Acta Biologica Hungarica 56 (3–4), pp. 205–214 (2005)

STIMULUS FREQUENCY DEPENDENCE OF THE CENTRAL AND PERIPHERAL SOMATOSENSORY EVOKED ACTIVITY IN RATS TREATED WITH VARIOUS PESTICIDES

ANDREA SZABÓ,* A. PAPP and L. NAGYMAJTÉNYI

Department of Public Health, Faculty of Medicine, University of Szeged,
Dóm tér 10, H-6720 Szeged, Hungary

(Received: July 7, 2004; accepted: November 12, 2004)

Rats were treated with a combination of insecticide agents in different timing schemes. In acute administration, 1/5 LD_{50} of the three insecticides: dimethoate, propoxur and cypermethrin, or their combination, was given once by gavage. In the developmental model, female rats received oral doses of 1/25 LD_{50} of the above insecticides in combination in three timing schemes including pregnancy and lactation. Responses in the somatosensory cortex and in the tail nerve, evoked by peripheral electric stimulation, were recorded in acute preparation under urethane anesthesia. It was tested whether the parameters of the cortical and peripheral evoked response are dependent on the frequency and whether this dependence is different in control and treated animals. The latency increase of the cortical responses with increasing stimulation frequency was significantly stronger in rats treated acutely with cypermethrin and the combination, and in rats receiving the combination during both intra- and extrauterine development. On the duration, the effects were less clear. Frequency dependent increase of the tail nerve action potential latency was significantly intensified by cypermethrin, and the amplitude decrease, by cypermethrin and dimethoate. Fatigue of this response during a stimulation series was also altered by the insecticides. Frequency dependence and fatigue possibly reflect the actual state of the nervous system and may have the potency to be developed to functional biomarkers.

Keywords: Insecticides – somatosensory system – cortical activity – peripheral activity – fatigue – rat

INTRODUCTION

In agriculture and in vector control, large amounts of insecticide agents are emitted into the environment so that these substances may be present in food and in drinking water. These are toxic, beyond the target organisms, for a broad spectrum of other organisms including humans. Of the main insecticides used today, organophosphates and carbamates are known to be neurotoxic in mammals and humans. Pyrethroids, used to replace them from the 80s on in order to avoid toxic risk, are regarded as non-poisonous for warm-blooded creatures [35].

Xenobiotics, entering the human body from the atmosphere and via food and drink, are a major source of exposure leading to chronic diseases. Although several

*Corresponding author; e-mail: szaboa@puhe.szote.u-szeged.hu

environmental compounds are known to be neurotoxic, little is known about their possible population effects in case of combined exposures.

Organophosphates (OPs; 33) are irreversible blockers of acetylcholinesterase [14]. Therefore, intoxication with OPs results, beyond the well-known general symptoms like bradycardia, salivation, etc. [14], in functional alterations of the central nervous system. EEG abnormalities have been observed in human subjects [10] and in animals [8, 11] together with alterations of the evoked cortical activity [3, 9]. Dimethoate (DIM) has been chosen as an OP to our study because it has a moderate human toxicity [36] and has been used in a number of countries. In our previous experiments with DIM, using different administration modes and timing schemes including a three-generation developmental model [8, 20], it was found that DIM causes electrophysiological changes even at low doses and can affect descendants.

Carbamates, including propoxur, are derivatives of carbaminic acid and inhibit acetylcholinesterase reversibly [2]. Propoxur (PRP), thanks to its long-standing residual activity, is used mainly in household pest control and for residual spraying in malaria eradication programs [34]. It acts quickly to paralyse the neural transmission in insects, producing a rapid "knockdown" effect. In rats, a single dose of ca. 1/10 LD_{50} caused a 60% drop in cholinesterase activity and marked disturbances in higher nervous functions [29]. In humans, the symptoms of PRP poisoning (diarrhoea, nausea, vomiting, abdominal pain, profuse sweating, salivation, blurred vision, temporary paralysis of the extremities, etc.) are typical for cholinergic overweight. Most of the reported illness has not exceeded a few hours, and the prognosis is generally better than in OP intoxication [37].

