Parkinson Disease

ERP sources in middle cingulate and precuneus differentiate Parkinson's patients from healthy controls and lingual gyri sources reflect human recombinant EPO effects in a Flanker task

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Abstract

We used EEG source analysis to identify which cortical areas were involved in the suppression of competing responses on a flanker task and compare the potential efficacy of recombinant-human erythropoietin (rHuEPO) in the performance of Parkinson' s Disease patients.
The samples were 18 medicated PD patients (9 with rHuEPO and 9 without rHuEPO) and 9 age and education-matched healthy controls (HCs) who completed the flanker task with simultaneous EEG recordings. N1, N2 and P3 event-related potential (ERP) components were identified and a low-resolution tomography (LORETA) inverse solution was employed to localize the neural generators.
Reaction times and errors were increased for the incongruent flankers for PD patients compared to controls. EEG source analysis identified an effect of rHuEPO on the lingual gyri for the early N1component. N2-related sources in middle cingulate and precuneus were associated with the inhibition of automatic responses evoked by incongruent stimuli differentiating PD and HCs.
From our results the rHuEPO seems to mediate an effect on N1 sources in lingual gyri but not on behavioural performance. N2-related sources in middle cingulate and precuneus differentiated PD and HCs.

Introduction

The basal ganglia structures particularly the striatum and the subthalamic nucleus are part of the fronto-striatal-subthalamic-pallidal network considered to mediate habitual/automatic and goal-directed inhibition as well as habitual and goal-directed action [1,2]. Thus, these circuits are hypothesized to coordinate the selection and suppression of competing responses. Parkinson's disease (PD), the prototypical basal ganglia disorder, is associated with deficits in inhibitory control on a number of experimental tasks such as the stop signal [3], go no-go reaction times [4], the Stroop and the Hayling Sentence Completion task [3], and the Simon task [5,6]. PD patients also have difficulty in suppressing interference arising from the

automatic activation of prepotent responses evoked by incongruent flankers in the Ericksen's Flanker Task [7]. Relative to controls, PD patients show increased reaction times (RTs) and errors on incongruent trials compared to congruent trials (eg. [8,9,10,11]).
In PD there is an ongoing search for neuroprotective agents which may slow down the progression of the illness and improve cognitive deficits [12]. The recombinant-human erythropoietin (rHuEPO) is studied with great interest due to its neuroprotector properties in neurologic diseases [13]. The anti-apoptotic, anti-inflammatory and cytoprotective effects of EPO in parkinsonism animal models have been described elsewhere [14,15]. The aim of our study is to use a flanker task to identify if rHuEPO produces beneficial effects on performance of PD patients and locate the neural generators involved in the selection and suppression of competing responses in comparison with healthy controls (HCs).

Materials and Methods

Methods: 18 PD patients (Hoehn and Yahr stages I to III, mean age 53.9, SD 3.2 years) were recruited at the Clinic of Movement Disorders and Neurodegeneration, Centro International de Restauracion Neurologica (CIREN) in La Habana, Cuba to participate in a safety clinical assay of Erythropoietin (rHuEPO) in PD. The design of this investigation, results, scheme of application and doses employed may be found in [16]. Inclusion criteria were: a clinical diagnosis of idiopathic PD according to the UK Brain Bank criteria and a good response to dopaminergic treatment and aged between 45-75 years [17]. Exclusion criteria were: manifestation or indicative signs of major cognitive impairment, psychotic symptoms, and/or presence of other chronic diseases. Nine of the PD patients through random allocation received additionally to their usual anti-parkinson medication, rHuEPO for five weeks and the other nine did not. rHuEPO approved and registered for its use in humans was obtained at the Centro de Inmunologia Molecular, La Habana Cuba (ior® EPOCIM). There were no significant differences in age, years of education or duration of illness between the two PD groups. To exclude dementia and major depression, the Mini Mental State Examination and the Hamilton Depression Scale were respectively administered [18,19]. All patients were assessed on the motor subscale of the Unified Parkinson´s Disease Rating Scale (UPDRS) both during "on" (mean 6.3, SD1.1) and "off" medication (mean 21.7, SD 4.3) states. Nine HCs matched in age (mean 51.2, SD 3.9 years) and educational level were recruited at the same clinic. The PD patients were tested on their usual anti-parkinsonism medication. The patients signed an informed consent to participate in this study as a complement of the clinical trial following the CIREN ethic's committee regulations.

