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Chapter 6

P300 after head injury: Pseudodelay caused by reduced P3A amplitude

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Abstract

**Objective:** We compared conventional P300 analysis with source analysis in normal subjects and head injury patients. Based on earlier findings of improved P300 component identification and reduced P3B latency variability with source analysis in normal subjects, our aim was to investigate whether source analysis could improve the distinction between these groups.

**Methods:** In total, 21 healthy control subjects and 21 patients with mild to moderate head injury were included in this study. A standard auditory 2-tone oddball paradigm was used. Latencies and amplitudes obtained with conventional P300 analysis were compared with source analysis results.

**Results:** With conventional analysis, head injury patients had delayed P300 latencies and reduced P300 amplitudes in comparison to controls, while source analysis showed no latency differences for both P3A and P3B components. Instead, source analysis indicated absence of P3A components in 43% of patients.

**Conclusions:** The P300 delay in head injury patients, observed with conventional analysis, is a pseudodelay caused by decreased P3A amplitudes. Consequently, the unaffected P3B component with its later latency determines conventional P300 latency in these patients. Conventional P300 latency cannot be used to conclude that there was delayed early stimulus processing in head injury patients.
Introduction

Soon after its discovery, the use of the P300 potential as a possible diagnostic test for cognitive dysfunction was evaluated. Several studies have reported significant group differences between normal subjects and patients with, for example, dementia or head injury. This was initially received with enthusiasm, since the observed P300 latency prolongation in dementia was consistent with cognitive theories of P300 latency as an index for certain mental processes, i.e. stimulus evaluation time. Despite these promising results, sensitivity and specificity in a clinical setting have been very variable, which has led to a debate regarding clinical utility. For example, the sensitivity of an increased P300 latency in dementia patients is reported to range from 7 to 83%\(^4\)\(^5\). The literature presents variable results for head injury patients. Increased latency\(^2\)\(^6\)\(^10\), reduced amplitude\(^11\)\(^13\) or no change in P300 parameters\(^14\)\(^15\) have all been reported. This is probably, at least in part, related to the fact that P300 is subject to considerable variability. Suggestions for improving and reducing variability have included the standardization of recording protocols and the identification of factors that contribute to the variability of P300 measurements, such as biological variables\(^16\). However, another important source of variability is the existence of overlapping P300 components.

Recently, efforts have been made to characterize the P300 potential in terms of its components. One of these methods is source analysis\(^17\), in which the scalp-recorded waveforms are modelled by a number of equivalent dipoles. By iteratively moving and rotating the dipoles, the best-fitting solution is obtained. Latencies and amplitudes of the dipoles can then be used to identify components of P300, which may not be visible as separate peaks in conventional P300 waveforms. The main P300 components are P3A, an earlier and more frontocentrally located component, and P3B, a later and more centroparietally located component. We demonstrated that, while conventional P300 analysis identifies P3A components in only a minority (c. 25%) of subjects, source analysis shows that both P3A and P3B almost always contribute to the P300 complex in normal subjects, but to varying degrees\(^18\). Furthermore, source analysis yielded a later mean P300 latency with a smaller standard deviation in a group of control subjects when compared with conventional analysis. Therefore, P300 latency in controls was less variable with source analysis. If a similar reduction in variability could be obtained in patients, the latency distributions of normal subjects and patients could show less overlap, if the same mean difference in latency persisted. This could then result in a better distinction between patients and controls, and ultimately in better diagnostic properties.