Pyrethroids are synthetic derivatives of pyrethrins, toxins contained in the flowers of some *Chrysanthemum* species, and are widely used as insecticides because of their high insecticidal potency, low mammalian toxicity and biodegradability [35]. Pyrethroids constitute today over 25% of the world insecticide market. The so-called type I or non-cyano-pyrethroids show generally peripheral activity while type II pyrethroids, including cypermethrin (CYP) incline to central action [16], leading to poisoning manifested in hypersensitivity, choreoathetosis, tremors and paralysis [28, 32]. In both vertebrate and invertebrate animals, sodium channels are the primary target of both types of pyrethroids [22, 23]. Other known effects include block of Ca^{2+} channels [12], inhibition of ATPases [18, 27], and of acetylcholine [1], GABA [15], serotonin [24] and benzodiazepine [7] receptors.

Due to the regular application, combined exposure by all of these insecticides, with both occupational and environmental origin, is possible.

Previous experiments showed that certain dynamic properties of the evoked activity, such as dependence of its measurable parameters on the frequency of stimulation, and "fatigue" defined as a change in the parameters of the individual evoked responses during a stimulus series, are influenced by application of neurotoxicants with environmental relevance [26]. In the present work, our aim was to test if the above mentioned insecticides have a detectable effect on the frequency dependence and fatigue of the cortical and peripheral somatosensory evoked response.

MATERIALS AND METHODS

Wistar rats were treated with the mentioned insecticide agents and their combination agents in two different timing schemes: an acute administration model, and a developmental model.

For acute administration, animals (in groups of 10) were treated by gavage with a single 1/5 LD_{50} dose of the three insecticides separately: DIM, 140.8; PRP, 17.0 and CYP, 110.8 mg/kg b.w. The combination group (DPC) received all the insecticides in the above dose. The substances were dissolved in sunflower oil of pharmaceutical quality, and the control group received the vehicle only. Here, the neurotoxicological investigations were made 24 hours after administration. At this time, the general toxic signs observed shortly after administration (rough fur, arched back, etc.) were no more seen.

In the developmental model, the combination of 1/25 LD_{50} doses of the three insecticides (DIM, 28.2; PRP, 3.4; CYP, 22.2 mg/kg b.w.) was given by gavage, to female Wistar rats ("P" generation). Treatment was done on day 5 to 15 of pregnancy (P protocol); on day 5 to 15 during pregnancy + in the 4 weeks of lactation (P + L protocol); or on day 5 to 15 during pregnancy + in the 4 weeks of lactation + the male offspring ("F1" generation) treated for further 8 weeks postweaning (P + L + P protocol). The treated offspring was, in each protocol, free of any sign of toxicity, in accordance with previous experiences [9]. The neurophysiological investigation was done in the F1 males at their age of 12 weeks. Again, 10 rats per treatment group were used.

For preparation and recording, the rats were anesthetized with 1000 mg/kg urethane i.p. [5], the head of the animals was clamped in a stereotaxic instrument and the left hemisphere was exposed. Wounds were sprayed with lidocaine and the body was covered in a warm cloth. After recovery from the surgery (30 min) silver electrodes were directly placed on the primary somatosensory projection area of the whiskers (barrel field; 30). The corresponding peripheral site (whiskery pad) was stimulated by electric pulses. In the rat's tail, the compound action potential of the tail nerve was recorded by means of a stimulating and a recording electrode pair. The NEUROSYS software (Experimetria Ltd., UK) was used for recording and analysis of electrophysiological signals.

