P300 brain potential among workers exposed to organic solvents

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SUMMARY

The P300 component of the auditory event-related brain potential was examined in a group of 11 workers exposed to low levels of organic solvents in a paint factory and 11 unexposed controls before and after 3 weeks of summer vacation. The P300 latency time was found to be prolonged among the exposed workers compared to the reference group before the summer vacation, and to be significantly longer before the vacation than after in the exposed group.

The P300 component was also examined in a group of 85 seamen from chemical tankers, experiencing peak exposures to organic solvents. They were compared to a reference group of unexposed seamen. Comparing these two groups, no difference was found in the P300 latency time. No relationship between the P300 latency time and exposure was found in a multiple regression analysis, including the variables age, alcohol consumption, smoking and cerebral concussions.

The study indicates the occurrence of an acute biological effect in the nervous system related to organic solvent exposure, expressed by prolonged P300 latency time. This was found at very low exposure levels and should be studied further.

INTRODUCTION

Central nervous symptoms in humans due to acute effects of organic solvents have been known for decades (1). The occurrence of chronic effects in the brain caused by long-term exposure to organic solvents has been suggested for several years, but are far more controversial and debated (2-6). However, the existence of a chronic organic solvent intoxication syndrome has been acknowledged by the WHO (7). The diagnostic criteria for the syndrome includes documented and sufficient exposure to neurotoxic organic solvents and the presence of typical symptoms and objective signs of damage in the central nervous system (8). Many studies have been performed to determine suitable tests for early diagnosis of brain damage due to organic solvents (9,10). Several studies have also been performed to find suitable screening tests, indicating early signs of damage before the exposed worker has become disabled (11,12).

The P300 component of the auditory event-related brain potential has been suggested to be related to cognitive events (13,14). The P300 latency time might be a useful indicator of brain dysfunction in organic solvent exposed individuals. In the present paper, the relationship between organic solvent exposure and the P300 latency time has been studied in two different occupational groups, one with low-level daily exposure to organic solvents, and one with exposure to high peaks of solvent for short periods. The purpose was to examine the occurrence of early signs of brain dysfunction in workers exposed to organic solvents.

MATERIAL AND METHODS

Subjects and their exposure

This study was designed in two parts. A group of 11 men working in a paint factory was chosen for a follow-up study. They were chosen because they were workers in a production department in the factory, present at work at the time of examination and had been working in the paint industry for at least 10 years (range 10–40 years, mean 25). The participation rate was 100%. Their mean age was 47 years. They had been exposed to a mixture of organic solvents at a low concentration generally within the Norwegian occupational standard at the time. Exposure measurements in their working environment had been performed during the same year as this study was performed, demonstrating e.g. median exposure to ethylbenzene of 3.0 ppm, *m,p*-xylene of 15.0 ppm, *o*-xylene of 2.8 ppm, toluene of 0.4 ppm and white spirit of 6.4 ppm (15). Their exposure had been continuous and daily, and they had not used personal respiratory protective equipment. Eleven age-matched men from a food plant were selected as controls. They had never been occupationally exposed to organic solvents.

In addition, a cross-sectional study was made by examining a second occupational group of 85 seamen in organic solvent exposed working sites from 20 randomly selected chemical tankers. A second reference group was established from 59 unexposed seamen from 26 dry cargo ships. They were all Norwegian males, and the mean age was 42 years in both groups. The educational level was comparable in the two groups. Details about exposure and health status is reported in previous papers (16,17). The seamen from chemical tankers had been exposed to several chemicals during loading, unloading and cleaning of the tanks. The main part of the transported chemicals had been hydrocarbon compounds, mostly organic solvents. The most commonly transported organic solvents had been benzene, ethanol, ethylene dichloride, *n*-hexane, methanol, naphtha, styrene, toluene and xylenes. The exposure had been irregular, with short periods (minutes to hours) of high exposure to the solvents one or several times daily, alternating with days without any exposure. Gas measurement from the respiration zones of mates during loading work on deck had e.g. shown values such as 80–3700 ppm ethanol, 218–629 ppm xylene and 15–374 ppm benzene. The group of seamen was chosen for this study as they had peak exposure type, different from the type of continuous exposure found in the painting factory. Due to limited resources, we were only able to examine the seamen once, and we chose to examine them in a period without chemical exposure.

