Abnormal P600 in heroin addicts with prolonged abstinence elicited during a working memory test

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The P600 component of event-related potentials, believed to be generated by anterior cingulate gyrus and basal ganglia, is considered as an index of aspects of second-pass parsing processes of information processing, having much in common with working memory (WM) systems. Moreover, dysfunction of these brain structures as well as WM deficits have been implicated in the pathophysiology of opioid addicts. The present study is focused on P600 elicited during a WM test in twenty heroin addicts with prolonged abstinence compared with an equal number of healthy controls. The results showed significantly prolonged latencies at right hemisphere, specifically at Fp2 abduction. Moreover, memory performance of patients did not differ from that of normal controls. These findings may indicate that abstinent heroin addicts manifest abnormal aspects of second-pass parsing processes as are reflected by the P600 latencies, elicited during a WM test. Additionally, the P600 might serve as a valuable investigative tool for a more comprehensive understanding of the neurobiological substrate of drug abuse.

Key words: Abstinence; Event-related potentials; Heroin addiction; P600; Working memory

INTRODUCTION

Chronic misuse of opiates such as heroin may lead to long-lasting impairments in brain function. For example, Krystal et al., using single photon emission tomography (SPECT) to evaluate the uptake of $[^{99m}Tc]d,1$-hexamethylpropyleneamine oxime (HMPAO) in the brain, a process measuring the regional cerebral perfusion, found that chronic opiate dependence is associated with lower activity ratios, of regional cerebral perfusion (regional count density/whole brain count density) in frontal and parietal cortices and greater activity ratios in the thalamus [1]. Similarly, Kouri et al. reported impairments of cognition and memory in heroin addicts after long-term dependence [2]. These findings were interpreted as evidence of the involvement of cortico-striatal circuitry in heroin abusers.

However, there have been relatively few detailed investigations of the nature of the possible neuropsychological changes associated with long-term opiate abuse, particularly when assessing profiles of impairment in a phase of long-lasting abstinence. Gerra et al. used SPECT to examine drug-free heroin addicts, who had been abstinent for 4 months, and nine healthy control subjects. They found significant hypoperfusion in the right frontal and left temporal lobes in addicts with comorbid depression and a significant decrease in cerebral blood flow in the right frontal lobe in those addicts with antisocial tendencies [3]. Convergent evidence from neuropharmacological, behavioural and neuroanatomical approaches suggests that a wide distributed brain circuit, including striatum and cingulate gyrus, is involved in addiction and craving [4,5].

Research on event-related potentials (ERPs) could provide information on the neurobiological correlations of such regions and the information that they are processing. The P600 component of ERPs (elicited between 500 and 800 ms or later after warning stimuli) is accepted as reflecting second-pass parsing processes that are of controlled nature. More specifically, it has been suggested that the P600 signals the completion of any synchronized operation immediately following target detection [6–8]. Psychophysiological research has argued that the P600 waveform is generated and/or modulated by both anterior cingulate gyrus [9] and striatum operation [7]. Moreover, Guillem et al. [9] argued that P600 has much in common with the central executive component of WM [10]. Similarly, Garcia-Larrea and Cezanne-Bert provided evidence indicating that the P600 component of ERPs may be related to WM [8]. In this context, more recently, Ornstein et al. observed that heroin abusers were impaired in spatial WM
test, in strategic performance of this test, and were also deficient on a test of pattern recognition memory [11].

The foregoing points motivated us to examine aspects of second-pass parsing processes of information processing in abstinent heroin addicts compared to those of healthy controls, as are reflected by the P600 elicited during a WM test. The aim of this study was to evaluate the effect of abstinence on cognitive function in abstinent addicts, with an almost exclusive drug preference for heroin, as compared with normal controls matched for age, sex and educational level, using the measurement of the P600 component of ERPs during a WM test.

