Author's response to reviews

Title: Cluster analysis of behavioural and event-related potentials during a contingent negative variation paradigm in remitting-relapsing and benign forms of Multiple Sclerosis

Authors:

Javier J. Gonzalez-Rosa (javgonros@us.es)
Manuel Vazquez-Marrufo (marrufo@us.es)
Encarnacion Vaquero (evaquero@us.es)
Pablo Duque (pablo.duque.nps@gmail.com)
Monica Borges (monica.borges@neuroinvest.net)
Guillermo I. Zquierdo (g.i.ayuso@gmail.com)
Carlos M. Gomez-Gonzalez (cgomez@us.es)

Version: 4 Date: 30 March 2011

Author's response to reviews: see over
October 16th, 2011

Sabina Alam, PhD  
Senior Executive Editor  
BMC series journals

Dear Sabina,

Following the referee’s suggestion, please find enclosed the corrected manuscript with the required changes. We thank the reviewer for carefully reading our manuscript and for giving valuable comments.

In the referee’s files, we address all of the reviewer’s comments (reviewer’s comments are bigger font size).

On the other hand, we have rewritten or moved different parts of manuscript as referees requested. Therefore, several changes have been carried out throughout the manuscript (please, note that more important changes have been highlighted with yellow coloured text).

The main corrections in the manuscript are detailed below:

Abstract:
– This part has been completely rewritten according different suggestions.

Introduction:
- Clinical background of MS has been changed and shortened;
- The hypothesis and aims have been rewritten and clarified;

Methods
- “Participants” section has been rewritten and different MS subtypes have been redefined according to second referee’s suggestions.
- “Stimuli and procedure” and “ERP measures” sections have been moved to the footnotes or referred to previous manuscripts;
- In “Statistical analysis”, a section with new correlation analysis has been also included.

Results
- A new section with correlation results has been included.
Discussion
- Important changes and reductions of text were also carried out in this part of manuscript in order to avoid repetitions of the conclusions of results in the “Conclusion” section, for instance:
  - The “Summary of the result” section was removed of manuscript;
  - Some evidences about neural generators of CNV and their relation with MS and different neurological and psychiatric diseases were removed;
  - Some examples of different slow waves related to post-P3 periods were removed;
  - Some conclusions and details about clusters characteristics have been moved from cluster-section to the footnote of figure 4.
  - The “Limitations and implications” section have been rewritten.

Conclusions
- Some final conclusions that were showed at the end of each part in “Discussion” section (amplitude, latency, clusters, etc) were shortened or removal since that a part of this information was already incorporated in the general “Conclusion” section;

References:
Please, note that as result of these changes, some references have been removed. As result, the most references have therefore changed now in the order in which they are cited in the text.

Tables:
- Table 1 has been modified, showing new variables and p values and footnote updated.
- In the Table 2 and Table 3 p values have been also showed. As result, footnotes were also rewritten.

Figures:
- Figure 1 has been completely changed.

Legends:
- Figure 1, a brief description of task have been now included.
- Figure 4, description of significance of results related to this figure have been now included.
A Competing interests section has been also included as requested.

Thank you for your consideration.

With warm thanks,

Javier J. Gonzalez-Rosa

Department of Experimental Psychology
University of Seville,
Seville, Spain
Reviewer's report

Title: *Cluster analysis of behavioural and event-related potentials during a contingent negative variation paradigm in remitting-relapsing and benign forms of Multiple Sclerosis*

Version: 2 Date: 12 January 2011

Reviewer: César Ávila

Reviewer's report:

This study is aimed to provide direct evidence about a possible reduction of information processing speed (IPS) in MS patients with different phenotypes. This is an interesting work since recent studies have observed that disturbances in IPS could be a primary cognitive deficit in MS related to the decline of other cognitive functions, such as attention and memory (this premise has been formalized by Deluca et al., (2004) in their Relative Consequence Model). Other positive aspects of this study include the appropriate methodological approach taken. Thus, event-related potentials (ERPs) might be a very appropriate tool to study IPS alterations. Further, the Posner visual-spatial cueing paradigm is a sensitive task to study selective attention deficits in MS patients; finally, cluster statistical analysis might be a useful multivariate statistical approach to classify MS patients in different subgroups according to their scores in different cognitive variables. On the negative side, some parts of this study are confusing and, to my opinion, need to be amended before recommending its publication in a journal such as BMC Neurology. I hope that the following suggestions will be helpful to improve a corrected version of this interesting study.

Major compulsory revisions

We would like to thank the referee for the careful, constructive and helpful comments and suggestions on this manuscript, as well as for sharing your opinion and advices. We address the referees' comments in the following answers.

A general comment refers to the language use and style throughout this manuscript. More specifically, the text is sometimes confusing and it is difficult to understand what the authors are trying to say in some paragraphs. In addition, authors inconsistently use labels to refer to the different groups involved in the present study (i.e. the labels “control”, “healthy participants” and “healthy controls” are used in different occasions to refer the same subjects’ group). Similarly, not proper attention was paid when trying to standardize some layout/style details (i.e. commas and dots were indistinctly used to separate decimal numbers see Table 4).

As requested, we have changed several parts and sections of manuscript in order to simplify and clarified the main results and conclusions.
- Now we have mainly used “control group” as label throughout the manuscript to refer healthy subjects.
- The indistinct use of commas or dots has been now corrected. Dot has always been used to separate decimal numbers.

