# AN ATTEMPT TO INTERPRET THE FATIGUE OF THE SOMATOSENSORY CORTICAL EVOKED POTENTIAL DURING A STIMULUS TRAIN AS A POSSIBLE BIOMARKER OF NEUROTOXIC EXPOSURE

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ABSTRACT: The ongoing exposure of human populations to a variety of neurotoxic substances points to the need of search for methods capable of early detection of the harmful effects. Sensory evoked potentials are readily recorded in experimental animals and in humans and have been shown to be sensitive of toxic effects. In the present work, rats were subchronically treated with alcohol (5% in the drinking water), with dimethoate (1/25 $LD_{50}$ per os) and with the combination of the two. It was tested whether the fatigue during a series of cortical somatosensory evoked potentials is reproducible and sensitive to the toxicants used. The first and last five potentials from a series of 50 were averaged and latency and amplitude of the main waves was measured. It was found that while the latency showed minimal changes over the series, there was always a decrease of amplitude, which was stronger in the treated animals.

KEY WORDS: Organophosphate, alcohol, rat, cortical activity, biomarker

## INTRODUCTION

Many cases of chronic ill health are considered to be of environmental origin. Xenobiotics, entering the human body from the atmosphere and via food and drink, are a major source of exposure leading to chronic illness. Although several environmental compounds are known to be neurotoxic, little is known about their possible population effects at low doses and long exposure times, first of all, concerning combined exposures.

Organophosphates (OPs; WHO, 1986) as irreversible blockers of acetylcholinesterase (Koelle, 1975) are used as insecticide agents. They are toxic, beyond the target organisms, for a broad spectrum of other organisms including humans. Along with the well-known general symptoms like bradicardia, salivation etc. (Koelle, 1992), intoxication with an OP results in functional alterations of the central nervous system. EEG abnormalities have been described in human subjects (Duffy et al., 1979)

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Received: 18 February 2002
Accepted: 21 March 2002

and in animals (Gralewicz et al., 1991; Dési et al., 1994) together with alterations of the evoked cortical activity (Arakawa et al., 1993; Dési and Nagymajtényi, 1999). Dimethoate (DIM), the OP chosen for our studies has a moderate human toxicity (WHO, 1989) and has been registered and used in a number of countries. Consequently, the chance of repeated exposure to DIM is considerable, first of all, in occupational settings but also in the general public via, e.g., food contamination.

Consumption of various amounts of ethanol is a general phenomenon in modern societies. Although not overtly neurotoxic at low consumption, alcohol has been known to potentiate the toxic effects of certain other substances. In our previous studies, it was found that ethanol increased the effects on neurophysiological outcomes (spontaneous and evoked cortical activity) of different OPs and heavy metals when given subchronically to rats in combination (Nagymajtényi et al., 1999).

The use of biomarkers (Grandjean et al., 1994) is a modern way of early detection of the negative effects of the above-mentioned exposures in individuals and populations. Most of the known biomarkers, however, are based on chemical samples, thus less suitable for detecting alterations in the central nervous system (Costa and Manzo, 1995). A consequent change in a form of nervous activity, readily recordable also in humans, would be a possible candidate for such a biomarker. The aim of the present investigation was to see whether the neurotoxic effect of dimethoate, ethanol and their combination was detectable in the fatigue of the somatosensory cortical evoked potential of rats.

## METHODS

The records were obtained from male Wistar rats (10 weeks old at the beginning). The animals were put into 4 groups of 10 rats each, and were treated for 12 weeks with 5% ethanol in their drinking water, or with 1/25 $LD_{50}$ (= 20 mg/kg b.w.) dimethoate (Dési et al., 1991) by gavage on a 5 times per week schedule, or with the combination of the two. Control rats had pure tapwater for drinking and distilled water in the gavage. At the end of the treatment, the animals were anesthetized with 1000 mg/kg urethane (Bowman and Rand, 1980), the head was fixed in a stereotaxic frame and the left hemisphere was exposed. Somatosensory cortical response was evoked by electric shocks (ca. 3 V, 0.05 ms) applied to the whiskery skin area via a pair of needle electrodes. One series of 50 stimuli at 1 Hz repetition frequency was delivered and the evoked potentials were recorded from the so called barrel field, the primary cortical projection site of the whiskers (Tracey and Waite, 1995). The recording was PC-based using the NEUROSYS software (Experimetria Ltd., UK).

For the evaluation in this work, "fatigue" was defined as the amplitude decrease and latency increase expected in the series of cortical responses evoked by the stimulus train. For quantification, the first 5 and the last 5 of the evoked potentials were averaged, and amplitude and peak latency of the main negative and positive wave components were measured (*Fig. 1*). The relative change (average of the last 5 divided by the average of the first 5) was taken as the measure of fatigue, which was averaged for the group of animals in question. Significance vs. control was

tested with two-sample *t* test. It was finally checked if there is a correlation between the fatigue and the neurotoxic exposure.