In the present study, we evaluated the P300 latency distributions of control subjects and patients with moderate to severe closed head injury, using both conventional P300 analysis and source analysis. We also analyzed amplitude parameters for both methods. Given the results in normal subjects, our hypothesis was that, with source analysis, the latency distributions of patients and controls would show less overlap due to a decreased variability.
Methods

Patients and Subjects
A total of 21 healthy control subjects (8 female, 13 male; age 30 ± 8 (years ± standard deviation) and 21 patients with moderate to severe closed head injury (5 female, 16 males, age 29 ± 11 years) were included in this study. None of the control subjects had a history of neurological illness or head injury. Glasgow Coma Scale (GCS) total scores ranged from 5-14 (mean 9.3 ± 2.5) and post-traumatic amnesia duration ranged from 7-60 days (mean 21.8 ± 12.6). CT scans were carried out on all patients on admission. In 7 patients CT scan results were normal, 7 patients showed abnormalities (edema or hemorrhage) in 1 frontal or temporal lobe, in 3 patients both the frontal and temporal lobe on one side were affected, and in 3 patients bifrontal contusional abnormalities were seen. In one patient, abnormalities were visible in the left parietal lobe only. In 2 patients, the GCS score could not be determined reliably due to the effects of sedatives and/or anesthetics. In all patients, a P300 recording was performed between 6 and 12 months after the injury. All subjects and patients gave their informed consent. Approval for this research was obtained from the Ethics Committee of the University Medical Center Groningen.

P300 recording and analysis
The entire recording and analysis procedure was described previously in our study on normal subjects. In short, we followed the recommendations on P300 recordings as outlined by Polich. A standard oddball paradigm was used, in which subjects were asked to silently count auditory target stimuli of 2000 Hz (probability: 0.15) and to ignore standard stimuli of 1000 Hz (probability: 0.85). All stimuli were 70 ms in duration with a 50 ms plateau phase and 10 ms rise and fall time. Inter-stimulus interval varied randomly between 1.5 and 2.5 seconds. The subject was asked to look at a fixation point during the recording, but no blink instructions were given. The paradigm was administered in 2 blocks of 100 tones and was repeated in cases of frequent blinking until a total of at least 20 blink-free target segments was obtained over all blocks. EEG was recorded from the scalp using a 128-electrode cap, which was connected to a 128-channel headbox (Twente Medical Systems BV, Hengelo, the Netherlands). We used 6 electrodes to monitor eye movements, 1 above and below each eye for vertical eye movements, and 1 lateral to each eye for horizontal eye movements. One electrode was placed on each earlobe for use as a linked-ears reference in the conventional P300 analysis. Impedance values were kept below 10 kΩ. We used Onyx software (Silicon Biomedical Instruments BV, Westervoort, the Netherlands) to capture the EEG data. Sample frequency was set at 1000 Hz. After storage of the raw data, further processing was performed off-line using Brain Vision Analyzer software (Brain Products GmbH, Munich, Germany). The low pass filter was 30 Hz (48 dB/octave) and the high pass filter was 0.16 Hz (48 dB/octave). We segmented the data in epochs of 1000 ms with a 50 ms pre-stimulus interval and a 950 ms post-stimulus interval. Segments with blinks were excluded from further
analysis. Artifact rejection was set at 100 μV. Next, a DC detrend procedure was performed on the individual segments using the first 100 ms as the starting point and the last 100 ms as the end point. A baseline correction procedure was performed using the first pre-stimulus 50 ms. Averaging was performed on individual channels, excluding those channels for which less than 20 segments were available due to the result of the artifact rejection. Conventional analysis and source analysis of P300 were performed by different investigators, and the analysis was ‘blind’ in terms of the subjects’ characteristics.

Conventional P300 analysis

Fz, Cz and Pz traces were produced using a linked ears reference. The largest positive peak occurring after the N1, P2 and N2 components that increases in amplitude from frontal to parietal scalp areas was identified as the P300 wave. In cases of bifurcated peaks, the second peak with a central/parietal maximum was selected for P300 latency and amplitude determination. In these cases, the earlier peak having a frontocentral maximum was termed P3A and was scored separately. Thus, the P3B component latency corresponds with P300 latency in this method.