Evoked potentials were recorded by applying a train of 50 stimuli (3–4 V, 0.05 ms) at various frequencies to each peripheral site. For determination of frequency dependence, stimuli were applied with 1, 2 and 10 Hz frequency to the whiskers, and with 1, 20, 50 and 100 Hz to the tail nerve. After averaging the 50 individual responses for each rat, the onset latency and the duration was measured on the evoked potentials, and on the nerve action potentials, peak latency and peak-to-peak amplitude (Fig. 1). The measurements were semi-automated: the measuring cursors were manually positioned to the critical points of the averaged curve on the screen, and the readouts (in ms and mV) were calculated by the software. To determine fatigue in the tail nerve (defined as amplitude decrease and/or latency increase during a train of stimuli) the first five and the last five of the tail nerve action potentials in the series

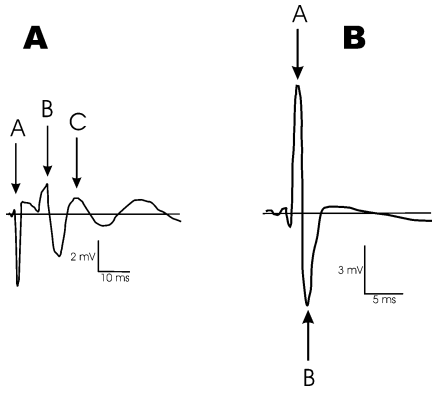

Fig. 1. A: Somatosensory cortical response evoked by whisker stimulation (average of 50 individual records). Latency was measured between arrows A (stimulus artifact) and B (onset of the main wave) while duration of the main wave between arrows B and C. B: Compound action potential of the tail nerve (average of 50 individual records). Peak latency was measured between time zero and arrow A, and peak-to-peak amplitude, between arrows A and B

were averaged and measured, and the relative change ([average of the last 5]/[average of the first 5]) of the above-mentioned parameters, indicating contingent fatigue, was calculated. Data of each rat in a group were then averaged and compared between the control and treated groups. Significance in fatigue and frequency dependence between control and treated was tested with two-sample *t*-test.

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

RESULTS

The general effect of increasing the frequency of the whisker stimulation was an increasing latency of the cortical responses while the effect on the duration of the responses was unclear. As seen in Fig. 2, a twofold increase in the stimulus frequency had moderate effect on the latency both in rats with acute (A) and developmental (C) exposure to the insecticides studied. Taking the latency obtained with the lowest frequency (1 Hz) stimulation as base, only the tenfold increase in the frequency caused a significant change: in the group treated acutely with CYP and DPC (Fig. 2A), and in the groups treated according to the P + L and P + L + P scheme (Fig. 2C). In the latter two groups, however, the cortical response latency obtained with 2 Hz stimulation was significantly longer than in the untreated controls.

In the duration of the cortical responses, comparison between differently treated groups showed no significant changes. Increase of the duration with increasing stimulation frequency was not seen in every group but where it was present, was mostly significant (Fig. 2B: DIM and DPC group; Fig. 2D: P and P + L group).