**MATERIALS AND METHODS**

**Patients:** The patient group comprised 20 right-handed heroin addicts, 16 males and four females (mean age 31.05 ± 5.3 years, mean educational level 11.35 ± 3.45 years) with a history of abuse of heroin alone of mean duration 9.00 ± 6.29 years before detoxification. Since substance misusers consist of a heterogeneous group, primary users of one class of drugs will probably have used drugs of another class. The subjects of the study were selected to meet DSM-IV criteria [12] for drug abuse/dependence, mainly for heroin abuse or dependence. The addicts studied were not characterised by double dependence or prolonged use of drugs other than heroin. Previous consumption of other drugs of abuse, use of psychotropic agents and excessive alcohol intake were criteria for exclusion. Participants were volunteers who sought treatment for substance and alcohol dependency from the Drug-Free Outpatient Clinic of the Psychiatric Department of Athens, University Medical School. After detoxification, patients were admitted to a long-term residential psychological rehabilitation program. On the ground that the validity of self-report data is a continual subject of debate, the subjects had undergone a urine examination in order to establish a possible presence of any substances of abuse. After the admission in this program, and for a period of 6 months, the patients underwent twice-weekly analyses for urine metabolites of the main substance of abuse. Only when these twice-weekly analyses excluded their consumption in the first 6 months after admission were the patients eligible for inclusion in the study. Furthermore, subjects were excluded if there was a DSM-IV Axis I diagnosis (excluding substance abuse and dependence) or a history of mental retardation, or major neurological/medical disorders (e.g. epilepsy, AIDS, etc.). Information for the latter exclusion was obtained from the subject’s medical record, which contained general medical, neurological, and psychiatric evaluations.

**Normal comparison subjects:** Twenty right-handed healthy volunteers (15 males and five females) matched to the addicts on age (30.7 ± 4.82 years) and educational level (12.15 ± 3.43 years) were recruited from the hospital staff and local volunteer groups. They were free of psychiatric and physical illness. All participants were right-handed as assessed by Edinburgh inventory [13] and had no history of any neurological or hearing problems. Written informed consent was obtained from both patients and control subjects.

**Stimuli and procedure:** Patients and controls were evaluated by a computerised version of the digit span Wechsler Test [14,15]. The subjects sat in an anatomical chair placed inside an electromagnetically shielded room. An outline of the procedure is provided in Fig. 1. A single sound of either high (3000 Hz) or low frequency (500 Hz) was presented to the subjects, who were asked to attend in order to memorize the numbers that followed. The warning stimulus lasted 100 ms. A 1 s interval followed and then the numbers to be memorised were presented. At the end of the number sequence presentation, the signal tone was repeated and subjects were asked to recall the given numbers as quickly as possible. The numbers were recalled in the same (low frequency tone) or in the opposite order (high frequency tone) than that presented to him/her.

ERPs were recorded during the 1 s interval between the warning stimulus and the first administered number at a sampling rate of 500 Hz. The electrophysiological signals were recorded through Ag/AgCl electrodes. Electrode resistance was kept constantly < 5 kΩ. EEG activity was recorded from 15 scalp electrodes based on the International 10-20 system [16], referred to both earlobes. An electrode placed on the subject’s forehead served as ground. The bandwidth of the amplifiers was set at 1 Hz to 35 Hz. During the administration of stimuli the subjects had their eyes closed, in order to minimize eye movements and blinks. Eye movements were recorded through EOG. Recordings with EOG higher > 75 µV were rejected. Warning stimuli as well as learning material, i.e. numbers to recall, were presented binaurally via earphones at an intensity of 65 dB sound pressure level. The evoked biopotential signal was submitted to an analogue to digital conversion and was averaged by a computerized system. Each recording session consisted of 26 repetitions of the trial.

Since the warning stimuli were of two different frequencies, one high and one low, it was not clear whether they could generate the same P600 waveform, although the P600 component is included in the array of late-endogenous ERP components, which are not normally modality specific [17]. We conducted an analysis of both sounds in each group (13 high and 13 low frequencies), in order to ensure that there were no differences in the P600 wave-
forms caused by frequency modalities. As was expected there were no differences in the P600 waveform by frequency in each group.