An exposure index was created for the seamen by multiplying years in various working sites on chemical and oil tankers with an intensity factor from 1 to 4 according to the degree of known exposure in these types of work (10). An alcohol-index was made by adding the previous year's alcohol consumption (in g absolute alcohol) to an estimated amount of alcohol consumption during periods of heavy drinking (heavy drinking is here defined as more than 60g absolute alcohol daily for more than a month). A smoking index showed the number of cigarettes smoked daily the past year. Another index showed the occurrence of cerebral concussions.

Time at examination

The group from the paint factory and their reference group were examined in the morning at their working place the last day before and the first morning after three weeks of summer vacation. This means they had recently been exposed to organic solvents at the first examination, but not at the second. The seamen were examined in a hospital. Their examinations were made at least 14 days after the seamen's last exposure to organic solvents, thereby avoiding acute effects of the solvents. All participants in this study were examined by the same physician and under standardised procedures.

Interview/Questionnaire

Data used in the analyses were obtained from the workers by a questionnaire given to the workers from the paint factory and a standardised interview of the seamen; exposure, previous illnesses, cigarette smoking, drug and alcohol consumption. No diseases with relevance to this study was reported among the workers from the paint factory. Some of the seamen had experienced cerebral concussions. None of the workers used any drugs which might influence the results of the study.

P300 potential registration

The preamplifier of a DISA Neuromatic 2000 C was used (sensitivity 5V, lower frequency 2 Hz, upper frequency 100 Hz). An Apple IIe computer was used as an averager and controller. The program, the interface and the stimulus-amplifier were constructed at the Department of Medical Technology, Haukeland hospital. The P300 potential was elicited by presenting a series of binaural 500 Hz or 2000 Hz tones at 70 dB through headphones (TDH-39P). The tones were presented in a random sequence with the high frequency tones making up 20% and the low frequency tones 80% of the total number of sounds. A high tone was always followed by a low one. The evoked potentials were computer-averaged. The averaged evoked potentials were computed separately for all of the rare and all of the frequent stimuli in each person. A time window of 750 ms after start of stimuli was used. The sampling rate was 333 Hz. The stimuli were presented until 80 registrations without artefacts were collected. The registration was rejected if the A/D-converter was saturated (125 V) for only one of the sampling values. The stimulus duration was 100 msec and the repetition rate was 0.7 Hz. The subjects were instructed to keep a mental count of the number of high tones and to report the number at the end of the run. After the test, the responses were digitally filtered with a second order filter (low pass, 30 Hz). Peak latency of the potential was made by determining the third positive peak of the averaged waveform. Electroencephalographic activity was recorded at the vertex (Cz-electrode site in the 10-20 system) referred to linked electrodes at the mastoids with a forehead ground electrode.

Statistical methods

Paired Student t-test was used to compare P300 latency time before and after summer vacation for the workers in the paint factory and their reference group. Two-sample t-tests were used to compare these two groups.

To detect the relationship between the P300 latency time, organic solvent exposure, alcohol consumption, cerebral concussions and smoking, stepwise multiple regression analyses were performed, with the P300

latency time as the dependent variable. The stepwise procedure was used to include only the variables with significant correlation in the final model, but adjustment for age was performed in the analysis by including this variable in all steps regardless of significance. The estimation of the parameters was based on the logarithm of the ratios of the maximised likelihood functions, MLR (18).

RESULTS

The P300 latency times found in the group of workers from the paint industry before and after summer vacation are shown in Table 1. In this group there was an significant decrease in mean score of 86 msec in latency time after the summer vacation, compared to the scores found before (Student paired t-test, $p = 0.005$). The latency time had decreased for all persons in the group, exept for one person who had no change in his latency time. A similar reduction in latency time was not found in the reference group (Student paired t-test, $p = 0.17$). Comparing the paint industry workers and the reference group before the summer vacation, revealed a significant longer latency time in the group of paint industry workers (Student t-test, $p= 0.01$). However, no significant difference in P300 latency time was found comparing the paint industry workers and the reference group after summer vacation (Student t-test, $p = 0.7$).

No significant difference was found between the group of seamen from chemical tankers and the group of seamen from dry cargo ships, comparing the P300 latency time.

In the group of seamen, the relationship between P300 latency time and exposure was also investigated. A multivariate analysis of these factors and age, alcohol consumption, cerebral concussions and smoking was made. Age was the only factor which was significantly correlated to the P300 latency time ($p = 0.05$). P300 latency time was not correlated to organic solvent exposure, alcohol consumption, cerebral concussions or smoking.

Table 1. P300 latency time (msec) among workers exposed and unexposed to organic solvents.