Eriksen´s Flanker Task

All participants completed the Eriksen´s Flanker task, while the EEG was simultaneously recorded. Each trial of the task consisted of the presentation of a set of 5 ordered letters (HHHHH or SSSSS) for the congruent condition and 5 letters with H or S at the center and different laterals or flankers (SSHSS or HHSHH) for the incongruent condition. Participants were instructed to respond to the central letter, whether H or S, by pressing a key with the index finger of the right or left hand respectively. Participants were instructed to respond as fast and as accurately as possible. A total of 480 trials in two blocks, each lasting 8 minutes were completed. In each block 80 stimuli were shown for the congruent condition and 160 for the incongruent, with the objective of provoking a greater number of errors. Reaction times (RTs) were measured to the nearest milliseconds and errors were recorded.
The physical characteristics of the stimuli were black letters on a white frame with an h = 1.5 cms and L= 7 cms, under 6 ^{0} a visual angle. The distance of the participant to the computer monitor was 60 cms. Each stimulus was presented at the center of the screen and kept for 190

msec., followed by an interstimulus interval of 1735 msec. A training block of 40 stimuli was designed to ensure task instructions were understood.

EEG:
The Electroencephalogram (EEG) was continuously recorded at a sampling rate of 512 Hz from 64 electrodes located at standard positions of the International 10/20 System using a Brain Vision system (Brain Products https://www.brainproducts.com/products_by_apps.php?aid=5 [20]. The electro-oculogram (EOG, horizontal and vertical) was recorded from electrodes placed 1 cm to the left and right of the external canthi, and from an electrode beneath the right eye. The ears were used as on-line reference and the front as earth.
Data were filtered using 1-30 Hz and a notch filter to eliminate the 60Hz powerline artefact. All data were referenced using an average reference to all the channels. The baseline was corrected between -400 to -200 msec. Epochs with electric activity exceeding baseline activity by 100 µV were considered as artefacts and were automatically rejected from further processing (15% of epochs related to hits and 11% of the epochs related to errors). For the analysis, several electrodes were excluded (EOG, ECG, TP9 and TP10).

ERP Components:
EEG recordings were analyzed for each participant within the two experimental conditions and averaged over the group using Analyzer software (https://www.brainproducts.com/productdetails.php?id=17). Epochs of 900 msec. (from -200 msec. (baseline) until 700 msec. post-stimulus onset) were analyzed time-locked to the stimulus. We selected three windows to examine the stimulus-locked ERPs, using only the correct response averages for the N1, N2 and P3 components in the expected time-windows (see ERPs guidelines in [21]).
In order to localize the generators of the ERP components, a lead field was constructed for each participant to calculate the inverse solution at the three selected latencies using LORETA (Low Resolution Tomography) (http://www.uzh.ch/keyinst/loreta)[22]. The significant specific source effects in each latency were independently confirmed by means of permutation methods [23]. The tomographic inverse solution was plotted using an average brain, (volume constraints) with the coordinates of the AAL (Automated Anatomical Labelling of Activations) 116 structures atlas of the Montreal Neurological Institute (MNI) [24].

Statistical analysis. The General Linear Model and *a priori* contrasts were used for statistical analysis, with Group (PD with rHuEPO vs PD without rHuEPO) as the between group factor and the experimental condition (incongruent versus congruent) as the within-subject repeated measures factor. The three windows for analysis were: 100-180, 180-300 and 300-450 msec. This was also applied for the neural sources using voxel-based analysis for the individual source matrices. For the second objective, we analyzed the difference between the two groups of PD vs Healthy controls in the same way. The resulting F statistic was corrected twice. First, using Bonferroni corrected according to the total number of points in the analysis window (700 milliseconds) and divided by α=0.05. The second correction was using FDR (false positives (FDR: false discovered rate) for a q-value=0.01, that is, controlling a 1% of the expected value [25]. Analysis was completed with STATISTICA 7.0 and the software (NEEST) from Neuronic http://www.neuronicsa.com/

Results and Discussion

Behaviour:

All groups, PD with or without rHuEPO and HCs had longer RTs and made more errors on the incongruent than the congruent trials (Table 1 and 2). However, when comparing PD patients with or without rHuEPO, there were no significant differences in performance between the task conditions (Table 1).

Table 1	**PD with rHuEPO n=9**		**PD without rHuEPO n=9**	
	congruent	**incongruent**	**congruent**	**incongruent**
	Means (SD)	Means (SD)	Means (SD)	Means (SD)
Reaction times msec.	459.33 (71.76)	479.89 (49.43)	460.22 (72.10)	488.22 (63.76)
Percent errors	13.22 (7.76)	43.22 (21.37)	8.78 (6.76)	32.00 (15.79)

Table 1 : The results of the reaction times and the percent of errors for the congruent and in congruent trials for the PD patients with and without rHuEPO. The values in the table are means with standard deviations in parenthesis.