The following parameters were calculated: (a) the P600 waveforms resulting from the leads Fp1, F3, C3, (C3-T5)/2, Fp2, F4, (C4-T6)/2, (C3-T5)/2, O1, O2, P3, P4, Pz, Cz and Fz. In this context, it should be noted that the positions (C3-T5)/2 and (C4-T6)/2 are used as electrode leads, because these positions correspond to brain areas serving verbal memory and language [18]. The amplitude of the P600 component of an ERP waveform in each average was identified as the most positive peak between 500 and 800 ms after the warning stimulus [6]. (b) The behavioural performance concerning recalled digits. Typical grand average of the ERP's waveforms are shown in Fig. 2

**Statistical analysis:** The normality of the distribution of all the variables was tested using the K-S test. Only the latency at the O1 abdution was found to be not normally distributed. Thus for comparisons concerning this variable, the Mann-Whitney test at the 95% confidence interval was used. Multivariate analysis of variance, accompanied by stepdown procedures, was chosen for determining significant differences between the two groups, concerning the mean latencies (exception being the latency of O1 abdution) and amplitudes of the P600. The t-test was conducted, regarding the mean values of memory performance for patients and control subjects. The significance level was set at 0.05.

The same procedure was applied for total latency means of assemblies of subgroups of abductions, corresponding to the right hemisphere (i.e. abductions Fp2, F4, C4-T6/2, C4, O2 and P4), left hemisphere (i.e. abductions Fp1, F3, C3-T5/2, C3, O1 and F3), and sagittal abductions (i.e. abductions Pz, Cz and Fz). The total latency means of the abduction subgroups were computed from new variables (right, left and sagittal), created for each subject by summing up the latencies at the abductions of interest and dividing by the particular number of abductions. Again the significance level was set at 0.05.

Finally, the binomial test was employed to test the prevalence of one group over the other in the frequency distribution of positive and negative differences between the mean latencies of two groups at the 15 abductions under consideration.

**RESULTS**

**Comparison of the P600 in abstinent heroin addicts and controls:** Figure 3 depicts the mean latencies of the P600 waveform for the two groups at each abdution. There are two striking features that accompany the visual inspection of the figure. First, the mean latencies of the addicts are greater than the corresponding mean latencies of the controls for all the abductions. The binomial test proves that this distribution is far from coincidental ($p < 0.01$).

Second, the mean latency of the Fp2 abdution for the addicts is abnormally prolonged. The mean latency for the Fp2 abdution for the addicts was actually significantly greater than that of controls (MANOVA univariate tests for between subjects effects, $p < 0.05$). The (C4-T6)/2 abdution also revealed significant difference between the two groups ($p < 0.05$), which, however, vanished when it was tested using the Fp2 mean latency as co-variate. The analysis used in this case was the multivariate analysis of variance using the stepdown procedure for the adjustment of the effects of abdution latencies, already found to be significant. It seems that the prolonged latency of the Fp2 abdution carries down a comoncomitant retardation of the latency of affiliated abductions. These results are shown in Table 1.

The above conclusion seems to be particularly true for the assembly of six abductions situated at the right hemisphere, (Fp2, F4, (C4-T6)/2, C4, O2 and P4), since the two-tailed t-test has shown significant differences of the total latency means of the six particular abductions between the two groups (see Table 2). This still holds true even if the Fp2 abdution is not taken into consideration (controls $585.0 \pm 60.1$, addicts $624.8 \pm 77.6$, one-tailed t-test, $p < 0.05$).

Application of one-tailed test is fully justified in this case, since it is known beforehand that the latencies of the addicts are greater than the latencies of the normal group, thus the rejection hypothesis could be formulated accordingly.

