences within each group. The mean duration of HVS episodes at the beginning of the experiment varied from 3.2 to 4.4 sec. It tended to increase during consecutive recording sessions. The differences between groups and between sessions as well as the Groups × Sessions interaction, however, were not significant.

Neocortical and hippocampal EEG before and after a change of biological significance of a sporadic stimulus

For the purpose of the analysis, the digital EEG records made during habituation (Session I), and during extinction (Session III), were separated, each into three 5 min sections. Sections 1, 2 and 3 were from Session I and sections 1', 2' and 3' were from Session III. Section 1 and 1' comprised the control periods (with no acoustic stimulus applied). Sections 2, 3, 2' and 3' encompased the periods during which the acoustic stimulus (1000 Hz tone) was presented every one min. A two-way ANOVA (Groups × Sections) was applied for comparison of the spectral power within the following frequency bands: 1.0–4.0 Hz, 4.0–7.0 Hz, 7.0–13.0 Hz, 13.0–21.0 Hz and 21.0–32.0 Hz in the case of the cortical EEG, and 1.0–4.0 Hz, 4.0–7.0 Hz and 7.0–9.0 Hz in the case of the hippocampal EEG. In the case of the cortical EEG statistically significant differences were found within band 1.0–4.0 Hz (effect of Sections: $F_{1,20} = 11.4$, $p < 0.005$, interaction: $F_{2,20} = 21.1$, $p < 0.001$), band 13.0–21.0 Hz (effect of Groups: $F_{2,20} = 4.5$, $p < 0.05$, interaction: $F_{2,20} = 9.7$, $p < 0.02$) and band 21.0–32.0 Hz (effect of Groups: $F_{2,20} = 5.7$, $p < 0.05$, interaction: $F_{2,20} = 14.9$, $p < 0.02$). In Group L, the percentage of 1.0–4.0 Hz activity in all 5 min EEG sections from Session III was found to be significantly higher than in the remaining groups during the same session as well as in the same group in corresponding sections from Session I. Moreover, in Session III the contribution of 13.0–21.0 Hz and 21.0–32.0 Hz activity in records of the Group L was significantly lower than in group H and C as well as in corresponding records of the same group in Session I. No differences were found between sections 1, 2 and 3 (habituation) as well as between sections 1', 2' and 3' (extinction) within any group. In other words, in each group the spectral power distribution of the cortical EEG remained relatively stable during a given session.

The analysis of the distribution of power in the spectrum of the hippocampal EEG (Fig. 6) revealed significant differences in the case of band 1.0–4.0 Hz (effect of Sections: $F_{1,20} = 5.1$, $p < 0.02$, interaction: $F_{2,20} = 5.05$, $p < 0.05$) and band 4.0–7.0 Hz ($F_{2,20} = 4.4$, $p < 0.05$, interaction: $F_{2,20} = 11.8$, $p < 0.005$). The content of 1.0–4.0 Hz activity increased in successive EEG sections. The differences, however, attained statistical significance in group H only ($F_{5,100} = 5.44$, $p < 0.002$); in this group the 1.0–4.0 Hz power in all sections of extinction was significanty higher than in section 1. Group H differed also from the remaining groups with respect to 4.0–7.0 Hz activity; during habituation the power of this band appeared to be significantly smaller in this group than in the remaining groups in the same session as well as in the same group in all sections of extinction. In group C and L the content of 4.0–7.0 Hz activity remained on a similar level in all sections. In all groups the activity within 7.0–9.0 Hz band was the highest in section 1. It decreased in section 2 and remained at a similar level in all the remaining sections.

In order to find out possible stimulus-related changes, averaged power spectra of 10 s sections of the EEG-s, made just before the stimulus