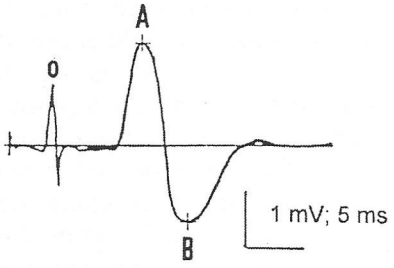
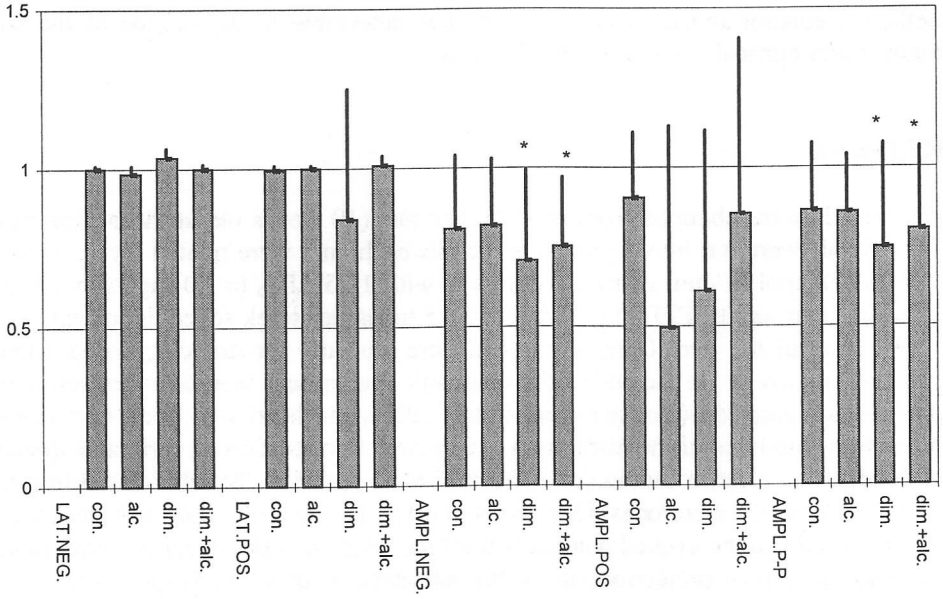



*Fig. 1. Somatosensory cortical evoked potential of the rat. The latency of the negative (A) and positive (B) peak was measured between the stimulus artifact (0) and point A or B. Peak amplitudes were measured between the isoelectric level before the artifact and point A or B. Peak-to-peak amplitude was the difference of A and B.*




*Fig, 2. Fatigue seen on the latency and amplitude of the somatosensory cortical evoked potentials. The columns are grouped by measured parameters and by treatments. Ordinate: quantified fatigue. Fatigue was defined as increase of latency or decrease of amplitude during a stimulus series and was calculated for each parameter as the average of the last 5 evoked potentials divided by the average of the first 5 evoked potentials. Columns and error bars represent mean ±SD (n = 10). * $p < 0.05$ vs. control.*

## RESULTS

Latency and amplitude of the main negative and positive wave components of the somatosensory evoked potential in the different groups of animals are shown in *Fig. 2*.

The latency of the wave components showed negligible changes during a series of stimuli, both in control rats and in those treated with DIM and/or alcohol. There was, however, a measurable decrease in the wave component amplitude between the first and the last evoked potentials during a series of stimuli. This was seen both in control rats and in those treated with DIM and/or ethanol, and was, according to the way described in Methods, interpreted as fatigue (*Fig. 2*).

In control rats and those treated with alcohol, nearly the same degree of moderate fatigue was seen. In the DIM-treated rats, the amplitude decrease was significantly stronger ($p < 0.05$ vs. control, for the negative wave and for peak-to-peak). In the DIM + alcohol combination group, the fatigue was not stronger than in the DIM-only group but still higher than in the control group.

## DISCUSSION

This kind of evaluation of cortical electrophysiological data showed that fatigue of a sensory evoked potential, as defined here, can be calculated from simple measurements and seems to be sensitive to certain toxic influences. There have been attempts to establish biomarkers of neurotoxicity. Manzo et al. (1996) described a number of potential neurochemical markers of neurotoxic effect but stressed that without a more complete understanding of the toxic mechanism the evaluation is difficult. The search for neurophysiological alterations in humans with long-term low level OP exposure brought ambiguous results. Engel et al. (1998) and London et al. (1998) found no significant functional differences but the association of symptoms and exposure in the latter study indicated that some kind of damage must have been present. This assumption is supported also by the findings of Azaroff (1999).

There are a number of data concerning cholinergic influence on cortical evoked activity, enabling a putative mechanistic explanation of the fatigue described above. ACh applied directly to the neurons of the primary somatosensory cortex of the rat altered the evoked sensory response (Donoghue and Carroll, 1987). In layer IV, receiving the majority of the thalamocortical input, the response was reduced. The visual cortical evoked response (in cats; DeBruyn et al., 1991) was also depressed by a cholinesterase inhibitor. The mechanism proposed was an influence on potassium channels, resulting in slowed an/or incomplete repolarization of the neurons after firing (Krnjević et al., 1971) which can impair the ability of the neurons to respond on repetitive stimuli. Increased ACh levels in the cortex, resulting from the exposure to an OP (Stavinoha et al., 1976) can this way cause an increased cortical fatigue.

Based on a former study of our laboratory, Dési and Nagymajtényi (1999) proposed to consider basic parameters of cortical electrical activity (e.g., average fre-

quency of EEG) as biomarkers. The approach presented by us above is more directed to the actual functional state of the cortical focus, and hence, is potentially more sensitive to disturbances by xenobiotic exposure. In the proposed calculation, the result does not depend on the absolute figures of the measured parameters which makes it potentially insensitive to the person-to-person variability of baseline values (often not known at all) and to changing recording conditions. Results obtained in another species and under experimental conditions (anesthesia, acute surgery) cannot be directly transferred to humans. The recording of cortical sensory evoked potentials is, however, a routine procedure in human neurology so that the calculation outlined above is, theoretically, applicable.

## ACKNOWLEDGEMENT

Supported by the National Scientific Research Fund of Hungary (OTKA) Grant No. 026467.

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