However, and according to other reviewer's suggestion, we have also reduced the manuscript or moved different parts. Therefore, several changes have been carried out throughout the
The major changes were:

**Abstract:**
- This part has been completely rewritten according different suggestions.

**Introduction:**
- Clinical background of MS has been changed and shortened;
- The hypothesis and aims have been rewritten and clarified;

**Methods**
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- A new section with correlation results has been included.

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**Conclusions**
- Some final conclusions that were showed at the end of each part in “Discussion” section (amplitude, latency, clusters, etc) were shortened or removal since that a part of this information was already incorporated in the general “Conclusion” section;

**References:**
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Legends:
- Figure 1, a brief description of task have been now included.
- Figure 4, description of significance of results related to this figure have been now included.
- A Competing interests section has been also included.

Introduction

Overall, this section is well written, although some minor changes should be introduced. Indeed, I have only a few suggestions in two paragraphs:

1) “These results have been interpreted, supported by neuropsychological and neuroimaging evidence, as a general objective index of cognitive impairment in MS patients, characterized by slow information processing, as a results of disconnections between cortical-subcortical structures”. Recent Studies (Au Duong et al., 2005; see also Leocani et al., 2010) have suggested that a reduction of IPS in MS patients is not only caused by disconnections between cortical and subcortical structures but also by disconnections within cortical structures (E.g. within structures of fronto-parietal networks). Further, the results of the present study also point out to a relevant role of this kind of disruptions at networks interconnecting different cortical structures.

The suggestion has been checked and corrected as requested.

2) “It is likely that the deterioration of different cognitive mechanisms is masked in both the early form of RRMS and the late form of BMS despite physical disability being same”. Is this a hypothesis of the authors? If so, it should properly noted and not presented as a proven fact. Moreover, RRMS patients participating in this study should not be described as “early” (disease duration ranged from 1 to 18 years in this group!).

The suggestion has been checked and corrected in the text as requested.

Methodology

Participants

1) When specifying the MS patients’ selection criteria, the authors state: “Lastly, we must highlight that overall MS patients included in this study had no immunomodulation record before beginning our study” Do the authors mean that none MS (including RRMS) patients had not received any treatment? If so, since the authors highlight this as a highly relevant variable, how it may affect for the interpretation of their results?

That sentence is totally true. MS patients included in our study had not any immunomodulation therapy at the moment of performing our study. Following suggestion of referee, we have highlighted this point with a phrase in “Discussion” section.
2) The educational level and the Intelligence Quotient (IQ) are important variables to be considered in any study assessing cognitive performance of MS patients (as well as of healthy subjects). In this way, and as stressed by the "cognitive reserve” hypothesis, educational level and IQ affect cognitive decline associated to MS and other neurological diseases (see Sumowski et al., 2010). Therefore, it seems crucial to assess, or at least to obtain a validated estimator of, the sample’s IQ (i.e. by using the matrix subtest of the WAIS battery). None of these measures were included, a fact that might be especially problematic given the specific aims of this study. Therefore, since it seems unfeasible to incorporate IQ measurements at this time point, I propose that at least educational level of the sample should be considered.

**ERP measures**

1) Authors say in: “For the measurement of CNV, two areas of interest were defined and their amplitudes were calculated with respect to a baseline 100 ms prior to the cue stimulus”. Which two areas? Were two or three the areas of interest used?. In fact, authors seem to specify three areas/variables: eCNV on parietal, cCNV on fronto-central and finally tCNV on occipital scalp locations.

There were “three areas of interest”; the suggestion has been corrected as requested.

Statistical analysis

1) Statistical analysis is correct and accurate. Only one suggestion: is it possible to use the educational level as a co-variable in the between subjects ANOVA?.

The answer will be given along with the answer to second point.

2) Another important co-variable to be introduced in the ANOVA might be the years of the disease evolution. This will be of major interest since, as stated by the authors themselves at the introduction section, it is one of the most important variables to understand cognitive impairment in MS patients. Indeed, this idea is further reinforced at the discussion section when referring to the BMS phenotype (“The increase of abnormalities such as delays in information processing, orienting, and behavioural accuracy for BMS patients, may be explained by a higher number of years of disease duration for this form of MS and by a silent but continuous progression of impairment in this disease”).

Regarding educational level, and first performing ANOVAs, we carried out different analysis in order to check the relation of demographic variables (age, sex, educational level) as well as MS clinical variables (EDDS scores, disease duration) between different groups of our study. Table 1 shows now p values of this one-way ANOVA. Please, note that except to disease duration, there were no statistical differences between different groups. Furthermore, partial correlations adjusted for age were also performed in order to describe the relationship between psychophysiological and MS clinical variables whilst taking away the effects of age on this relationship. As result, new sections have been added in the text (please, see in the Method and Result sections “Correlation” headlands). Some conclusions have been also clarified to the light these results.

Cluster analysis


1) Please, specify the variables included in the cluster analysis at the following sentence: “The variables introduced in the cluster analysis were those which most clearly discriminated between groups in terms of statistical significance (p < 0.035)” Why authors did write the p<0.035 in the cluster analysis and no p<0.05? I suppose that is in reference to the ANOVA results but is not clear for the reader.