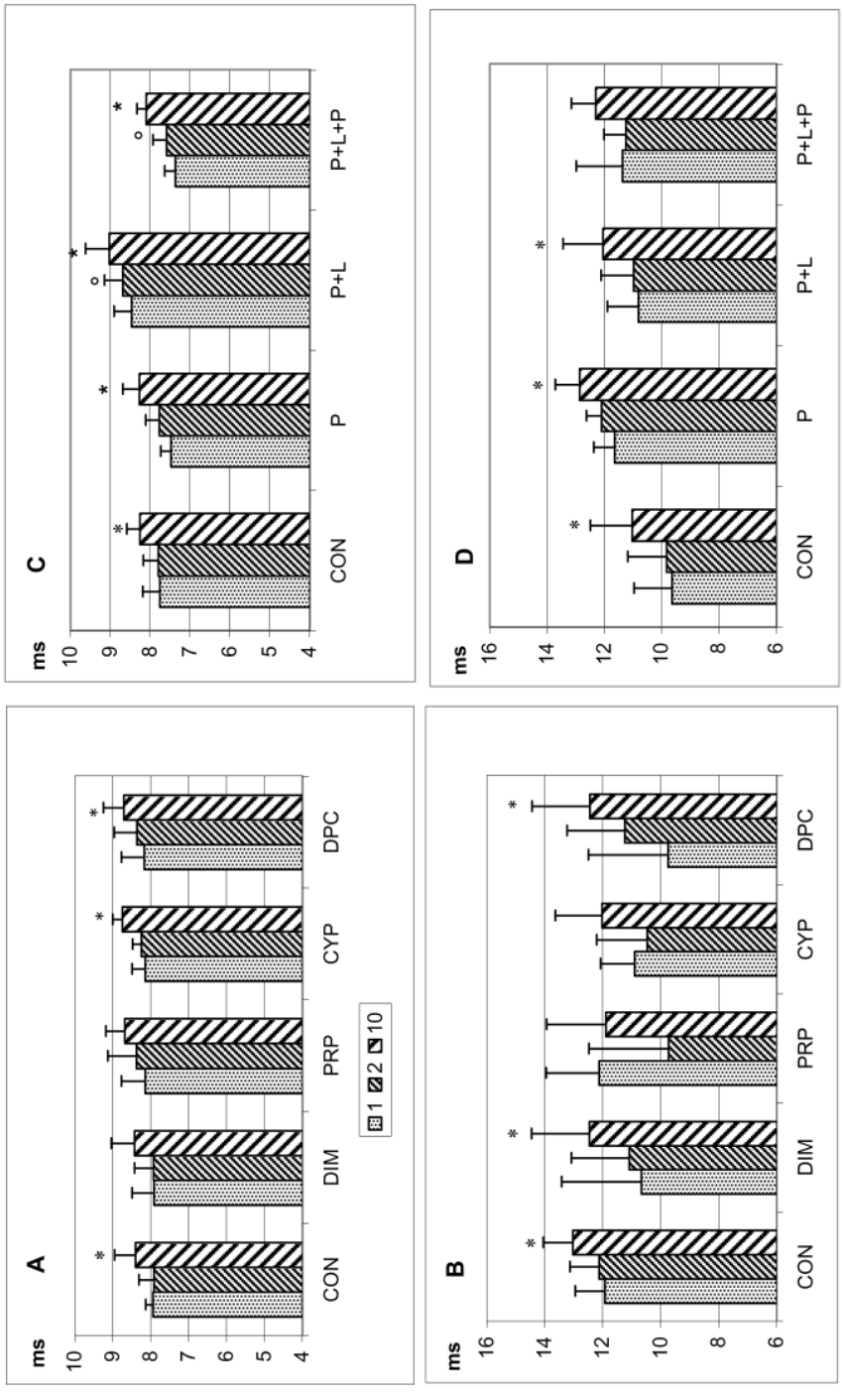

Fig. 2. Frequency dependence of the latency (A, C) and duration (B, D) of the somatosensory cortical evoked potential. Abscissa: groups. For acute treatment (A, B): CON, control; DIM, dimethoate; PRP, propoxur; CYP, cypermethrin; DPC, triple combination. For treatment during ontogenesis (C, D): CON, control; P, treated during pregnancy; P + L, treated during pregnancy and lactation; P + L + P, treated during pregnancy, lactation and post-weaning. Ordinate for all: milliseconds of latency or duration (mean + SD, n = 10). Insert: stimulation frequency (Hz). * $p < 0.05$ higher frequency vs. lowest frequency stimulation within one group. ° $p < 0.05$ treated vs. control, for responses with identical stimulation frequency

