Author's response to reviews

Title: P300 amplitude is insensitive to working memory load in schizophrenia

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Author's response to reviews: see over
Dear Miss Angelina Iliesvska MSc.
The BioMed Central Editorial Team.

Dear Editor:

Herein we submit the revised version of our article, now entitled “P300 amplitude is insensitive to working memory load in schizophrenia” to BMC Psychiatry. We have addressed all concerns raised by the reviewers and are confident that this is a much clearer and direct version of the article. Basically, we have attempted to clarify some theoretical issues that were especially confusing, like the relation between P300 amplitude and memory load, and the interpretation of our results. These are now more focused on P300 invariance at different memory loads in schizophrenia, which suggests that in this group, the P300 potential may reflect the activation of attentional rather than memory processes. We have also modified the methodological section to make clearer the experimental setup, the recording and the analysis procedures.

Sincerely yours,

Pablo Gaspar
Francisco Aboitiz
RESPONSE TO REVIEWERS

We are grateful for the helpful comments. Below we will address the points made by both reviewers, one by one.

Reviewer 1

**Several major points in the writing are unclear or very weakly laid-out:**
1. The conceptual descriptions of the WM domain and resource allocation theory.
2. The relationship of WM to ERP markers,
3. complex nature of cognitive and neurobiological contributions to the P300, and

We have worked to clarify these issues by following recent reviews and interpretations by Kok and others (Kramer AF). There is no agreement on the cognitive or even neurobiological significance of the P300 component, besides being a marker of attentional processes or decision-making mechanisms. In our revised article we provide a general interpretation of this potential, reflecting the activation of a widespread parieto-frontal network involved in attentional as well as memory processes (P. 3, 1st par.; see also P.10 last paragraph and P 11 first paragraph). According to one model, in high-demanding conditions, other networks, preferentially involved in memory rehearsal processes, contribute to the task at the expense of activation of the networks contributing to the P300 amplitude. In this context, we have modified our interpretations and the article’s title by emphasizing that our main finding is the lack of P300 modulation between attentional and memory tasks in schizophrenia.

4. biological significance of impaired load-induced P300 suppression in SZ.

It is not clear what this paper uniquely adds to the literature and our understanding of the neurobiology of SZ. We already know that SZ show 1. WM deficits, especially at high WM loads, and 2. P300 deficits. Authors need to state clearly what this paper uniquely add to the literature.

We interpret our findings as an inability to contribute memory resources to the P300 in the schizophrenic brain, which explains both the lack of difference between the 0-back and the 1-back conditions in schizophrenics, and the difference in the 1-back condition between schizophrenics and controls. Our hypothesis is that schizophrenics use, at low working memory loads, the same cognitive strategy that normal subjects use at high working memory loads, i.e. relying on rehearsal memory mechanisms that do not contribute to P300 amplitude. These considerations are, we believe, clearly stated in the Abstract, the Discussion (P. 11, 2nd par.) and the Conclusions (P. 13) sections.
Discussion. The alternative theories offered to explain the data (i.e., task-closure) are unclear. The last point suggesting that SZ exhibit deficits of ‘...WM updating even at low loads, and an incapacity to reallocate resources at high WM demands’ should be further fleshed out, very simply and with plenty of evidence from the literature (references).

As mentioned above, we restated the task-closure hypothesis by emphasizing the lack of difference in P300 amplitude among all conditions in the schizophrenic group. In schizophrenics P300 amplitude never reaches values much higher than those obtained in the pure attentional condition, i.e. 0-back, suggesting that memory-related processes contribute little to the P300 amplitude in these patients.

p.5 The sample size is small (n=13 SZ v 13 controls), limiting generalizability of the findings. This may contribute to the lack of significant expected differences in some of the comparisons. This should be mentioned in a limitations sub-section of the discussion.

We have made a comment on sample size in the Discussion, indicating that larger samples will be needed to confirm our findings (P. 11, bottom).

p.6 is ref #s12 and 16 the correct citations for the N-back task used?

We have changed the citations used.

The task description should be written more clearly and the English needs improvement.

The task has been described in different, we hope clearer terms (P. 6, 1st par.). We also sought advise form a fluent english speaker.

Tables.
Commas are used where periods should appear (periods are standard in English journals).

We changed commas to periods in the Tables.

Why is EQZ used as an abbreviation for schizophrenia?