Regarding this point, only variables with \( p \) minor of 0.035 was chose as statistical criteria and then introduced in the cluster analysis. On the other hand, in the phrase “The variables introduced in the cluster analysis were those which most clearly discriminated between groups in terms of statistical significance (p > 0.035) we have added “after performing ANOVA analysis” in order to clear the suggested issue.

**Results**

**Behavioral data**

1) Table 1: EMRR and EMB are not English acronyms.

“EMRR” and “EMB” acronyms have been corrected using appropriated English acronyms. Please note that new variables and \( p \) values are showed now in this table.

2) Table 2: It would be clearer for the reader if authors would specify \( F \) and \( P \) values as additional columns in the table.

As requested, \( p \) values have been included in the tables. However, due to there are a lot statistical comparisons and effects, and in order to simplified the information showed in the tables, we show \( F \) values only in the Result section.

**CNV period and ERP amplitude**

1) The same comment applies for Table 3 as well Cluster analysis

As requested, \( p \) values have been included in the tables.

2) Table 4 is not readily comprehensible and it actually confuses more than clarifies the obtained pattern of results. Accordingly, this table (as well as the paragraph reproduced below) should be re-elaborated in a form that allows its comprehension by readers not familiarized with some of the statistics described here. Also to facilitate comprehension, please specify all the acronyms in all the legends.

On the other hand, the fact that all subjects identified as “outliers” belong to BMS patients deserves a comment, specially, when considering that the number of subjects falling in this category is rather high (20% of BMS patients were considered as outliers).

We have added new comments related to the outliers in Discussion section and in the footnotes of Figure 4. In “Discussion/Cluster analysis” section, it is possible to read:

“Finally, a specific analysis for outlier cases removed from the clustering procedure revealed that these outliers only belonged to BMS patients and they showed severe impairment in terms of behavioural performance (RTs and %CRs) and psychophysiological parameters
We would like to have some extra comments related to this point. Owing to the fact that outliers might severely distort the results of cluster analysis and according to general recommendation for this type of analysis [32,33], in this first step some participants were identified as outliers (those showed highest square Euclidean distances with regard to the others clusters) and then removed from follow statistical step. K-means clustering method is very sensitive to outliers, since they will usually be selected as initial cluster centers. This will result in outliers forming clusters with small numbers of cases. Before starting a cluster analysis, it is normal to screen the data for outliers and remove them from the initial analysis [35,36]. Other important reason why outliers are frequently removed of cluster analysis is because precisely their removal “homogenizes” the sample, being more difficult then to identify clusters with highly homogeneity intra-clusters and high heterogeneity inter-cluster.

In general, in the Discussion and Conclusion sections, whenever we referred to “benign” group, we are actually talking about all benign patients (including these outliers benign patients), since benign patients appear to form a more heterogeneous in terms of their psychophysiological abnormalities.

Discussion

Behavioral performance

1) In this part of the discussion section, authors said that, in general, MS patients are slower than healthy controls. However, to my personal opinion, such a conclusion cannot be really extracted from the subjects’ behavioral performance but from the ERPs-based results. In this regard, one might argue that BMS had a higher number of errors due to “missed” errors but, without further information, “missed” errors cannot be considered as due to a reduction of information processing speed. Further, this would apply only to the BMS group and would not be applicable for “all MS patients”.

We decide to use a time limit (700 ms) as deadline for the response. It is true that this could explain why BMS patients (who had the slowest RTs) had significantly more misses than the other two groups: they did not only miss the targets, but they were too slow for the available response deadline. It could be the case that many responses were made, but they were slower than 700 ms in this special population of MS that is known to suffer from general slowing. This could be a limitation of our study, as we admit in “Limitations” section actually. According this, missed errors could be considered then like an “extra” speed information slowing.

In any case, this is a fact per se: MS patients performed slower responses than controls, which was statistically confirmed both for RRMS and BMS. Therefore, and using RT response as an evidence (see for an example the references 39 and 36), we have conclude saying that MS patients are slower than control subjects in terms of information processing speed.

2) In this paragraph: “The findings of our study suggest that subtypes of MS disease may be associated with a moderate/severe attentional impairment and with information processing deficits. BMS patients were, compared to RRMS patients, slower and less accurate, which suggests that BMS patients also show a deterioration in visual-motor processing which is potentially greater than other subtypes of MS (such as RRMS) despite an apparent milder
physical disability”. I do not agree with this conclusion, especially regarding the last sentence. First, it is true that BMS patients showed reduced accuracy (higher number of “missed” errors) and that this could be related with a reduction of IPS. However, this is not necessarily a consequence of “visual-motor processing deterioration […] despite an apparent milder physical disability”. This seems incompatible with the fact that, according to what is said at the methods section, patients without visual or motor impairment were selected as well as with the fact that BMS patients exhibit lower EDSS scores. Alternatively, it should be considered that IPS is a trait underlying several cognitive processes (i.e. attention, working memory, learning) rather than a cognitive function per se. Therefore, the reduction of IPS could be affecting the attentional functions (and not “visual-motor deficits”). This alternative interpretation seems more compatible with three interesting and specific results of the present study: First, using and attentional paradigm, reduced IPS in MS patients compared to control group was observed. Second, BMS patients showed a higher degree of IPS impairment than RRMS patients. Third, these cognitive differences seem to be related to a disruption between frontal and parietal areas (recruited during the Posner paradigm). Therefore, IPS reduction might be affecting a controlled process (attentional process) rather than a consequence of visual-motor processing deficits (which is an automatic process). In conclusion, the observed deficits might be due to disruptions in the fronto-parietal network necessary to execute and attention spatial task rather than in visual or motor pathways. A similar comment deserves MSRR results. Thus, although authors state “However, both MS groups appear to have the amplitude of the final period of CNV intact, which is traditionally related to sensory preparation for the imperative stimulus as well as to preparation for the motor act”, it seems that deficits observed in MSRR patients could be related to attentional problems derived of IPS reductions rather than an affectation of the pathways carrying the sensorial input/ the output responses.