When comparing all PD patients and HCs (Table 2), the results were consistent with previous findings and RTs increased with incongruent flankers compared to congruent for both groups. This RT cost of incongruence was significantly greater among PD patients (p=0.026) than healthy controls (p=0.91). The percent of errors in the PD group was significantly higher (p=0.0003) for both (congruent: p=0.006) and (incongruent: p=0.0001) trials than HCs, indicating reduced efficiency in the suppression of competing responses (Table 2).

	PD n=18		**HC n=9**	
	congruent	**incongruent**	**congruent**	**incongruent**
	Means (SD)	Means (SD)	Means (SD)	Means (SD)
Reaction time msec.	459.78 (69.79)	484.06 (55.51)	411.22 (52.00)	431.33 (43.47)
Percent errors	9.00 (3.81)	37.61 (19.12)	3.33 (2.40)	11.00 (7.42)

Table 2: The results of the reaction times and percent of errors for the congruent and incongruent trials for the Parkinson's. disease (PD) patients and healthy control (HCs) groups. The values in the table are means with standard deviations in parenthesis.

Electrophysiology:

N1. The only significant difference for the N1 component was found between the two groups of patients ($p<0.01$), but not between conditions. Higher mean negative amplitudes for rHuEPO group (-4.72 μV) relative to the non rHuEPO patients (-1.2 μV) was located in occipito-parietal electrodes ($p<0.01$).

N2. The N2 component only statistically differentiated between HC and all patients ($p<0.001$) with higher mean negative amplitude for controls (-2.45 μV) than PD patients (-0.72 μV) in the Cz location.

P3. The P3 component did not show any statistical differences for group or condition ($p>.05$).

Source analysis of the ERP differences between PD patients with and without rHuEPO:

The comparison between patients showed that PD group with rHuEPO showed a larger N1 component at the lingual gyri than the other patient group ($p< 0.05$). The N2 did not show any significant differences. See figure 1.

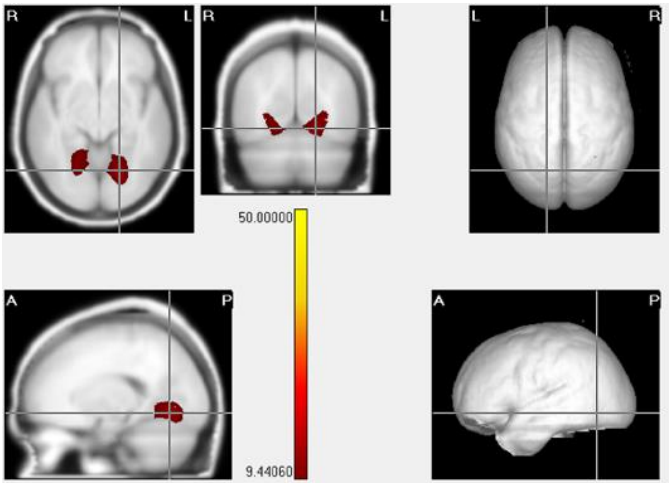

Figure 1: The Lingual Gyri are the sources of the N1 component according to AAL coordinates (X=92, Y=76, Z=172). The scale of statistical significance is self generated using the real values of the original data. All the voxels plotted were significants at p< 0.05).

Source analysis of the ERP differences between all PD patients and HCs:

HCs showed a higher activation in N2 component at the sources at the middle cingulum and precuneus bilaterally compared with PD patients. See Figure 2.

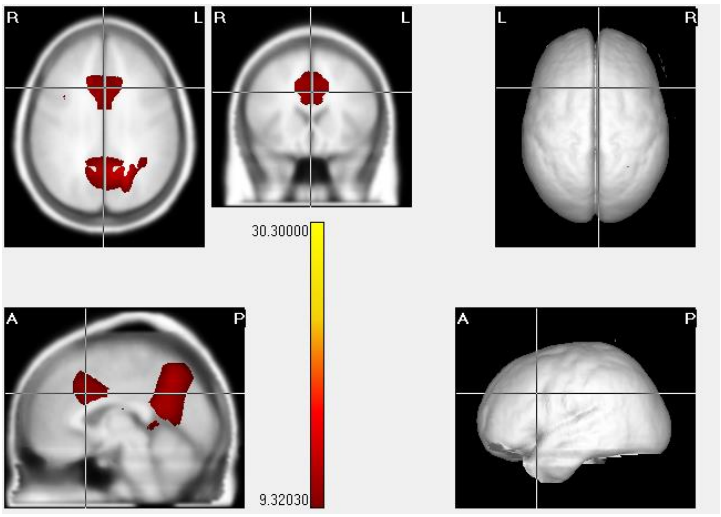

Figure 2: The N2 component showed maximal activation at middle cingulum and precuneus bilaterally (left located at X=92, Y=108, Z=156). To the right the localization of the precuneus left. The bicolor scale is showing all the signifcant values after Bonferroni correction and using permutations.