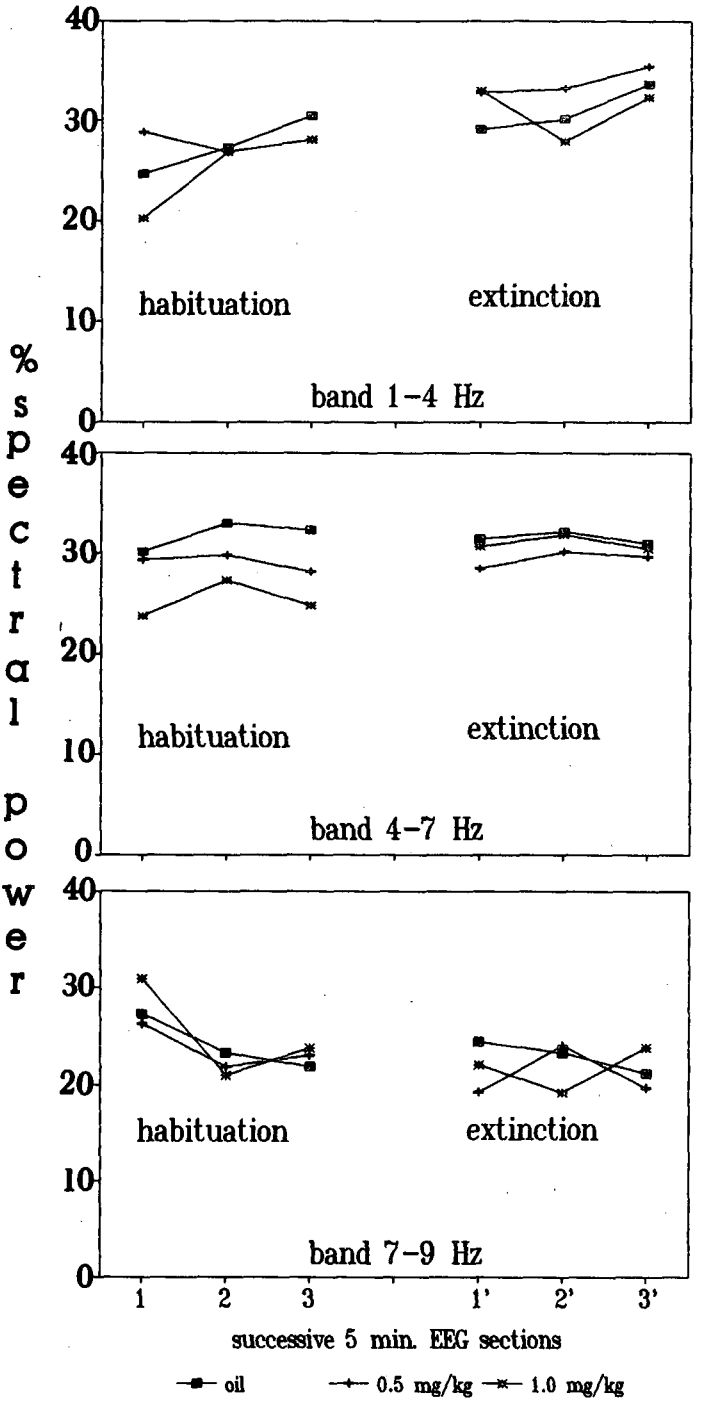


Fig. 5. Distribution of power within the spectrum of the cortical EEG of control and CVP exposed rats before (habituation) and after (extinction) association of a 1000 Hz tone with unavoidable footshock. Remaining explanations as in Fig. 4.

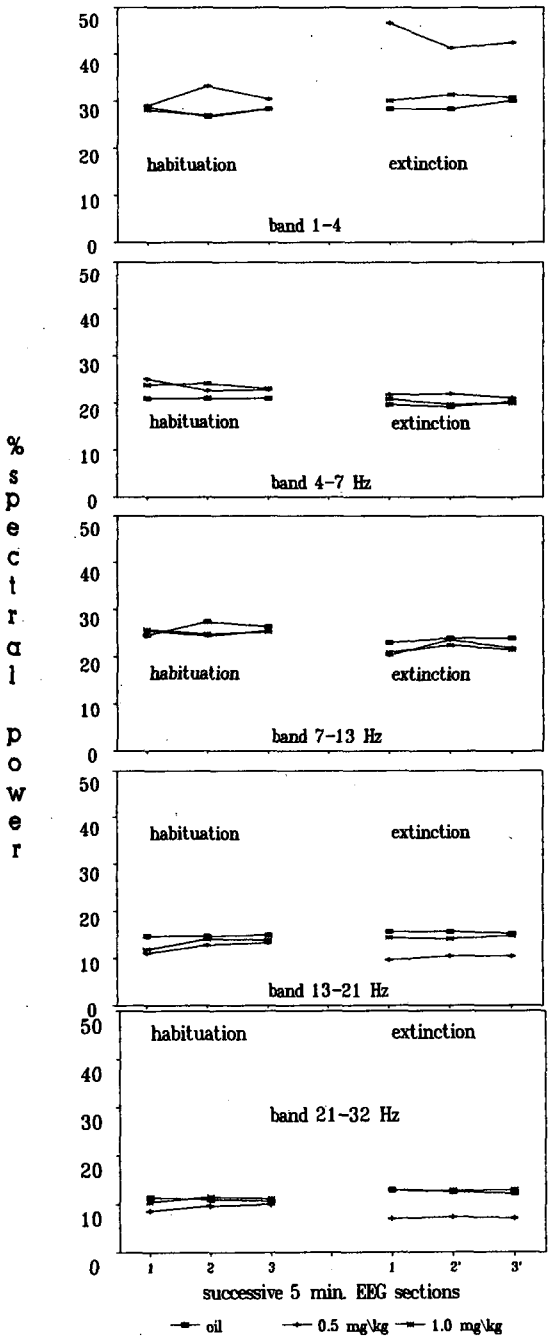


Fig. 6. Effects of CVP exposure on the development of HVS activity. Sessions 1, 2 and 3 were performed at seven day intervals. Session 4 and 5 were performed on the 14 th and 60th day, respectively, after exposure.

presentation (section a), during the stimulus (section b) and after the stimulus (section c) were subjected to statistical analysis. Comparisons of spectra from Session I revealed no differences between groups as well as between analysed EEG sections. Comparisons of data from Session III confirmed a significantly higher level of 1.0–4.0 Hz activity in group L. They also showed that this activity increased during stimulus presentation in all groups, but only in group L did this increase attain statistical significance. Comparisons of power within other frequency bands confirmed a generally decreased power of 21–32 Hz activity in group L. Comparisons between corresponding sections of cortical EEG from Session I and III once again confirmed an increase in power of 1.0–4.0 Hz activity and a decrease in power of 21.0–32.0 Hz activity in group L. Both these effects, however, were seen in all analysed sections.