No significant differences between the two groups regarding the P600 amplitudes were found. Similarly, the Mann-Whitney test did not reveal any significant difference between the two groups concerning the latency of the P600 at the abdution O1.

**Behavioral data:** The memory performance of abstinent addicts, concerning the recalled digits, was lower than that of normal controls but did not differ significantly. The mean and s.d. values for two groups were 60.8 $\pm$ 15.5 and 68.1 $\pm$ 8.69, respectively. It should be noted that the total digits presented exceed 149.

**DISCUSSION**

The present study is focused on P600 elicited during a WM test in twenty heroin addicts with prolonged abstinence compared to equal number of healthy controls. The results showed that the patient group, as compared to healthy controls, was characterised by significantly prolonged latencies at right hemisphere specifically at the abdution Fp2.

The observed differences of the P600 latency in the present study may be discussed in the light of recent views about its psychophysiological significance. Taking into account that the latency of the P600 is considered as an index of onset and duration of parsing processes [7], the particular results seem to be in agreement with neuropsychological research indicating that heroin addicts are deficient in monitoring processes [19]. This assumption appears to be compatible with neuropharmacological studies demonstrating long-lasting changes in dendritic branching in brain regions, such as the nucleus accumbens, parietal and prefrontal cortex, after repeated injections of single doses of morphine in ranges relevant to reinforcing effects [20].

Moreover, the present findings appear also to be consistent with functional neuroimaging methods assessing CBF changes associated with behavioural effects of opiate. For example, Jones et al., using PET scans acquired from a patient in pain, reported that induced morphine had increased CBF in the prefrontal and anterior cingulate cortex.
Schlaepfer et al., employing SPECT methods, reported increased CBF in temporal lobes after administration of Butophranol, while the administration of hydromorphone (μ opioid agonist) resulted in increases of CBF in the anterior cingulate cortex, the thalamus and both amygdalae [22].

The importance of the observed differences at the particular abductions may be understood considering the...
Table 1. Mean (±s.d.) latencies (in ms) of the P600 waveform for the two groups, at each abduction. The last column indicates statistical significance.

<table>
<thead>
<tr>
<th>Abduction</th>
<th>Controls</th>
<th>Addicts</th>
<th>Univariate F-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fp1</td>
<td>631.8 ± 107.0</td>
<td>682.5 ± 117.1</td>
<td>0.161</td>
</tr>
<tr>
<td>F3</td>
<td>613.3 ± 112.7</td>
<td>646.2 ± 108.3</td>
<td>0.353</td>
</tr>
<tr>
<td>(C3-T5)/2</td>
<td>612.3 ± 92.8</td>
<td>619.9 ± 101.0</td>
<td>0.806</td>
</tr>
<tr>
<td>C3</td>
<td>603.7 ± 85.9</td>
<td>605.7 ± 83.3</td>
<td>0.941</td>
</tr>
<tr>
<td>Fp2</td>
<td>617.9 ± 92.6</td>
<td>691.2 ± 101.0</td>
<td>0.022*</td>
</tr>
<tr>
<td>F4</td>
<td>619.1 ± 90.4</td>
<td>655.7 ± 117.8</td>
<td>0.277</td>
</tr>
<tr>
<td>(C4-T6)/2</td>
<td>585.3 ± 77.9</td>
<td>658.1 ± 116.5</td>
<td>0.026*</td>
</tr>
<tr>
<td>C4</td>
<td>584.6 ± 76.3</td>
<td>624.8 ± 104.1</td>
<td>0.172</td>
</tr>
<tr>
<td>O1</td>
<td>563.9 ± 39.3</td>
<td>620.1 ± 108.3</td>
<td>—*</td>
</tr>
<tr>
<td>O2</td>
<td>576.4 ± 74.5</td>
<td>598.9 ± 99.4</td>
<td>0.423</td>
</tr>
<tr>
<td>P4</td>
<td>559.8 ± 60.1</td>
<td>586.3 ± 79.8</td>
<td>0.243</td>
</tr>
<tr>
<td>P3</td>
<td>578.5 ± 66.3</td>
<td>606.9 ± 96.8</td>
<td>0.286</td>
</tr>
<tr>
<td>Pz</td>
<td>577.8 ± 81.0</td>
<td>597.5 ± 93.4</td>
<td>0.48</td>
</tr>
<tr>
<td>Cz</td>
<td>590.8 ± 90.5</td>
<td>628.8 ± 107.5</td>
<td>0.234</td>
</tr>
<tr>
<td>Fz</td>
<td>605.2 ± 101.8</td>
<td>630.9 ± 114.7</td>
<td>0.459</td>
</tr>
</tbody>
</table>