Authors are very grateful to reviewer for the extra comments displayed in this point. After thinking about these sentences, and according general results found in this study, we have carried out some changes in this paragraph. Thus, “visual-motor processing” phrase has been removed from text and replaced with a new phrase as follows:

“The findings of our study suggest that subtypes of MS disease may be associated with a moderate/severe attentional impairment and with information processing deficits. Compared to RRMS patients, BMS patients were slower and less accurate, suggesting that BMS patients could incur greater information processing impairments. These deficits could be affecting attentional functions in terms of attentional orienting and reorienting affecting the BMS disease subtype more than other subtypes of MS (such as RRMS) despite an apparent milder physical disability”.

3) I do not agree with the authors’ interpretation of the Mainero et al., 2006 study (“On the other hand, MS patients may have functional cortical changes when performing cognitive tasks because of damage strategic to white matter structures, limiting the adverse clinical consequences of structural brain damage and improving behavioural performance”). In this sentence authors seem to introduce the existence of compensatory mechanisms. However, the study of Mainero et al., 2006 might not be the best one to support such a possibility. Indeed, the MS patients recruited in Mainero et al. 2006 show a worse performance in the PASAT task than healthy controls and, therefore, one should conclude that the activation of additional areas in these patients did not were “compensating” their behavioral deficits. Thus, I would suggest
citing other studies that do actually show that additional activations in MS patients might actually have a “compensatory role” as they are observed in the absence of differences in cognitive performance (see Audoin et al., 2003; Forn et al., 2006; 2007).

More accurate references have been cited in this part of discussion section according to suggestion of referee. Specifically, we have removed the Mainero’s study and included in this sentence Audoin et al (2003) as well as Forn et al (2006). Please, note that now numeration of references have been modified.

**ERP latency.**

1) To my personal opinion, because authors did not include an appropriate a neuropsychological assessment of BMS patients they are not really legitimated to conclude: “Increased ERP latencies in BMS as well as RRMS led us to suggest that ERPs may indicate subtle degrees of cognitive dysfunction that are not always detected by more traditional clinical measures”. If this evaluation would had been performed (i.e. using Rao’s Battery) perhaps these deficits would had been identified. Further, the conclusion that BMS patients do not show cognitive impairments is in contradiction to those of the Amato et al., (2008) and Rovaris et al., (2008) who observed cognitive dysfunctions in this clinical population.

As requested, the following sentence has been corrected, as follows:

In results, authors use differences in the ERP as proof of cognitive impairment which is not right. In this setting, they can talk about altered cognitive processing, but not claim cognitive impairment because they have not performed a neuropsychological assessment and the relationship between ERP and cognition is not straightforward.

Our ERP findings allow us to talk about altered cognitive processing, but not claim cognitive impairment because we have not performed a neuropsychological assessment and the relationship. Attending the referee’s suggestions, we have rewriting these issues in the manuscript. Thus, concepts as such “cognitive impairment” or “cognitive dysfunction” have been replaced by “altered cognitive processing” in most of cases.

**Cluster analysis**

1) Which parameters do have greater weight to separate healthy controls and MS patients in regards to cognitive impairment? It seems clear that they must include some ERP components but it is unclear which ones of them have higher significance.

ANOVA computed after clusters analysis revealed that, in general, ERP latencies had a greater weight to split healthy controls and MS patients but also amongst patients. Specifically, as referee can check in the figure 4 and considering Z-scores, latency of middle- and long-latency ERP components (P2 and P3) had greater weight to split controls and MS patients. We have revised in the discussion section and tables legend some phrases to reinforce this point.

For instance, in the “Discussion/Cluster analysis” section:

“The new ANOVA performed with the clusters as between-subjects factors and psychophysiological scores as within-subjects factors revealed that, in general, latency
parameters (N1, P2 and particularly P3 components) were the strongest scores for clustering patients."

2) In several occasions authors refer to age as an important variable (i.e. Did the authors use the age as a variable of interest in the cluster analysis?), why exactly? Did the authors use the age as a variable of interest in the cluster analysis? Could the effects of age be separated from disease progression?, In this way authors refers the age in two times: “Cluster analysis revealed two more interesting findings: firstly, two control groups could be created based on their younger age…” “Cluster 1 was relatively younger than healthy control of cluster 2…”

We thank the referee for the extra comments in this point. We did not use age as variable to be introduced in the cluster analysis. However, after cluster analysis, we subsequently performed new descriptive analysis and ANOVAs with the clusters as between-subjects factors and the new Z-scores and some clinics variables (age, EDSS and disease duration) as within-subjects in order to verify which variables controlled in our study could explain clustering differences. In this regard, we have observed that there had a statically trend related to age by which subjects of Cluster 1 were relatively younger than subjects of Cluster 2 and that this finding seems to be related to shorter latencies for ERP components. In this regard, we had previously performed in the current version of manuscript partial correlation analysis to control the age effect.