Source analysis

The averaged data were exported to ASA software (ANT software BV, Enschede, the Netherlands) using an average reference. All P300 topographical maps were first visually inspected in ASA to identify P300 components fields, i.e. the frontocentral P3A component and the centroparietal P3B component. The standard realistically shaped head model in ASA was used. It takes into account the conductivity of the brain, skull and scalp. A standard Talairach coordinate system with 3 perpendicular axes intersecting at x=0, y=0 and z=0 was used. The x-axis had a frontal-occipital orientation, the y-axis ran through both pre-auricular points, and the z-axis was oriented towards the vertex. In order to model both P3A and P3B, a 2-dipole model was applied using x=60, y=0 and z=0 as a starting position for dipole 1, and x=−60 y=0 and z=0 as starting position for dipole 2. A 1-dipole model was also applied, which can also identify both components if multiple peaks in dipole activity are found. This means that if, at the moment of dipole peak activity, there is a frontocentral P3A field, this peak activity is attributed to the P3A component, and if a centroparietal EEG field is seen, it is attributed to the P3B component. A starting position of x=0, y=0 and z=0 was used. For both dipole models, rotating dipole models were used without any constraints. Dipoles were fitted over the interval from 250 ms until 500 ms after target stimulus onset. The following criteria were used:

1. Dipoles had to account for at least 75% of the variance in individual EEG data, so residual variance up to 25% was accepted.
2. Component latency and amplitude were determined using dipole time course information in combination with topographical mapping. Dipole peak activity was attributed to P300 component activity only if a clear frontocentral P3A or centroparietal P3B positive field was seen in the topographical maps. Conversely, components were only scored as clearly absent if, in the topographical maps, no clear component field was visible. This prevents a faulty dipole model from resulting in an incorrect interpretation of absent P300 components.

3. For the 2-dipole method, crossed dipole solutions (the frontal dipole explains P3B and the posterior dipole explains P3A) and solutions in which two dipoles are equal (i.e. rotate exactly in the same manner and are in close proximity) were not accepted. Instead, the 1-dipole method was used for latency and amplitude determination.

4. After both dipole methods had been tried, the method with the lowest residual variance was used for latency and amplitude determination.

Note that comparison of dipole peak activity with topographical maps is an essential feature, especially if there are multiple dipole peaks.

As was demonstrated earlier \(^{18}\), this strategy is a functional source analysis method, which is aimed at separating temporally overlapping P300 components. The rotating dipoles in our method can be viewed as resultant vectors representing the mean effect of several simultaneously occurring bilateral cortical processes. When the exact locations of generators of P300 are of interest, this is obviously incorrect \(^{17}\) and more than 1 or 2 dipoles are needed to obtain a credible dipole solution. However, to describe P300 components in a functional way (latency, amplitude), exact source localization is not needed and bilateral activity can be described using 1 or 2 dipoles only.

Statistics

Normality of P3A and P3B latency and amplitude distributions was verified using the Shapiro-Wilk statistic. Levene’s statistic was used to test differences in latency variability of P300 between groups. T-tests or the Mann-Whitney U-test were performed in order to compare mean P3A and P3B latencies between groups. Chi-Square tests or Fisher’s Exact Tests were used to compare proportions between groups. In order to compare latencies between methods, one-sample T-tests were applied to the intra-individual latency differences between both methods.
Results

In the remainder of the text, P3A and P300 always indicate scalp measures when referring to the conventional method, and P3A and P3B dipole estimates when referring to the source analysis method.

Comparisons between groups

In all subjects, a P300 potential could be obtained. The results for the conventional and source analysis for both groups can be found in Table 1.