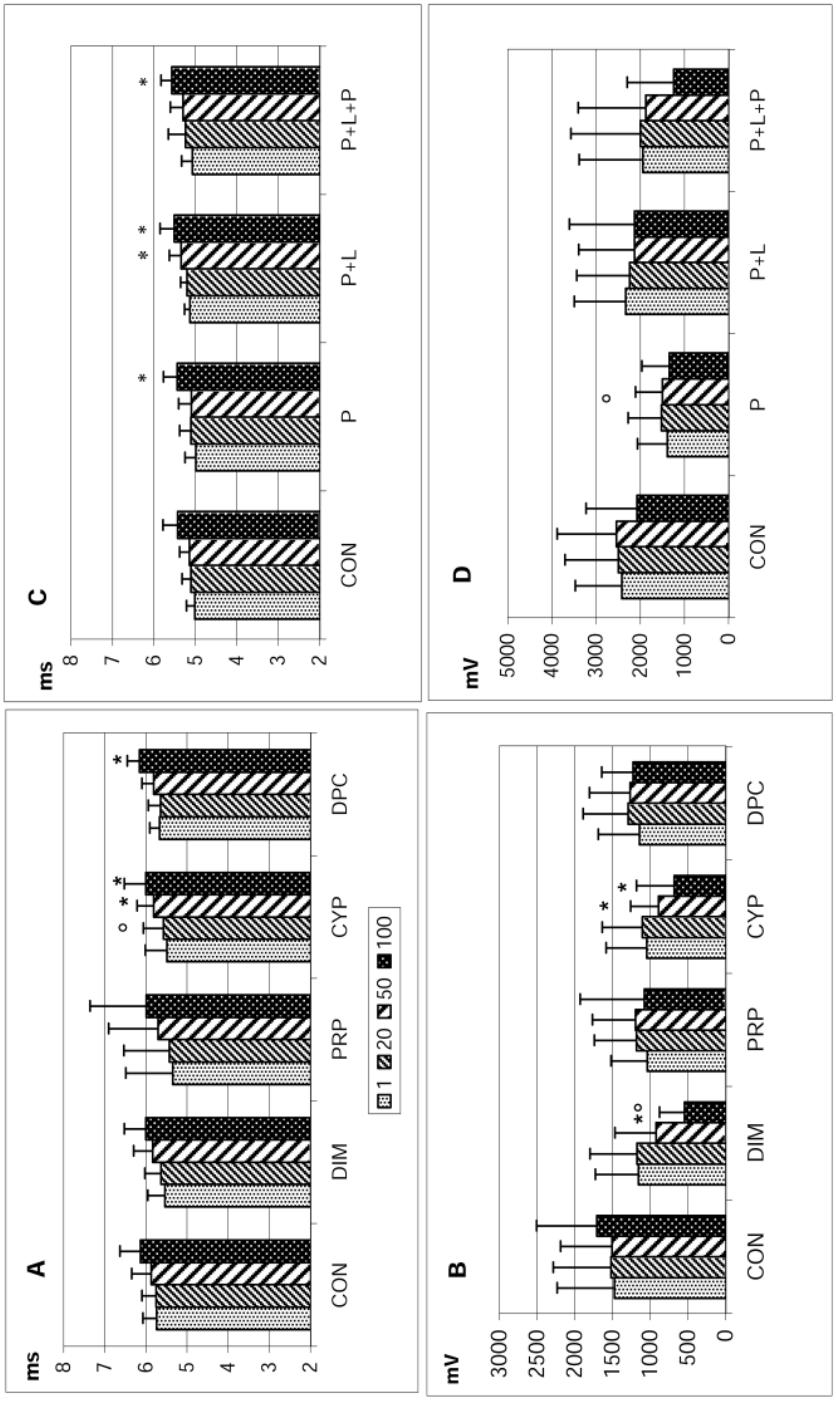

Fig. 3. Frequency dependence of the peak latency (A, C) and peak amplitude (B, D) of the tail nerve compound action potential. Abscissa: groups as given in Fig. 1. Ordinate for all: milliseconds of latency (A, C) or millivolt of amplitude (B, D; mean+SD, n=10). Insert: stimulation frequency (Hz). * $p<0.05$ higher frequency vs. lowest frequency stimulation within one group. ° $p<0.05$ treated vs. control, for responses with identical stimulation frequency