Similarly performed analysis of the hippocampal EEG from session I revealed no differences between analysed EEG sections in any analysed frequency band and in any group. In Session III effect of sections and the group × sections interaction were significant in the case of 1.0–4.0 Hz band (effect of sections: $F_{1,15} = 14.58$, $p < 0.002$; interaction; $F_{2,15} = 6.65$, $p < 0.01$). In groups C and H the power within the 1.0–4.0 Hz band decreased significantly during the stimulus presentation. In group L this effect was less pronounced. A comparison between corresponding sections from both sessions revealed that in group H the power of 1–4 Hz band before and after the stimulus presentation was significantly higher in Session III than in Session I.

In the case of the 4.0–7.0 Hz band only a significant group × section interaction was found ($F_{2,15} = 6.94$, $p < 0.01$). In group H the content of this activity increased significantly during the stimulus presentation, whereas in the remaining two groups it was on a similar level in all analysed EEG sections. It should be noted that in group H in all analysed EEG sections from Session III the content of 4.0–7.0 Hz activity was significantly higher than in corresponding sections from Session I.

The content of 7.0–9.0 Hz activity increased during the stimulus presentation (effect of section: $F_{1,15} = 5.24$, $p < 0.05$; group × section interaction: $F_{2,15} = 3.21$, $p < 0.05$). The differences between periods were most pronounced in group H.

In the case of the 9.0–13.0 Hz band, differences between sections were not found.

DISCUSSION

Generally, the data obtained in the present experiments on rats are in agreement with those on rabbits from our previous work. Similarly, as in rabbits, the normalization of ChE activity in the brain, except cerebellum, proceeded at a slower rate than in the blood. It appears that in the case of repetitive exposure, neither plasma nor erythrocyte ChE activity makes a good indicator of neurotoxicity. The significance of this fact was discussed in detail in our previous work (7).

The changes in the cortical EEG, i.e. an increase in content of low frequencies and a decrease in high frequencies, found previously in the exposed rabbits, was also present in the exposed rats, although in the latter case this effect appeared only in group L. In the exposed rabbits, an acoustic stimulus associated with pain induced stronger hippocampal theta response (7). An increase in power of 4–7 Hz and 7–9 Hz activity during stimulus presentation in the exposed rats of group H suggests the same type of change. In the averaged spectra, however, from the whole 5 min hippocampal EEG samples of this group, the proportion of 4–7 Hz activity during Session III was smaller and that of 1–4 Hz activity higher than in the remaining groups.

The exposure exerted only a slight influence on the development of HVS, a form of activity not present in the rabbit EEG. The differences were seen two weeks after the exposure and consisted in a lack of increase in the HVS number in group H. It might be related somehow to ChE activity; according to the biochemical data two weeks after the exposure, ChE activity in the brain might have been reliably depressed only in group H.

The decrease in the HVS number seen eight weeks after the exposure in group L and C might be causally related with earlier conditioning i.e. association of some situational stimuli with pain. This might result in an increased level of arousal, a state unfavourable for HVS activity (5). It is likely that the same effect could be seen in group H, if the HVS measurements were performed more frequently.

Contrary to those in HVS, the differences seen in cortical and hippocampal EEG during test 2 were not attributable to differences in ChE activity; at the time of this test ChE activity in the blood and brain was most probably normal in all groups. An increased content of low (1–4 Hz) frequencies in cortical and hippocampal activity usually denotes a decreased level of arousal (3, 10). Changes in the content of 4–7 Hz hippocampal activity reflect changes in the cholinergic theta – a correlate of arousal with no overt movements (freezing), while those in the 7–9 Hz band changes in the noncholinergic theta – a correlate of voluntary movements (3). Accordingly, the higher content of 1–4 Hz activity in the cortical EEG of group L suggests merely a lowered level of arousal. The rats of group H, however, may be described as less aroused generally but more responsive to threatening stimuli than the rats of the remaining groups. (The effect seen in exposed rabbits during our earlier experiments could be defined in a similar way). It is not clear at present why, in group H, the effect of the exposure was manifested only in the hippocampal but not in the cortical EEG.

Summing up, the results of our present and previous studies allow us to conclude that, in the rat and the rabbit, repetitive exposure to this OP pesticide at subsymptomatic doses may result in long lasting effects, detectable after normalization of ChE activity in the blood and brain. At the EEG level they manifest themselves in the form of changed electroencephalographic response to stressful stimuli. Thus, the data suggest that changes in the EEG may appear not only after exposure to warfare OP-s (4), but also after exposure to less toxic compounds of this group, used as pesticides in agriculture.

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