**Figure 1. Experimental paradigm.**

In this legend a short description of the Posner task might facilitate the comprehension of the study for the readers. Indeed, I suggest to include this description here and, if needed, to remove it from text.

As requested, a brief description has been added in the footnote. In the Method section, we have referred to previous manuscripts for a more detailed description of paradigm. Please, note that Figure 1 has been completely changed.
Reviewer's report

Title: Cluster analysis of behavioural and event-related potentials during a contingent negative variation paradigm in remitting-relapsing and benign forms of Multiple Sclerosis

Version: 2 Date: 25 January 2011

Reviewer: Pablo Villoslada

Reviewer's report:

In this study authors assessed attentional evoked potentials, using Posner paradigm, for studying patients with MS compared with controls. In addition, they performed a comparison between typical RRMS cases and RRMS with benign course. Finally, they attempt to identify subgroups of patient based in the attentional ERP performance that may be related with different degree or type of brain damage based in cluster analysis. They found that MS group showed impaired responses in terms of amplitude and latencies of ERP compared with controls. When analyzing RRMS subtypes, they found poorer performance by the so-call “being” group compared to the typical RRMS cases. Finally, they were able to identify 4 different clusters, two of then specific for MS cases and with different frequency among RRMS subgroups, suggesting different patterns of brain damage, being more severe in benign MS. The study is well designed and conducted and results reinforce the concept of widespread brain damage in MS, even at early stages of the diseases or in subgroups with low physical disability, challenging the concept of benign MS and even the concept of low disability in RRMS.

We would like to thank the referee for the careful, constructive and helpful comments and suggestions on this manuscript, as well as for sharing your opinion and advices. We address the referees’ comments in the following answers.

Comments:

1. The article is very long. Even if there is not a formal limitation for length in BMC Neurology, it is important to keep in mind the reader. For this reason, it is always very convenient to keep manuscript in a range below 6,000 words. I recommend to short all sections taking in consideration that clinical aspects about MS are well known by readers, methods previously published can be shortened and referred to previous papers, for results, avoid the overlap between text and tables and figures. In general is very easy to grasp the info from tables and figures more than text. Discussion should be
focus in what is new and what is challenging this paper. Same for references, select the most important ones; readers are going to appreciate this work.

As requested, we have reduced the manuscript and several references were also removed where necessary. However, we could not keep manuscript in the range of 6000 words. The main reason is due to a lot of ERP components were analyzed using a cognitive paradigm with different experimental conditions and in three different groups. As result, many results were found and they have to be explained. Moreover, the use of cluster analysis to detect possible psychophysiological patterns and cognitive profile needed of additional explanations both in the Methods, Results and Discussion sections. Furthermore, and according to other reviewer’s suggestion, we had to perform new analysis and additional comments in different parts of manuscript. As a result, and despite we have shortened the manuscript the manuscript has been only reduced in the range of 2000 words in comparison to the previous version.

However we have removed, rewritten, or moved different parts of manuscript as requested. Therefore, several changes have been carried out throughout the manuscript (please, note that more important changes have been highlighted with yellow coloured text).

The major changes were:

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- A new section with correlation results has been included.

Discussion
- Important changes and reductions of text were also carried out in this part of manuscript in order to avoid repetitions of the conclusions of results in the “Conclusion” section, for instance:
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Conclusions
- Some final conclusions that were showed at the end of each part in “Discussion” section (amplitude, latency, clusters, etc) were shortened or removal since that a part of this information was already incorporated in the general “Conclusion” section;

- References:
Please, note that as result of these changes, some references have been removed. As result, the most references have therefore changed now in the order in which they are cited in the text.

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Figures:
- Figure 1 has been completely changed.
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- Figure 1, a brief description of task have been now included.
- Figure 4, description of significance of results related to this figure have been now included.
- A Competing interests section has been also included.

Finally, and according to the other reviewer’s suggestion, we have also computed correlation analysis between psychophysiological measures (behavioural data and ERP latencies and amplitudes) and MS clinical variables (EDSS and disease duration). As result, new sections have been added in the text (please, see in the Method and Result sections “Correlation” headlands). Some conclusions have been also clarified to the light these results.

2. Abstract should provide more details about results, providing some numbers. Specifically, if you say patients have more “important” deficits, illustrate it with some numbers. Also, split background from methods with subheadings. You can add a sentence for background (e.g. ERP may distinguish tissue damage or phenotypes in MS…).

New comments and details have been provided in the Abstract as requested. Further, abstract is now structured into more separate sections. Due to large numbers of results as well as limitations of words we have not added numbers of main effect. However, we have detailed the main effects and have offered some variables or parameters as example where statistical differences between different groups were found.

3. The hypothesis is descriptive and not clearly state. They state as the objective “to investigate the information processing…”. Based in the authors understanding they can formulate a clearer hypothesis at the end of the introduction in order to help reader to know the question this article address (e.g. RRMS patients have significant attentional impairment that do not correlates with physical disability…).