Discussion

Behavioural results

PD patients showed significantly increased reaction times and a higher number of errors to the incongruent stimuli during the performance of the flanker task in comparison to age and education matched HCs. These higher error rates in PD than controls are consistent with the proposal that the basal ganglia together with the anterior cingulate [26] participate in the monitoring of incongruence and error monitoring [27,28] which may be impaired in PD due to the dopamine deficiency (for a recent revision how the progressive dopamine deficiency reduces striatal cholinergic interneuron activity see [29]). But we did not find the expected beneficial effect of rHuEPO on the behavioural performance (RT and accuracy) in PD patients who received the drug in comparison with the others. Nonetheless, the differences

between groups of patients were found in the electrophysiological results. For example in the N1 component.
This component reflects selective attention, linked to the basic characteristics of a stimulus, and also to the recognition of a specific visual pattern [30]. In terms of spatial localization, the N1 amplitude is greater in occipital regions as well as in discrimination tasks [30,31]. On the other hand, Bokura et al (2001) using LORETA identified additional sources of the visual N1 in the inferior temporal lobe [32]. We localized the generators of N1, also using LORETA, in the lingual gyrus of the occipital lobe of both hemispheres, with the PD patients who received rHuEPO having larger amplitudes than the PD group who did not. This is suggestive of a probable neuroprotective effect of rHuEPO on the lingual gyrus, a region associated with the early processing of visual stimuli.

The second component N2 has been found in several studies of incongruence using the Flanker task and its latency was unaltered in medicated PD patients (for a review see [33]). In our study, we did not find any differences between experimental conditions or in the N2 latency, but the HCs had significantly higher N2 amplitudes than PD patients. The neural generators of this difference was localised to the posteromedial portion of the parietal lobe, the precuneus, a structure involved in the processing of perceptual ambiguities of stimuli [34]. In PD, relative to HCs Van Eimeren found dysfunction of the default mode network and particularly deactivation of the posterior cingulate cortex and the precuneus[35]. These changes in PD may be closely related to higher errors in executive tasks in PD compared with healthy controls. The other region showing significant N2 differences between PD and HCs was the middle cingulate cortex, probably related to monitoring of conflict in the Flanker task [36].

Contrary to our expectation, rHuEPO was not associated with a significant improvement in behavioural performance and did not influence the neural generators of the N2. Since this study was completed as part of a safety trial, the doses employed were small, nevertheless, the early N1 at the lingual gyrus could be reflect the differential effects of rHUEPO. This incremental amplitude, reflecting more allocation of neural resources, could possibly be related to the neuroprotector effect of rHuEPO on central cholinergic neurons, which have been demonstrated in vitro and in vivo studies [13].

Limitations of this study are the small sample size and the inverse solution restricted to cortical structures. Thus, the results require confirmation with larger samples in future studies. However, the results highlighted the role of EEG source analysis and advantages of electrophysiology with its high temporal resolution and insensitivity to placebo effects, in identifying brain changes after an intervention such as rHuEPO.

Conclusions

-Electrophysiology could be a tool able for identifying potential effects of neuroprotective compounds.
-rHuEPO did not improve behavioural performance but had an effect on the N1 component at the lingual gyrus.
- Relative to HCs, PD patients had slower RTs and more errors on the incongruent trials, and N2 sources in the middle cingulate and precuneus bilaterally differentiated patients and controls.

Data Availability

The tables with the behavioural performance of the samples was submitted in the supplementary material 1, described below. The raw EEG recordings in BrainVision format and the latencies for the N1, N2 and P3 components are in text files, stored in a local server in Cuba, but can be available under request to the corresponding author.
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Conflicts of Interest

The authors declare that there is no conflict of interest regarding the publication of this paper.

Funding Statement

This paper received support from the NSFC (China-Cuba-Canada) project (No. 81861128001) and the funds from National Nature and Science Foundation of China (NSFC) with funding No. 61871105, 61673090, and 81330032, and CNS Program of UESTC (No. Y0301902610100201).

Acknowledgments

The authors would like to thank to the Centro de Neurociencias de Cuba, specially to Valia Rodriguez and Indira Alvarez for the support during the recordings of the ERPs and the Centro Internacional de Restauracion Neurologica for the recruitment and neuropsychological evaluation of the patients. We are in debt with all the PD patients and their caretakers who volunteer to participate in our study.

Supplementary Materials

The supplementary material 1 consisted in one excel table with the behavioural performance of the subjects during the Flanker task. Tab “Answers”: the hits, errors and non-answers in the congruent and incongruent condition. Tab “Reaction Time”: the mean and standard deviation (SD) of the hits, errors of each subject in each group for congruent and incongruent trials.

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