We now use the term SZ to abbreviate “Schizophrenia”.
I completely do not understand what the authors are trying to say with the sentence describing ‘exclusion criteria’: page 5: “Exclusion criteria for this study were any current or past psychiatric diagnosis (excluding schizophrenia in the schizophrenia patients)” – I am sure the authors did not try to exclude schizophrenia diagnosis in schizophrenia patients – please clarify.

We have revised and corrected this sentence: “Exclusion criteria for this study were any current or past psychiatric diagnosis (excluding schizoaffective disorder diagnosis in the schizophrenic group)...” (page 5, 1st paragraph, lines 11-12)

This is really odd to perform a clinical evaluation 1 month after the subject was included in the study – please clarify.

We agree with the reviewer that the meaning of this sentence is also obscure, we have changed it as follows: “Clinical rate evaluation (PANSS and WAIS) was performed no more than 1 month since the EEG recording.” (P5, end of 1st par.)

One of the major issues with the study is the description of the task. The authors need to provide a more detailed description so that one could make a judgment about exactly what cognitive processes took place during the task. As it stands right now, we are not told how the two equal numbers – I assume identical digits - were displayed. Were they displayed simultaneously, or in a sequence. If in sequence, what was the ISI. The authors need to give the ISI for all their conditions as this variable influences the task in a crucial way. The subjects made a response – was the use of right and left hands counterbalanced across subjects?

We agree with the reviewer and we reworded the description of the task, hopefully making it clearer:

“WM was assessed using an implicit verbal N-back task, in which subjects were presented a sequence of digit numbers, and had to determine whether the currently displayed stimulus at any given time had been already displayed in the previous presentation (1-back condition, low WM load); or in the second-to-previous presentation (2-back condition, high WM load) (see figure 1). There was also a control condition (0-back), in which the subject had to recognize a specific digit –zero- when it appeared on the screen. Subjects had to distinguish between targets and non-targets, by pressing two buttons localized in a response palette. Reaction times were recorded after pressing the button. A trial was defined by the presentation of one number followed by the subject’s motor response. Trials were presented in 3 blocks; each block representing either the control (0-back) or WM conditions (1- and 2-back). 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. from the face) located in the center of a black background screen in a 21’ CRT monitor. Interstimulus intervals
(ISI) were 1700 msec in all the conditions studied. All the subjects used the dominant hand to respond.” (P6, Task and stimulation procedures, 1st paragraph).

The decision to create epochs with 500 msec baseline is rather strange – the typical baseline is 100-200 msec unless there is a methodological/theoretical good reason to extend it – what was the authors’ reasoning? In addition, this baseline in especially problematic given that we are not told what the ISI is and how it relates to the length of the baseline.

We agree with the reviewer about this. We defined a 500 msec prestimulus interval in the context of additional frequency analyses not published here. However, for the ERP analysis we used only a 200 msec prestimulus interval to determine the baseline.

“The continuous EEG was segmented between 500 ms. previous to the appearance of a target stimulus to 800 ms. after stimulus onset. Nevertheless, the baseline used for the ERP analysis was 200 msec. previous to the appearance of the target stimuli.” (P7, Event-related potential analyses, 1st paragraph, lines 5-7).

Please report unadjusted DF only.

This has been done (P8, Line 3).

The use of ‘virtual electrodes’ is strongly discouraged. With 80 electrodes it is perfectly legitimate to select a subset of electrodes that represent maximal voltage/group separation for a given paradigm and use these electrodes in the stats analyses. Otherwise, one can use a grouping factor of ROI or region and use actual electrode values within each region. The use of virtual electrodes largely obscures the real voltages on the scalp and often creates a false impression of what is going on in the data. Thus, for this paradigm, it is hard to say exactly what effects the authors obtained.

We agree with the reviewer about the use of virtual electrodes. Therefore, as the reviewer suggested, we performed an ROI analysis. We reworded that part of the text in order to clarify the methodology used:

“Additionally, we performed an analysis of regions of interest (ROI) by averaging fifteen neighboring electrodes for each one [20]. The 5 resulting ROIs were labeled as follows: central midline (CMROI), frontal right (FR-ROI), frontal left (FL-ROI), parietal right (PR-ROI) and parietal left (PL-ROI).” (P. 8, last par.)

In addition, the way to go about the stats analysis is to start from an omnibus M/ANOVA with 1 between factor of group (SZ, NC) and two within factors of condition (3 levels: 0, 1, 2 n-back) and region/electrode (number of levels dependent on approach/electrodes chosen) and proceed from there. The main effects and interactions should dictate follow up analyses.

