Differences in working memory load produce an abnormal pattern of P300 amplitude in schizophrenia

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Abstract

Background
Impairments in multiple working memory (WM) domains might represent a core deficit of cognitive dysfunctions observed in schizophrenic patients. High WM load conditions render a reduction of the P300 ERP amplitude in normal subjects. So far, this effect has not been studied in schizophrenia. The aim of this study is to evaluate the amplitude and latency of the P300 wave in schizophrenic patients at different WM loads, compared with normal subjects.

Methods
We recorded 26 subjects (13 schizophrenic patients and their respective aged-handedness matched controls) with an 80-channel electroencephalogram. These subjects performed an N-back task, a WM paradigm that manipulates the number of items to be stored in memory.

Results
The ERP analysis showed an abnormal pattern of P300 amplitudes comparing control subjects and schizophrenic patients. While in healthy subjects, a significant decrease of the P300 amplitudes was observed in relation to increments in WM load, the schizophrenic group did not show differences between conditions (from 0- back to 2- back). Moreover, a significant difference between groups was observed in P300 amplitude at the low WM load condition (1 –back), being smaller in the schizophrenic group.

Conclusions
The abnormal pattern of P300 amplitudes presented in schizophrenic patients at high versus low WM demands might reflect WM impairments in this disease, perhaps partly related to the incapacity to redistribute memory resources at high WM load conditions.
Background

Cognitive deficits have become a central topic in schizophrenia, because they might reveal underlying patho-physiological mechanisms of interest [1]. For instance, impairments in multiple working memory (WM) processes have been proposed to be among the core cognitive deficits in this condition [2, 3]. In this line, verbal and spatial WM dysfunction has been consistently reported at different clinical stages, including first episode patients and groups of healthy subjects at high risk of developing the disease [5, 6]. Thus, the study of WM mechanisms and their impairments in schizophrenia might contribute to elucidate some of the underlying mechanisms that are affected in this disease.

WM refers to a set of executive and memory processes that allow an active maintenance and manipulation of stimuli or contents once they are no longer available [4]. Several models suggest that there is a limited set of resources for storing different items in a given working memory domain. As a consequence, these limited WM resources are reallocated into different WM subcomponents during high memory demands, in order to keep adequate levels of functioning [7].

In this context, several Event Related Potential (ERP) waveforms have been proposed as cognitive markers of increasing demands during the execution of WM tasks during both normal [8] and altered WM processing [9]. For instance, changes in the amplitude of the P300 wave may be related to the reallocation and updating of WM, predicting a decrease of P300 amplitude at high WM loads [10]. Studies performed in normal subjects have confirmed these predictions [3, 11] using an implicit verbal WM load paradigm called n-back task [12].
In schizophrenia, alterations in P300 amplitude and latency have been consistently reported [13, 14], yielding this potential as an important marker of the cognitive state in this condition. However, to the best of our knowledge, there are no studies that test the P300 pattern at high WM load conditions in schizophrenic patients. In this study, we compared the behavioral performance and the elicited P300 wave under the execution of a verbal N-back task at different WM loads in a sample of paranoid schizophrenic patients and matched controls.
Methods

Participants

Thirteen chronic schizophrenic outpatients (paranoid type) were recruited from the mental health service of the Pontificia Universidad Católica de Chile. Two psychiatrists confirmed the diagnosis of schizophrenia (according to the DSM-IV-TR clinical version). Patients were matched by sex, age and socioeconomic status with 13 healthy controls. All subjects in this study were right handed and had normal or corrected-to-normal vision. Every subject underwent a medical and a psychiatric interview, which included the Mini international neuropsychiatry interview 5.0 (M.I.N.I plus). Demographic data were recollected and a local-validated structured socioeconomic scale was performed. A trained psychologist performed an IQ scale (WAIS) to both patients and controls. Severity assessment of schizophrenic patients was determined with the positive and negative syndrome scale (PANSS) for schizophrenia and the Clinical Global Impression Scale (CGI-S) [15]. Exclusion criteria for this study were any current or past psychiatric diagnosis (excluding schizophrenia in the schizophrenic patients), substance abuse/dependence, the use of benzodiazepines or anticonvulsive drugs, mental retardation, a clinically significant medical illness or any history of neurological disease. We also excluded any control with family records of schizophrenia, psychosis or bipolar disease. Figure 1 shows all relevant clinical and demographic data for patients and controls. Clinical evaluation was performed no later than 1 month after the EEG recording.

This protocol was approved for the ethics committee of the Pontificia Universidad Católica de Chile. Every subject signed an informed consent. In case of schizophrenic patients, this consent was also signed by a well-informed relative.