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Head Injury</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>P3A (Conventional Analysis)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lat (ms)</td>
<td>277±19</td>
<td>304±22</td>
</tr>
<tr>
<td>Amp (μV)</td>
<td>22±5</td>
<td>14±2</td>
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<tr>
<td>% identified</td>
<td>19</td>
<td>10</td>
</tr>
<tr>
<td><strong>P300 (Conventional Analysis)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lat (ms)</td>
<td>331±21*</td>
<td>350±28*</td>
</tr>
<tr>
<td>Amp (μV)</td>
<td>19±7*</td>
<td>15±6*</td>
</tr>
<tr>
<td>% identified</td>
<td>100</td>
<td>100</td>
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<thead>
<tr>
<th></th>
<th>Control</th>
<th>Head Injury</th>
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<tbody>
<tr>
<td><strong>P3A (Source Analysis)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lat (ms)</td>
<td>312±17</td>
<td>315±16</td>
</tr>
<tr>
<td>Amp (nA)</td>
<td>220±122*</td>
<td>149±153*</td>
</tr>
<tr>
<td>% identified</td>
<td>100**</td>
<td>57**</td>
</tr>
<tr>
<td><strong>P300 (Source Analysis)</strong></td>
<td></td>
<td></td>
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<tr>
<td>Lat (ms)</td>
<td>359±10</td>
<td>365±11</td>
</tr>
<tr>
<td>Amp (nA)</td>
<td>177±78</td>
<td>244±128</td>
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<tr>
<td>% identified</td>
<td>86</td>
<td>81</td>
</tr>
</tbody>
</table>

Table 1. Results for the conventional and source-analysis methods for both groups. Values are means ± SD. % identified = percentage of subjects in which the component of interest was identified. In head injury patients with absent P3A components, values were left-censored at 34 nA. Lat=latency, Amp=Amplitude, nA=nano-ampere. * indicates p<0.05 ** indicates p<0.01.

With the conventional method, head injury patients had delayed P300 latency (350 vs 331 ms, p=0.016) and reduced P300 amplitude (15 vs 19 μV, p=0.049) compared to controls. P3A components could be identified only very infrequently, precluding statistical comparison. With the source analysis method, neither P3A nor P3B component latencies varied between patients and controls. However, 43% of head injury patients had absent P3A components, whereas a P3A component could be identified in all control subjects (Fisher’s Exact Test: p<0.001). A P3B was present in all cases where no P3A could be identified with source
Figure 1. An example of source analysis (upper half) and conventional P300 analysis (lower half) for a control subject (left-hand column) and a head injury patient (right-hand column). In the control subject, a 2-dipole solution identifies both P3A (291 ms) and P3B (346 ms) components. In the conventional method, only one P300 component is identified at 296 ms. Closed arrowheads correspond with source analysis P3A, open arrowheads with source analysis P3B. In the head injury patient there is no clear frontal P3A field, and a 1-dipole solution is obtained with a P3B at 358 ms. In the conventional method, the P300 reaches a maximum at 357 ms. The difference in P300/P3B latency between both subjects is much larger for the conventional method when compared to the source-analysis method (61 ms vs 12 ms).
analysis. If only patients with identifiable P3A components were included (n=12), there was no difference in P3A amplitude between the groups (235 vs 220 nA, p=0.76). Because no P3A field could be seen in the topographical maps in these cases, all absent P3A values were conservatively left-censored with a value of 34 nano-ampere (nA, lowest P3A value: 35 nA). After left-censoring, P3A amplitude was lower in patients compared to controls (149 vs 220 nA, Mann-Whitney U-test: p=0.020). P3B amplitude did not vary between the groups in the source analysis method. Figure 1 shows an example of both analysis methods for a control subject and a head injury patient, illustrating the result typically obtained in this study. Figure 2 shows the conventional P300 target and standard grand averages for both groups.

Figure 2. Grand average waveforms for controls and head injury patients. Grand average P300 latency in head injury patients is prolonged and P300 amplitude is lower when compared to control subjects.
Comparisons within groups

Variability in terms of standard deviation of P300 latency was significantly smaller with source analysis than with conventional P300 analysis, for both the control group (10 vs 21 ms, Levene’s test p=0.006) and the head injury group (11 vs 28 ms, Levene’s test p<0.001). Within both groups, the difference in component latency between the two analysis methods was calculated for each subject. In view of the low percentage of identified P3A components with conventional analysis, only P3B (source analysis) – P300 (conventional method) latency differences were calculated. In the control group, the mean within-subject P3B-P300 latency difference was 28.7 ± 24.7 ms (p<0.001), while in patients this difference was 7.6 ± 24.4 ms (p=0.22). The mean within-subject P3B-P300 latency difference was higher in controls than in patients (p=0.016).