An asterisk marks differences between the two groups that are statistically significant. *This abduction was not included in the particular analysis.

Table 2. Mean (±s.d.) latencies (in ms) of the P600 of the total latency means of subgroup abductions for the two groups, corresponding to right hemisphere (e.g. abductions Fp2, F4, C4-T6/2, C4, O2 and P4), left hemisphere (e.g. abductions Fp1, F3, C3-T5/2, C3, O1 and P3), and sagittal abductions (e.g. abductions Pz, Cz, Fz). The last column indicates statistical significance.

<table>
<thead>
<tr>
<th>Total latency</th>
<th>Controls</th>
<th>Addicts</th>
<th>Two-tailed t-test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Right</td>
<td>590.5 ± 60.5</td>
<td>635.8 ± 76.0</td>
<td>0.044*</td>
</tr>
<tr>
<td>Sagittal</td>
<td>591.3 ± 79.4</td>
<td>619.1 ± 83.4</td>
<td>0.287</td>
</tr>
<tr>
<td>Left</td>
<td>600.1 ± 51.8</td>
<td>630.2 ± 75.0</td>
<td>0.160</td>
</tr>
</tbody>
</table>

* p < 0.05.
role of the two hemispheres in integrating information about rewards and punishments. According to the valence hypothesis of information processing theory, the right hemisphere is conceptualised as a key part within a brain circuit subserving negative valence of information, mediating withdrawal associated behaviour, while the left hemisphere is considered as a main part within a brain circuit subserving positive valence of information, mediating approach associated behaviour [23].

The post hoc assignation of psychological function to regional activation is somewhat speculative and more experiments are required addressing the specific role of a particular psychological process in the functional anatomy of heroin abstinence. Furthermore, the present study was concentrated on a heroin addicts group under a specific clinical protocol. To draw general conclusions about auditory P600 elicited, during a single WM test, in prolonged abstinence heroin addicts, our study may be thought preliminary. Future work is required involving different tasks or different stimulation modalities. The conjugation of classical clinical data (semiology and demographic data) with other kinds of neuroimaging (MRI) and/or metabolic techniques could prove useful in order to assess the functional alterations associated with localised brain dysfunction and may provide valuable information about the functional interactions between brain systems involved in drug abuse. Such combined approaches might offer the opportunity to a more comprehensive understanding of neurobiological substrate of drug abuse.

Finally, as far as the memory performance is concerned, our results showed that abstinent heroin addicts did not differ in comparison to healthy controls. Present findings seem to support previous research which indicates that long term abstinence may improve neuropsychological second-pass parsing processes as reflected by auditory P600 waveform and secondly, there are important implications of this type of research for addiction. ERP’s provides a means for assessing patterns of changes in cognitive brain function associated with drug abuse, and might offer the opportunity to a more comprehensive understanding of neurobiological substrate of drug abuse.

CONCLUSION
The aim of the present study was to investigate aspects of second-pass parsing processes of information processing in abstinent heroin addicts compared to those of healthy controls, as are reflected by the P600 component of ERPs elicited during a WM test. The results showed significantly prolonged latencies at right hemisphere, specifically at the abduction Fp2. Considering the above stated limitations, it could be suggested that, firstly, these findings may indicate that abstinent heroin addicts manifest abnormal aspects of

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