Task and stimulation procedures

WM was evaluated in schizophrenic patients and healthy controls using an implicit verbal N-back
task [12], which assesses the load of WM in terms of the numbers of items stored in memory [16]. The task consisted in a control condition and two different levels of working memory load. In the control condition, subjects identified the zero (“0”) number (target condition) among a random presentation of other digits sequentially presented (0-back task). There were two different WM tasks, which consisted in the identification of two equal numbers (target condition) separated by none (1-back, low WM task), or one (2-back, high WM task) random digit (no target). A trial was defined by the presentation of one number following by the motor response of the subject. Trials were presented in 3 blocks, each block representing either the control (0) or WM (1 and 2) conditions. Each block consisted in 180 trials with a 1:1 target/no target relation [12]. Stimuli consisted in a 200 ms. presentation of a gray digit (size: 2.6 x 5.2 deg at 65 cm. of distance from the face) located in the center of a black background screen. Stimuli presentation were implemented using the STIM 1.0 software (Compumedics-Neuroscan®). The stimuli (2.6x5.2 deg of visual angle at a distance of 65 cm, presented over a period of 200 ms) were presented in a 21’ CRT monitor. A head-rest system was used in order to avoid head movements. Subjects had to distinguish targets versus non-targets, by pressing two buttons localized in a response palette. Reaction times were registered after the button pressing.

**Acquisition data**

Continuous EEG activity was recorded with an 80-channel electroencephalographic system (Neuroscan® EEG Nuamps device). Electrodes were placed using a QuikCap (Neuroscan® Inc) according to the 10/20 extended system. During recordings, references were placed at vertex by default, but were subsequently off-line re-referenced to averaged mastoids. Impedance values were kept at 5 KΩ for all electrodes. We used three external flat electrodes to monitor eye movements (two above and below the left eye and one 3 cms. next to the outer canthus of the right eye). Recordings were sampled at 1000 Hz and band-pass filtered between 0.1 and 100 Hz using an on-line amplifier.
Event Related Potential analyses

Trials with undesirable eye movements and eye blink artifacts were eliminated from the analysis using a semi-automatic and manual block rejection procedure. To remove unwanted ERP components, such as the CNV like wave evoked in this kind of task [3], we executed an offline digital band-pass filter from 2 to 30 Hz (zero phase shift filter). Continuous EEG was subsequently segmented ranging from 500 ms. previous to the appearance a target stimulus to 800 ms. after stimulus onset. Then, we included in our analysis only trials where the subject performed successfully the task (defined as match condition). Individual segments were excluded if the absolute voltage of each channel was greater than 80 $\mu$V. In each subject, successful, artifact-free trials were averaged in each task (0 -1- and 2- back tasks) to obtain the corresponding ERP waves [17]. Subjects with less than 30 epochs to average for each task were excluded. The mean and standard error of the mean (in parenthesis) of averaged trials of each condition and groups were: Control group: 0- back: 46.5 (4.2), 1- back: 47.3 (3.9), 2- back: 43.3 (4.5); Schizophrenic group: 0-back: 50.5 (4.8), 1- back: 41.8 (4.5), 2- back: 38.6 (4.5). Finally, we executed a group average over the ERPs obtained across subjects for visualization purposes. All of these analyses were processed using Scan 4.3 (Compumedics-Neuroscan®), Matlab 7.0 software (The Mathworks Inc.) and the EEGLAB 4.5 toolbox.

Statistical analyses.

Behavioral performance: The behavioral response was characterized by hit rate (HR) and reaction times (RTs). HT was defined as percentage of correct responses, while RTs were defined as the first response of the subject 200 ms. after the appearance of target stimulus. The behavioral effects of WM load (0-, 1- and 2- back tasks) and group (patients and controls) on were statistically evaluated using a repeated measures analysis (general linear model ANOVA). Greenhouse-Geisser and Bonferroni methods were used to correct compound symmetry violations in the ANOVAs. Post
Hoc analysis and main-effect comparisons of were adjusted using the Bonferroni correction. Corrected and uncorrected DFs were reported for each F statistics following the order F (uncorrected DF, correct DF). 