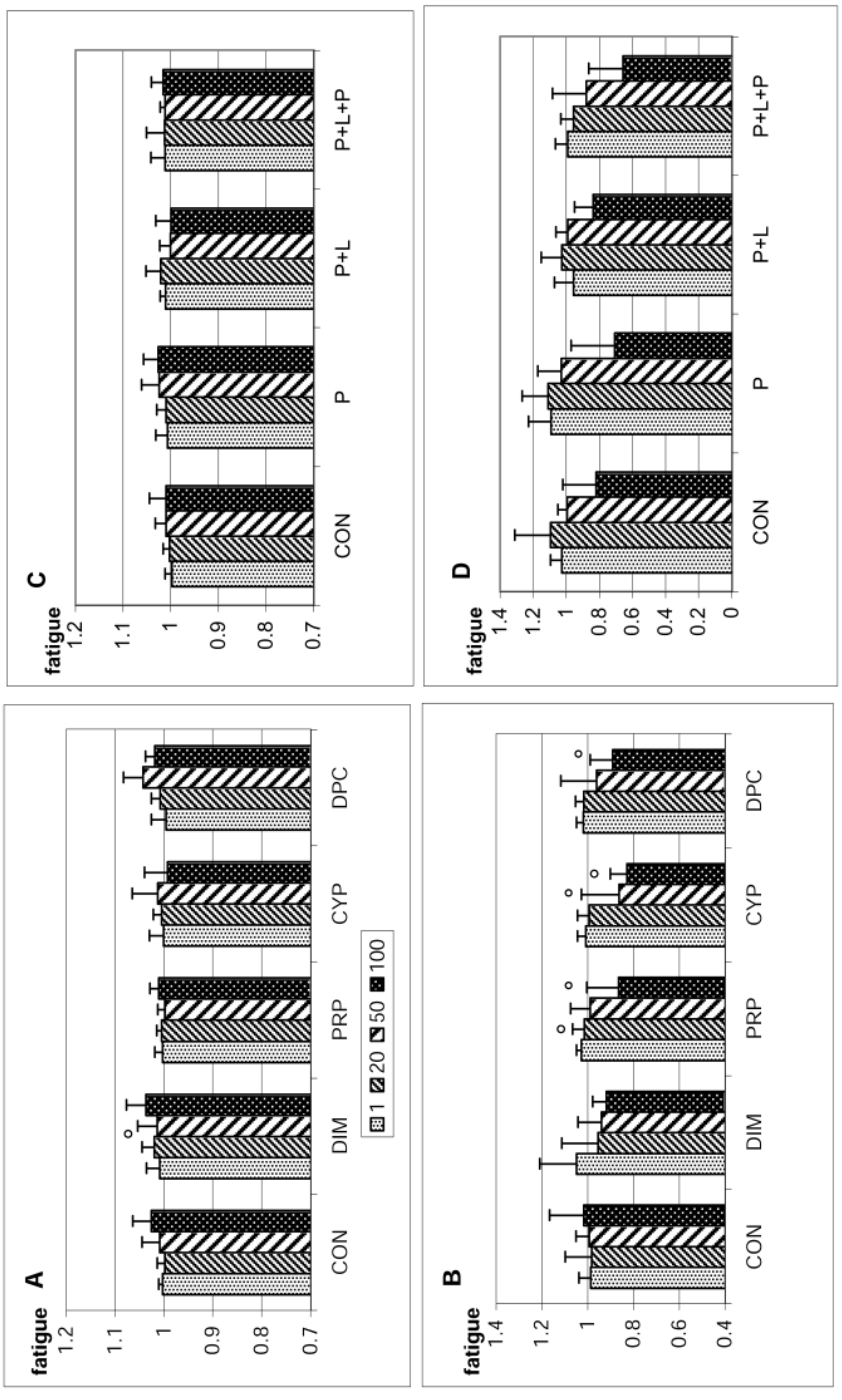

Fig. 4. Fatigue in the peak latency (A, C) and peak amplitude (B, D) of the tail nerve compound action potential. Abscissa: groups as given in Fig. 1. Ordinate: measure of fatigue (as defined in Methods, mean + SD, n = 10). Insert: stimulation frequency (Hz). ° $p < 0.05$ treated vs. control, for responses with identical stimulation frequency