Group of workers	mean	SD	range
Paint industry workers before vacation (n = 11)	379	47.9	303–400
Paint industry workers after vacation (n = 11)	293	31.8	255–348
Reference group before vacation (n = 11)	272	25.1	230–309
Reference group after vacation (n = 11)	272	25.9	231–309
Seamen from chemical tankers (n = 85)	279	29	226–310
Seamen from dry cargo ships (n = 59)	273	24	229–309

DISCUSSION

An acute effect of organic solvent exposure on the P300 latency time is clearly demonstrated in this study, by showing a reduction in latency time after summer holiday in the exposed workers from the paint factory. No long-term increase of the P300 latency time among the exposed workers was found, neither in the workers from the paint factory nor in the group of seamen. No relationship was found between the P300 latency time and the estimated exposure to organic solvents among the seamen.

Age is an important factor regarding the P300 latency time (19-21). This was shown in our study as well. Alcohol affects the nervous system, and alcohol abusers have poorer tests than non-abusers in examinations of P300 latency time (22). In the present study, no such effect was found. The reason may be that few of the seamen reported a high alcohol consumption. They were all working seamen, and probably not incapacitated because of alcohol abuse. The occurrence of cerebral concussions was also considered as a potential confounding factor, as head traumas may lead to posttraumatic encephalopathy. A correlation between the occurrence of cerebral concussions and the P300 latency time was, however, not revealed in this study. The effect of smoking was analysed as well, as smoking may influence the central nervous system (23,24). No such effect was found. However, no other studies has demonstrated any relationship between smoking and the P300 latency time so far.

The P300 latency time was significantly prolonged only in the paint industry workers with recent occupational exposure to organic solvents. A similar effect has been found in an American study of 35 painters recently exposed to mixtures of organic solvents (25). The mean score of P300 latency time in this study was 380 msec in the group of exposed painters, and this is quite comparable with the findings in our present study.

A few studies of the P300 latency time have been performed in groups of solvent exposed adults defined

as patients with cognitive deficits. The results from two American studies indicate that longer P300 latency time is associated with poorer cognitive test scores (26,27). The first of these two studies demonstrated this relationship in a group of 12 patients, and the second study had similar result in a group of 30 patients (which included the first 12). However, none of these studies included any reference group, and no conclusion could be made concerning the relationship between exposure and the P300 latency time.

To our knowledge, few studies – if any – have demonstrated permanent changes in the P300 potential related to long term exposure to organic solvents. A recent Swedish study of 12 patients did not show any prolonged latency time in these patients compared to a reference group (28). However, this study showed lower P300 amplitudes among the patients compared to controls. A study of 16 persons with a history of organic solvent exposure indicates that the P300 latency time improves less among persons who have been exposed to organic solvents for longer periods, compared to persons who have been exposed for shorter periods (29). The study is unfortunately difficult to interpret due to a complex methodology.

We were not able to demonstrate any permanent delay in the P300 latency time in the present study either. However, the results must be interpreted with caution, as a healthy worker effect may be present. This may in particular be the case in the studied group of seamen. Seamen are in general a healthy group of workers, as they have a health certificate which must be renewed every second year. In addition, these results are collected as part of a cross-sectional study, with the limitations cross-sectional studies do have. However, the healthy worker effect was not any problem for the group of paint factory workers, as they were examined by a follow-up study.

It must be stressed that the P300 latency time is a test with limitations. The P300 latency time is mostly known to show deficits in cognitive functions, and has been showed to be prolonged in e.g. patients with dementia. The test of P300 latency time is by no means a complete test of brain functioning. A person can have several types of disturbances in the brain that do not affect the results of this test. Also, the testing was not performed blindly, and this might also have influenced the results.

The results from the paint factory workers clearly indicates that the organic solvents do cause an acute prolongation of the P300 latency time that disappear after cessation of exposure. This was found even though the exposure level among the factory workers was low, and far below the Norwegian occupational standard. The clinical implications of a prolonged P300 latency time are not totally clear. However, it is likely that a delay in this response is an expression of cognitive disturbances in the brain, and that these disturbances might be noticeable for the workers during the exposure period and also some time after work. If this is the case, it is hardly acceptable working conditions.

With the scarce number of studies in this area, it is too early to draw specific conclusions from the present study. However, the P300 latency time is probably a sensitive indicator of acute effects in the brain caused by organic solvents. As these effects were revealed even at low concentrations of organic solvents in the air at the workplace, this shows that air monitoring of organic solvents has limitations concerning the evaluation of the working environment. Such monitoring probably ought to be supplemented with tests of biological effects. Further studies of P300 latency time in workers with different levels of organic solvent exposure will be of large interest.

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