Event-related potential statistical analysis: Statistical analyses of ERPs were performed on the subjects average waveforms. Peak amplitude and latency of the P300 component were detected between 290 and 400 ms. post-match stimulus. To avoid loss of statistical power due to the multiple comparisons problem, the 80 electrode array channel was compiled into 5 regions of interest (ROIs) averaging fifteen neighbour electrodes for each ROI [18]. The resulting regions represent relevant regions of the scalp and were denominated as follows: central midline (CMROI), frontal right (FR-ROI), frontal left (FL-ROI), parietal right (PR-ROI) and parietal left (PL-ROI). Using these measures, two relevant kinds of analyses were performed. First, to assess the question about changes in P300 amplitude/latency related to WM load, we compared ERPs from low WM load (1-back) and high WM load (2-back) conditions in both groups (schizophrenic and healthy controls). Second, to compare schizophrenics with controls, we compared the averaged ERP values for each condition in both groups. In addition, we subtracted the high WM load condition from the low WM condition to each subject, and resulting differences were compared across groups. In all cases, Mann-Whitney tests were used for comparison. The Kolmogorov-Smirnov test confirmed that the data could not be distinguished from a normal distribution.
Results

Behavioral responses

In each WM condition, HR percentages were more than 90% and 70% in control and schizophrenic patients, respectively. As expected, we found increments in RTs and decrements of HRs due to the increase of WM load (from 0- to 2- back tasks) in both groups. In the control group, only the differences in RTs among 0- to 2- and 1- to 2- back tasks were significant. In the schizophrenic group differences were significant from 0- to 2- back in both HT and RT (figure 3). In general, schizophrenic patients presented more errors and delayed responses than controls in each condition (figure 3). These intergroup differences were significant in both the low and high WM task (1- and 2- tasks) for the HR [F(2.1, 5) = 16.7, p <.001, Greenhouse-Geisser corrected] and RT [F(1.5, 5) = 4.7, p =.028, Greenhouse-Geisser corrected].

EEG data analyses.

A large P300-like positive deflection peaking between 300-340 ms. post target (matched) stimulus was in all subject of both groups (normal controls and schizophrenic patients) and all conditions of the n-back task. This P300 component shows a typical parietocentral topography, consistent with previous reports [17] (see figure 4).

Within-group analyses: The control group presented a significant decrease of P300 peak amplitude from 1-back to 2-back (p = 0.018, Mann-Whitney test) and 0- to 2- back condition (p=0.016, Mann-Whitney test). On the contrary, schizophrenic patients did not show a significant difference in P300 peak amplitude among any condition studied (from 0- to 1- back, p value = 0.56; 0- to 2-back p value = 0.34; and 1- to 2- back p value = 0.76, data not shown). Besides, we did not find significant differences in P300 latency between 1- and 2- back tasks inside each group (figure 2).

Between-group analyses: The mean P300 peak amplitude was higher in the control group for every
WM condition (figure 2 and figure 4), but this difference was significant only in the 1-back task (Mann-Whitney test, $p = 0.019$, see figure 2). We also performed a subtraction between P300 peak amplitudes in low and high WM load conditions, as explained above (see methods and figure 4). Schizophrenic and normal groups comparison shows that the net decrement in P300 amplitude is higher in the control group ($p = 0.023$; Mann-Whitney test, see figure 2). Finally, a delay in P300 latency was found in schizophrenic patients compared with controls in all the tasks studied. However, this difference was not significant (see figure 2).

**Discussion**

Consistent with previous studies, the behavioural performance of the N-back task is better in healthy controls compared with schizophrenic patients [19]. As we expected, the difference in hit rate and reaction times between schizophrenic and controls becomes larger with higher WM demands. In addition, our ERP findings are in general consistent with previous proposals suggesting that alterations in the P300 wave represent markers of cognitive dysfunction in schizophrenia, possibly representing an endophenotype of the illness [20]. These proposals have been confirmed by several other studies using a wide range of tasks, including WM paradigms [14]. Likewise, in our sample of patients, different demands of WM load produced abnormal patterns of P300 amplitude. As opposed to the control group, we did not find a decrease of P300 peak amplitude from low to high WM load (from 1- to 2-back condition, See figure 2 and 4). Interestingly, the comparison of each condition studied (0-back, 1-back and 2-back) between groups showed a significant difference only at the low WM load condition (1-back).

Different factors contribute to P300 amplitude, such as mistakes in cognitive processing and overlap with other ERP components [10]. It has been proposed that in normal subjects, the reduction of P300 amplitude at high WM loads relates to the reallocation of WM resources in different cognitive
domains [10]. Thus, at high WM loads, an important amount of WM resources might be dedicated to sustain the phonological loop, at the expense of other processes that could contribute directly to the generation of the P300, thus decreasing the potential’s amplitude [10]. Supporting this notion, the P300 wave amplitude normally decreases during tasks that require to temporally store an increasing number of items [3, 12].