Discussion

In this study we have shown that different results were obtained for conventional and source analysis methods when analyzing P300 results from head injury patients. In line with our initial hypothesis, source analysis resulted in reduced P300 latency variability compared with conventional analysis in both head injury patients and control subjects. However, contrary to our initial hypothesis, the mean P300 latency difference between controls and head injury patients was much smaller with source analysis than with conventional analysis. In fact, source analysis did not reveal any significant latency differences between patients and controls for both P3A and P3B components. Thus, the latency distributions of patients and controls actually showed greater overlap with source analysis, despite reduced variability, which was exactly the opposite of what we were expecting to find.

With the conventional method, both decreased mean P300 amplitude and increased mean P300 latency were found when compared to controls. Comparable results have been reported in other studies that evaluated P300 parameters in head injury patients[^10][^23]. With source analysis, only decreased P3A amplitude was found, with no decrease in P3B amplitude. This decrease in mean P3A amplitude was distributed inhomogeneously within the patient group, with some having no identifiable P3A components, while the subgroup of patients with identifiable P3A components had normal mean P3A amplitude. We hypothesize that the exact location of the contusional injury is important in determining the effect on P3A amplitude. Damage to areas containing important P3A generators (i.e. anterior cingulate) are likely to result in a more dramatic decrease in P3A amplitude than, for example, orbitofrontal damage, which was recently associated with increased novelty P3A amplitude[^24]. The interpretation of absent P3A components with source analysis as lower brain activity in the P3A time window is reasonable since no clear P3A field could be seen in the topography maps. However, our
method may be relatively insensitive for detecting low P3A amplitude, leaving the exact difference in amplitude rather imprecise, which is a limitation of this method. However, alternative explanations for absent P3A components in terms of generalized decreased signal-to-noise ratios seem unlikely because in all patients with absent P3A components, a normal P3B could be identified. When source analysis of P3B latency and conventional analysis of P300 latency results were compared within each group, differences were present only in the control group and not in the patient group.

Bearing in mind the results obtained in normal subjects, it is now questionable whether the observed latency difference between head injury patients and controls in the conventional method is genuine. Any decrease in P3A amplitude will result in a more prominent contribution of P3B to the P300 complex. Consequently, the unaffected P3B component with its longer latency determines conventional P300 latency in these patients. It is therefore likely that, with the conventional method, the P300 delay in head injury patients is a pseudodelay, caused by a decrease in P3A amplitude.

Similar results from other studies also show significant P300 amplitude reductions in the absence of a significant delay in P300 latency \(^{11, 13, 25-27}\). This may be explained by the fact that these studies included patients with only very mild concussion \(^{11, 13, 25}\), which may indicate that a substantial P3A amplitude reduction is needed before significant group differences in P300 latency are found with the conventional method.

The results of the present study have important implications for the neurophysiological interpretation of a delayed P300 potential in head injury patients. One of the most consistently observed deficits after head injury is mental slowness, which may be an essential factor in various other cognitive disturbances after head injury \(^ {28, 29}\). Although it has been recognized that cognitive slowing may be more pronounced at the response end of information processing, several authors have concluded that delayed P300 latency after head injury must reflect slower stimulus processing, which contributes to slower cognitive performance \(^ {9, 30, 31}\). This conclusion seems valid across studies based on meta-analyses of P300 latency and reaction times after head injury \(^ {32}\). By contrast, our data show that slowing may not occur at all in early processing stages, which strongly supports the theory of stage-specific cognitive slowing, occurring at the response end of processing \(^ {32}\). Thus, while impaired and disorganized stimulus processing may still be a valid interpretation given the decreased P3A amplitudes, it is not justified to conclude that there is a slowing of stimulus processing, solely on the basis of a delayed P300 latency in conventional P300 analysis.
References