We are grateful with the reviewer for this comment. As the reviewer suggests, the parametric analysis with ANOVA test is one of the most common analyses used for ERPs statistics. We performed the proposed analysis and confirmed our results.
However, we found a trend to statistical significance for the intergroup comparison (1 back of SZ– to – 1 back of Controls) with this method. We assessed this problem testing with an alternative non-parametric methodology that had been recently suggested by Maris et al. (2007, J Neurosci Methods) (see sub-section methods page 9). This method is based on a permutation test (Montecarlo distribution). Therefore, we are able to assess two major concerns on the statistical analysis in electrophysiology datasets. First, because it is based in a resampling distribution of the studied population, the assumption of normal distribution is no longer necessary under the null hypothesis of no differences between populations. Second, it offers a satisfactory solution of the multiple comparisons problem, by pooling the evaluated statistics of neighboring electrodes. We described the methodology, in the Methods section:

“the cluster–level test statistics is defined by pooling the z scores of neighboring electrodes showing the same effect (pooled z scores >1.96) in a given time window of interest. The type I error rate for the complete set of electrodes was managed by evaluating the cluster–level test statistics under the randomization null distribution of the maximum cluster–level statistics. This was obtained by randomly permuting the data between conditions and between groups. By creating a distribution from 100 random sets of permutation, statistical significance (p < 0.05) was estimated as the proportion of elements in the randomization null distribution exceeding the observed maximum–cluster level test statistics.”. (P.8, last Par.)

I would also like to see group comparison for the 0 back – in spite of this paradigm presenting WM demands, it is a visual P300 paradigm and reports often do not find a reduced visual P300 in schizophrenia.

The intergroup comparison for the 0- back did not report reduced amplitude in the schizophrenic group using the non-parametrical premutation test (see the explanation above) and Mann-whitney test

Finally, while reporting stats results, it is OK to say that there is a trend level difference in amplitude/latency but it is not OK to say that, e.g., latency was more prolonged in patients but the difference was not significant – the result is that there was no difference in latency – or it was prolonged at trend level.

We agree with the reviewer and we have erased that paragraph of the text to avoid any confusion.

In the Discussion, the authors make a claim that different WM demands produced abnormal patterns of P300 amplitude in schizophrenia. This is exactly what not happened. In fact, this is what is noteworthy in the study: patients did not modulate P300 as a function of a WM load.

The reviewer is correct. Schizophrenia patients were characterized by their inability to modulate the P300 potential, being it under predominantly attentional tasks, or at different WM loads. We have modified our interpretation, by considering that in schizophrenics, the contributors to this potential may reflect the engagement of predominantly attentional rather than memory resources, which are allocated to
rehearsal networks even at low load conditions. This interpretation is consistent with the inability to find P300 differences between 0-back and 1-back conditions in schizophrenia (see Discussion, P11, paragraph 2).

Kok (2001) is cited in the context of a notion of a ‘phonological loop’ taking away from resources devoted to the generation of the P300. Kok never mentioned this concept: please read his review carefully.

We have changed our statement by suggesting that one of the rehearsal circuits proposed by Kok might be the phonological loop.

“When memory demands exceed the capacity of such frontoparietal circuits, recruitment of additional rehearsal circuits (which perhaps include the phonological loop) may contribute to performance by allocating additional memory resources, a mechanism that is proposed to be reflected in a decrease of the frontoparietal P300 potential [3] [10].” (Background, P3, end of first paragraph, and other sections).

The authors discuss ‘a trend’ in the group differences in the high memory condition. However, no trend is reported in the stats section. One can discuss a trend if one reports it – otherwise – there is no trend to discuss.

This sentence has been deleted.

I do not understand what the authors are trying to say when they discuss a WM update process which ‘fails to be fully executed’ in normal controls at high WM load – please clarify. In conclusion, I am not convinced at all by the explanation offered by the authors regarding the lack of P300 modulation in a WM task in a patient group.

We agree that our interpretations have been somewhat confusing. This is why we restated this interpretation in terms of the P300 reflecting predominantly attentional processes and not specifically memory-related processes (see above, and Discussion, P11, paragraph 2).

Minor Problems:
Please, edit the paper in terms of English grammar and word use.

We sought advice from a fluent english speaker.

People typically talk about P300 component not wave or waveform which is used to describe the entire epoch.

We have changed the words used accordingly.

What do the authors mean by ‘altered WM processing’ – page 3 – please explain the concept better.

We agree with the reviewer and we have eliminated this sentence from the text.
Please report your results in past tense.

We have changed the text accordingly.