Accordingly to reviewer’s suggestion, we have rewritten the hypothesis clarifying our aims as follows:

“The motivation for the present study was to investigate the information processing patterns and abnormalities of attentional mechanisms associated with the Posner paradigm
both in benign and typical relapsing-remitting forms of MS disease. To our knowledge, there are no published studies regarding the temporal dynamics of CNV associated to a Posner task in MS disease. According to previous psychophysiological studies [28,29], we suggest that patients with a benign profile of MS also manifest altered cognitive processing that may be different from that which patients with a typical relapsing-remitting course demonstrate. Furthermore, and consistent with current evidence about the natural course of MS disease [1,2], we suggest that the deterioration of different cognitive mechanisms is masked throughout the relapsing-remitting course of MS despite minimal physical disability. Additionally, and in an effort to explore natural subgroups of MS patients from a different perspective, we conducted a cluster analysis to establish particular grouping of individuals on the basis of their common psychophysiological pattern and cognitive profile and whether this particular grouping might also be related to clinical parameters.

4. The disease subtypes are not clearly defined. 1) Benign MS is always RRMS: then I suggest describe the cohort as RRMS and divide it between benign MS and “common or typical” RRMS. 2) definition of benign MS is not clear in the literature but there is the consensus that at least should be a EDSS < 3.0 (better < 2.5) after 10-15 years disease duration. EDSS 3.5 is not benign MS and using disease duration of 8 years is less than previous definitions. Because the majority of the benign MS patients in this cohort fulfill these criteria, I suggest to remove the few of them not fitting this criteria and provide a reference for benign MS definition (e.g. Amato et al. J Neurol 2006).

Regarding to the first point, we have modified the previous version of the manuscript and include the term “typical” for the RRMS patients and some times the term “benign profile” for the BMS patients. The updated text stays as follows:

“Clinical, behavioural and ERP data were collected from twenty-seven MS patients, clinically defined as per Poser criteria [30], and classified in two clinical subgroups: (1) seventeen had typical relapsing-remitting MS (RRMS), defined as a history of several relapses and remissions and with disability on the Kurtzke Expanded Disability Status Scale (EDSS) [31] less than 3.5; (2) ten had a profile of benign MS (BMS), defined by a relapsing-remitting onset, but a disease course of more than 8 years without relapses and remissions and with an EDSS of less than 3.”
Concerning to the BMS definition of our study, first of all, please note that we had made a mistake in the previous version of manuscript describing the EDSS score of our cohort of BMS patients. Regarding the BMS group patients included in our study, none of them had a higher EDSS ≤ 3. Please, this point can be checked both in the table 1 of the previous version and in the new one: the median EDSS score were 1.9 (range 1 to 3).

<table>
<thead>
<tr>
<th>Groups</th>
<th>CONTROLS</th>
<th>RRMS</th>
<th>BMS</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>18</td>
<td>17</td>
<td>10</td>
<td>-</td>
</tr>
<tr>
<td>Sex(female)</td>
<td>15 (83%)</td>
<td>12 (80%)</td>
<td>6 (60%)</td>
<td>0.716</td>
</tr>
<tr>
<td>Education (years)</td>
<td>10.67 ± 2.3</td>
<td>9.9 ± 3.9</td>
<td>9.2 ± 3.1</td>
<td>0.537</td>
</tr>
<tr>
<td>Age</td>
<td>36.54 ± 8.73 [26-54]</td>
<td>38.88 ± 9.04 [24-63]</td>
<td>42.30 ± 7.21 [28-56]</td>
<td>0.247</td>
</tr>
<tr>
<td>Age at onset</td>
<td>-</td>
<td>34.12 ± 6.5 [23-44]</td>
<td>30.90 ± 6.9 [21-42]</td>
<td>0.414</td>
</tr>
<tr>
<td>EDSS</td>
<td>-</td>
<td>2.1 ± 1.3 [0-3.5]</td>
<td>1.6 ± 0.9 [1-3]</td>
<td>0.609</td>
</tr>
<tr>
<td>Disease Duration</td>
<td>-</td>
<td>4.67 ± 4.13 [1-18]</td>
<td>12.09 ± 4.77 [8-16]</td>
<td>0.001</td>
</tr>
</tbody>
</table>

Second, referee also questions our current definition of “benign” in this study in relation to disease duration. Although the definition of “benign” course is still arbitrary in general, there is a relatively recent consensus about benign definition in MS (EDSS score remains below 3 and mild disability after at least 10 or 15 years from the disease onset). Therefore, it is also true that the benign criteria used in our study do not match exactly with more recently consensus.

However, we decided to include some benign patients with at least 8 years of disease duration for several reasons:

1) Benign forms are a cohort of MS patients enough difficult to recruitment;
2) During our study, criteria for definition of benign course in MS were still highly controversial in our opinion. It was relatively frequent to find in the recent literature a definition of benign form of MS with a EDSS score ranging from 0 to 2 (Lauer & Firnhaber, 1987), from 0 to 4 (Benedikz et al, 2002) and with a variation in disease duration from 5 years (Kalanie et al, 2003) to 15 years (Perini et al, 2001).
Therefore, we decided to include some patients (indeed only two patients) with at least 8 year of disease duration, but with a compatible progression as benign profile of MS, including symptom-free after a long follow-up period, with no or infrequent relapses history, and a low disability score (EDSS ≤ 3) during a period of eight years. Furthermore, our cohort of patients with a benign profile is clearly different of those with a typical course relapsing-remitting in terms of years of disease duration (mean in the typical RMMS group: 4.6; Benign: 12 years).