The compound action potential of the tail nerve reacted on increasing stimulation frequency with modest latency increase (Fig. 3). In the controls, the increase was minimal. In the acutely treated animals (Fig. 3A), only CYP and DPC caused significant increase vs. lowest frequency in the same group. Significant change vs. control, namely decrease, was seen with CYP. In the rats treated during development (Fig. 3C), the latency of the responses obtained with the highest frequency (100 Hz) was significantly longer than of those with the lowest frequency in each treated group (Fig. 3C) but not in the controls.

The effect of increasing stimulation frequency on the tail nerve action potential amplitude was markedly different in the control and treated animals in case of acute treatment (Fig. 3B). In spite of the variability of the individual measurements, the effect was significant with DIM and CYP. The effect of the insecticides one by one seemed to be dissimilar and the effect of the combination was dominated by PRP. In the rats treated during development, the frequency dependence seemed to be stronger in the P + L + P group than in other animals (Fig. 3D) but the difference was not significant.

In the latency of the tail nerve action potential, the fatigue, calculated as given in the Methods, was minimal (Fig. 4A and C). It showed no dependence on stimulation frequency or insecticide treatment. The peak amplitude of the action potential showed, in the acute treatment groups, practically no fatigue in the untreated controls (Fig. 4B). In the treated animals, the amplitude decrease was stronger at higher stimulation frequencies and this difference was, in the groups treated with PRP, CYP and DPC, significant vs. fatigue in the control group at identical stimulation frequency. In the animals treated during development (Fig. 4D), the increase of fatigue with increasing stimulation frequency was not significant in the treated vs. control groups.

DISCUSSION

There have been numerous reports in the literature on the effects of neurotoxic exposure reflected in evoked activity in humans – e.g. mercury [31]; lead [25] or OPs [4] – and in animals – e.g. OPs [9] or ozone [6]. In the present experiments, duration of the somatosensory cortical evoked potential seemed to be more sensitive to the insecticide agents applied than latency. In earlier experiments, with partly the same agents applied during ontogenesis, both latency and duration showed dose-dependent changes [8, 21].

Increase of latency and duration with increasing stimulation frequency, indicating a less sharply delimited cortical activation, was seen both in control and treated groups. In the rat whisker sensory pathway, stimulus frequency seems to be an important input parameter, and its processing seems to depend on the level of cortical activity [19]. Regarding the cholinergic mechanism of the ascending cortical activation system [17], cholinesterase inhibitors would be expected to influence the frequency dependence of the cortical evoked potential. In our results, this was seen with DIM and the DPC combination (but not with PRP alone). In the developmental treat-

ment model, significant difference in the frequency-dependent change between treated vs. control rats, possibly due to a functional difference between the sensory pathway, was found in the latency, but not in the duration.

On the frequency dependence of the tail nerve compound action potential, CYP exerted the most marked effect by strengthening frequency-dependent latency increase and amplitude decrease – effects possibly resulting from the action of CYP on the nerve sodium channels [32]. The difference in frequency-dependent amplitude decrease between the control and CYP-treated animals was, all the same, not significant, most likely due to high variability of the individual measurements. Acute CYP treatment also resulted in significantly greater fatigue of the tail nerve action potential amplitude compared to the controls.

The investigated dynamic properties of the cortical and peripheral evoked activity proved to be sensitive to the tested neurotoxicants. Cortical evoked potentials showed more pronounced frequency dependence but the difference between treated and non-treated animals was most characteristic in the frequency-dependent fatigue of the tail nerve. These parameters probably reflect the actual functional state of one or another part of the nervous system, and were shown here to be sensitive to disturbances by xenobiotic exposure. As recording of cortical and peripheral evoked sensory activity is a routine procedure also in human neurology, the approach outlined above could lead to development of standard measures of effects for animal experimentation, and possibly biomarkers for human effect screening and follow-up.

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