Functional neuroimaging [21, 22] and electrophysiological approaches [23] suggest that schizophrenic patients present important dysfunctions in the prefrontal cortex and in areas specifically involved in the phonological loop. Consequently, in high WM load conditions, they might not be able to reallocate their limited resources into the phonological loop as normal subjects apparently do. Thus, the maintenance of P300 amplitude at high WM loads in these patients could be partly explained by a difficulty to redistribute their WM resources. Note that there is a trend in the inter-group comparisons of high WM load conditions (2-back), which might reach significance in a larger sample (See figure 2). This would indicate that these patients do have some ability to redistribute cognitive processing into the phonological loop or other circuits, although their capacity to do so is more limited than normals.

However, the above explanation does not account for the decrease in P300 amplitude at low WM load conditions. Another hypothesis is that the P300 is reflecting a task-closure, or a successful WM update process, which in normal subjects fails to be fully executed at high WM load conditions, and is reflected in a smaller potential amplitude. On the other hand, schizophrenics might be unable to properly “close” the task at both low and high WM load conditions. In fact, the inter-group comparisons of P300 amplitude in each WM condition show significance only at low WM loads (1-back, see figure 2). The P300 amplitude at the 0-back condition, although not reaching significance, is much lower in schizophrenics than in controls, something that again might reach significance in a larger sample and could reflect a general attentional impairment as well.
our view both processes are likely to contribute to the observed findings in schizophrenics: a deficit in WM updating even at low loads, and an incapacity to reallocate resources at high WM demands. Studies that point to this critical difference are needed to assess both hypotheses in more detail.

The latency of the P300 waveform was also evaluated in this study. The observation of a prolonged latency in schizophrenic patients is controversial. A few studies have described a P300 latency prolongation in schizophrenics and their siblings [24, 25], but other reports failed to find this effect [20]. Here, we found a P300 latency prolongation in all tasks studied, in accordance with slower stimulus processing. However, these differences did not reach significance.

Clinical factors have to be considered in our results. Our patients made up a homogeneous sample, paranoid-type, clinically stable, “moderately ill”, and using moderate doses of antipsychotic medication. In spite of the fact that the results of the PANSS were not correlated with the P300 amplitude, larger samples of patients will be needed to confirm the possible contribution of these and other clinical factors (such as sex or progression of the disease) in these results.
Conclusions

Characteristic patterns of P300 are observed in healthy subjects and schizophrenic patients as a function of WM-load. Although these findings are partly consistent with the hypothesis of an alteration in resources reallocation, alternative possibilities are also likely. Since this study used a numerical N-back task that is likely to recruit the phonological loop, especially at high WM loads, it would be interesting to use other, visuospatial WM tasks that activate other WM domains. For example, the spatial version of the N-back task might recruit less neuronal networks associated with the phonological loop due to its lesser dependence on articulatory rehearsal. In these conditions, P300 amplitude may not depend so strongly on the WM load in normal subjects, and in schizophrenic patients it may maintain a low amplitude at all difficulty levels.
Competing interests

The authors declare that they do not have competing interests.
Authors' contributions

PAG participated in the conception and design of the study, acquisition of the data, analysis and interpretation of the results, and in drafting the manuscript. SR participated in selection of the participants, acquisition of the data, and in drafting the manuscript. FZ and CAB participated in the acquisition of the data, analysis and interpretation of the results and drafting the manuscript. MA and CP helped in the acquisition of the data and selection of the participants. FA participated in the conception and design of the study, interpretation of the results, and in drafting the manuscript. All authors read, critically revised, and approved the final manuscript.
Acknowledgments

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Figures

Figure 1. Socio-demographic variables.
Socio economic scale includes: education, employment status, family economic status and graduate living conditions.

Figure 2. Statistical analysis of the P300 wave in the CM-ROI.
Comparison of amplitude and latency measures among schizophrenics and controls. * p value < 0.05 (Mann-Whitney test).

Figure 3. Results from a group analysis examining behavioral effects of load for all n-back conditions.
Hit rate (upper panel) and reaction time (lower panel) for schizophrenics and control subjects. Filled circle represent controls subjects; empty circle represents schizophrenic patients. * indicates p < 0.05 and ** indicates p < 0.01 (evaluated by an ANOVA repeated measures test).

Figure 4. Grand averages and topographical distribution of the evoked P300 potentials elicited by 1- and 2- back tasks in the CM-ROI.
The third column represents the subtraction of the P300 amplitudes at 1- and 2- back conditions for controls and schizophrenics. The differences were significant for controls (p <0.05), but not for schizophrenics (p >0.05). Color bar indicates amplitude (uV).
References


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Figure 2
Figure 3

- Hit rate (%)
- Reaction time (sec)

**Comparisons and Statistical Analysis**

- Controls
- Schizophrenics

Significance levels:
- *: p < 0.05
- **: p < 0.01