In light of all the above, we have decided to keep our small sample of benign patients given that there are still a controversial definition of benign course (Kalanie et al, 2003; Ramsaransing & De Keyser, 2006). Therefore, our benign MS group will be defined in the manuscript as “benign profile” instead of “benign form” (we would like to thank to the referee about this comment). As a result, we have also added in some parts of manuscript (Method and Discussion sections) the concept of “benign profile” in order to clarify this issue and to avoid confusions.

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5. In the introduction, current standard of disease subtypes are based in Lublin criteria (Lublin F Neurology 1996). Please, use this reference and not ref 1,2 that are very general.

We have replaced reference 1 with Lublin and Reingold (1996) as requested.
6. A main limitation of the study is the lack of a formal cognitive assessment of patients in addition to the attentional task included. This would be critical in order to correlate with attentional performance and for proving that MS subtypes based in EDSS do not correlated with cognitive ERP, such as in the case of the so call benign MS. Have authors data from BRB-N battery or other neuropsychological tests? Similarly, lack of MRI quantification is another significant limitation. Cognitive performance would be more associated with brain atrophy or lesional load than physical scales used for stratifying patients. You should comment it in limitations of the study.

New comments about the potential limitation of our study related to not-inclusion of a standard neuropsychological assessment or conventional MRI quantification has been added in the text as requested (in “Limitations and implications” section).

7. The two disease subtypes differs in disease duration and age, because in order to be define as benign MS it requires longer observation period than for RRMS. This also affects the validity of the control group, because it cannot match with both subgroups at the same time.

The lack of validity of control group, due to cannot be totally match with different MS subtypes in terms of age or disease duration, is a common fact in most studies which MS patients with diverse disease course. This is even true in several studies which assessing only diverse subtypes of MS groups without a control group. However, participant of our control group were carefully matched for age in order to find a balance between control group and both MS patients. Please, note that there is no statistical significance for age between different groups. Now, these p values can be checked in the table 1.

8. Methods: please, state whether patients are the same one or if they differs from the one reported in Gonzalez-Rosa et al. 2006.

This information was already given in different sentences at the Result section. Moreover, we have added an additional sentence in Methods/Participant section as requested.

9. Methods. Sample size is small (27MS/18 controls) for the many comparisons planned. You should comment it in limitations of the study.

This comment was included at “Limitations and implications” section. as requested.
10. Methods: The use of the disease onset definition based in the first visit to neurologist is fine for natural history studies, but in a cross-sectional study it do not provide any value compared to the disease duration variable. Since score of the disease onset definition based in the first visit to neurologist is not used or analyzed in our study, we have removed it of text. Therefore, standard MS clinical variables (disease duration, age at disease onset and EDSS scores) were finally included as MS clinical variables after assessment by our neurologists’ team.

11. Methods: list the clinical variables collected in all patients: sex, age, EDSS, (BRB?)…

Clinical variables collected in all patients in this study have been listed in Method/Participants section as requested.

12. Methods: authors performed many statistical tests and for this reasons it is required to adjust for multiple tests (you can use FDR because Bonferroni is going to be very exigent at this time). Then, authors must indicated which result survived correction and which are significant but not after correction.

Multiple post-hoc comparison procedures are commonly used in an analysis of variance after obtaining a significant result, for instance, a significant interaction between two main conditions in a repeated measure ANOVA. Due to that, referee suggests adjusting alpha levels for multiple comparisons.

We would like to have some extra comments related to this point.

First, in our study, post hoc t-tests with Bonferroni’s correction were always computed for comparisons of the between-subjects factors where necessary. For instance, if we found that there was a statistical significant group effect then posthoc comparison were always adjusted with Bonferroni correction. This is because the term "comparisons" in correction for multiple testing typically refers to comparisons of two or more groups (Perneger, 1998; Howell, 2002), such as a treatment group and a control group. Therefore, this adjusting was already carried out as was described in the Method section.

Secondly, in our study, effects found for main within-subject factors or for interactions between within-subjects and between-subjects variables were not corrected for multiple
comparisons according to recommendations of some authors (Rothman, 1990; Perneger et al, 1998; Howell, 2002). Following these recommendations, we have not adjusted alpha levels in this type of multiple post hoc comparisons.

In this respect, adjustments for multiple comparisons are recommend to avoid rejecting the null hypothesis, reducing the Type I error (i.e., incorrectly deducing a difference, when in fact there is no significant difference). However, reducing the risk of making a Type I error increases the chance of making a type II error (i.e., incorrectly deducing no difference, when in fact there is a significant difference). Furthermore, if alpha levels of post hoc comparisons are adjusted for within-subject factors and, moreover, a large number of independent tests are performed, there is the risk to increase also the type II error, so that truly important differences are deemed non significant.

We have studied psychophysiological indicators of potential attentional impairment and information processing deficits. Thus, there are clear a priori hypothesis on the effects of MS disease on based on previous studies.

Therefore, and following the guidelines of Rothman (1990) and Perneger (1998), interaction effects were not correct for multiple comparisons to minimize the risk of false negative. Moreover, and as Rothman points, while the Bonferroni correction for multiple comparisons is often considered de rigueur in clinical research, its uncritical use is based on the faulty notion that type II statistical errors (failing to detect a real difference between groups or conditions) are less problematic than type I errors. In our study, despite that main a priori hypotheses are well known, we were also interested to explore new “sensible effects” or findings in order to conduct additional and new psychophysiological researches to establish whether the current significant findings can be confirmed. Therefore, a reasonable alpha level is an easily justified compromise.

13. Methods: how to manage outliers is always a problem. By removing them, it is easier to identify patterns that explain most of the data, but at the price of losing generalization. Then, validation of the identified patterns would be required by crossvalidation.

We agree to manage outlier cases is always a problem. According to standard statistical procedure to perform clusters analysis [32,33], and as we described in Method/Statistical analysis section, we carried out a 2-stage cluster analysis to build up the cluster solution. First, we computed a hierarchical clustering in order to explore and to determine the number of clusters and thus to provide the initial solution for the second step. Owing to the fact that outliers might severely distort the results of cluster analysis and according to general recommendation for this type of analysis [32], in this first step some participants were identified as outliers (those showed highest square Euclidean distances with regard to the others clusters) and then removed from follow statistical step.

$K$-means clustering method is very sensitive to outliers, since they will usually be selected as initial cluster centers. This will result in outliers forming clusters with small numbers of cases. Therefore, before starting a cluster analysis, a screening data to detect outliers should be carried out [32,33]. Other important reason why outliers are frequently removed of cluster analysis is because precisely their removal “homogenizes” the sample, being more difficult then to identify clusters with highly homogeneity intra-clusters and high heterogeneity inter-cluster.

We also agree with the referee that a validation method of the identified patterns or clusters would be required in this study. We have not computed this validation by cross-validation since this procedure is mainly used in settings or analysis where the goal is prediction, and one wants to estimate how accurately a predictive model can be in practice. The aim of our study performing the cluster analysis is not obtained a predictive model related to new groupings. Nevertheless, in the present study, we have indeed performed other validation procedures more according with clusters method [35,36] in order to validate the proposed clustering solution. Thus, as it is indicated in the “Method/Statistical analysis/Cluster” section, we have carried out partitioning of clustering variables and profiling the clustering solution with others variables other than those in the clustering procedure (for examples, using other ERPs components, clinical variables, etc). After validation cluster procedures were performed, minimum changes were observed for clustering solution in terms of numbers of
cases into each cluster, moving of subjects from a cluster to other one, relevance of variables to split clusters, etc...

14. Avoid priority claims (“the first study….”). They decrease value of the study and have no added value.

This sentence have been removed and changed in the text as requested.

15. Results: when authors claim that patients have a slowing of information processing, indicate the parameters used for such statement based in ERP results.

The following paragraph in the Result section has been corrected as follows:
“The present study shows that cognitive impairment observed in MS patients do not only correspond to the general slowing of information processing in terms of slower behaviour responses or ERP latencies”.

16. The ERP recording method and analysis are right. However, authors highlight many small differences considering the small sample size and lack of correction for multiple testing. Moreover, the most significant differences are between patients and controls and not between patients subgroups.

We have replied in part this point in the question number 12 and some comments can be found in the “Limitations” section. As referee points out, in our study and with our patient samples, several small differences between three experimental groups were found after a psychophysiological assessment using a Posner paradigm. It is also true that many of these statistical “small differences” were revealed by ANOVA in terms of double or triple interactions, which underlines the powerful of effect. However, one of the goals of performing a cluster analysis using psychophysiological measures was precisely to explore new particular grouping of individuals and patients on the basis of their common psychophysiological pattern.

17. In results, authors use differences in the ERP as proof of cognitive impairment which is not right. In this setting, they can talk about altered cognitive processing, but not claim cognitive impairment because they have not performed a neuropsychological assessment and the relationship between ERP and cognition is not straightforward.
Attending the referee’s suggestions, we have changed the concept “cognitive impairment” in the manuscript as a result of differences found in the ERP by “altered cognitive processing” and others similar concepts where necessary.

16. Discussion: authors argue about a new reclassification of disease subtypes based in ref 93. But this is mainly focused in progressive MS and it is not really new (1999). I think the issue of benign MS is still pending to be redefined.

We have decided to remove this reference in the text since the main goal of this study is not to underline a new reclassification of disease subtypes. Our findings suggest rather that benign MS is still pending to be redefined, which is commented in several occasions throughout the manuscript.

17. Limitations of the study should indicate also the issue of small sample size, the lack of patients with progressive MS, the lack of MRI and neuropsychological data.

As requested, we have highlighted these issues in the “Limitations” section.

18. Table 1. Indicate also the sample size (n), sex, age at onset and the use of disease modifying drugs and the p value for comparisons between groups.

As requested, additional variables and data (p values) has been added in the Table 1.

19. Tables: it is easier for readers to identify which results are significantly different if you indicate the presence of significant differences by putting the superscript after the numerical value and not in the name of the variable, because it requires a lot of checking the legend and the corresponding subgroup.

Accordingly to reviewer’s suggestion, we have carried out some modifications in the tables, specifically related to show p values. Please, note that we have tried to show in the different tables as much information as possible about results (particularly tables 3 and 4). However, due to the large number of variables, experimental conditions and interactions between within-subjects and between-subjects variables, we have finally opted for this type of tables.
20. Figure 1 is the same than in Gonzalez-Rosa et al. 2006. They should decide whether it should be kept or just referred.

We have decided to keep Figure 1 according to the other reviewer’s suggestion. In addition, this figure has been modified and updated as requested. Most details related to the experimental paradigm, in one way, have been moved from Method section to footnote of Figure 1, and in another, have also referred to previous studies.