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The restoring of interhemispheric brain connectivity following carotid endarterectomy: an exploratory observational study.

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List of abbreviations

- AAL = automated anatomical labelling
- ALFF = amplitude of low frequency fluctuations
- BOLD = blood oxygen level dependent
- CAS = carotid stenting
- CEA = carotid endarterectomy
- CSF = cerebrospinal fluid
- DWI = diffusion weighted imaging
- EEG = Electroencephalografy
- eICA = extracranial internal carotid artery stenosis
- EPI = echo-planar imaging
- ESC = European Society of Cardiology
- GLM = general linear model
- GM = Grey matter
- HRF = Hemodynamic Response Function
- HS = healthy subjects
- ICA = internal carotid artery
- IMT = intima-media thickness
- MMSE = mini-mental state examination
- MNI = Montreal Neurological Institute
- MRI = magnetic resonance imaging
- p-FDR = p-value corrected for false discovery rate
- p-unc = uncorrected p-value
- PG = Patient group
- ROI-to-ROI = Region of Interest to Region of Interest
- rs-fMRI = resting-state functional magnetic resonance imaging

- rs-fc MRI – resting-state functional connectivity MRI
- T1-W = T1 weighted
- T2-W = T2 weighted
- TFE = turbo Field Echo
- TIA = transient ischemic attack
- US = Ultrasound
- WM = White matter

Abstract

Purpose: To evaluate the differences in terms of brain connectivity between healthy subjects (HS) and patients with extracranial internal carotid artery (eICA) stenosis before and after carotid endarterectomy (CEA).

Methods: An exploratory observatory prospective study was designed. The study population consisted of a patient group (PG) of 20 patients with eICA stenosis eligible for CEA, and a control group (CG) of 20 HS, matched for age and sex, were recruited. The subjects of the PG group performed a Magnetic Resonance Imaging (MRI) for resting-state functional connectivity MRI (rs-fc MRI) analysis one week within the surgical procedure of CEA (pre-CEA) and 12 months following CEA procedure (post-CEA). The CG performed a single MRI for rs-fc MRI evaluation with the same MRI protocol adopted for the PG. The following three region-of-interest to region-of-interest (ROI-to-ROI) rs-fc MRI analyses were conducted: analysis 1 to compare pre-CEA PG and CG; analysis 2 to compare pre-CEA PG and post-CEA PG; analysis 3 to compare post-CEA PG and CG. The Functional Network Connectivity multivariate parametric technique was used for statistical analysis, adopting a p-uncorrected (p-unc) < 0.05 as connection threshold, and a cluster level False Discovery Rate corrected p (p-FDR) < 0.05 as cluster threshold.

Results: Analysis 1 revealed two clusters of reduced interhemispheric brain connectivity of pre-CEA PG when compared to CG. Analysis 2 and 3 showed no statistically significant results.

Conclusion: Patients with eICA stenosis show a reduced interhemispheric connectivity when compared to HC, and this condition tend to restore 12 months after CEA.

Keywords
Carotid endarterectomy; resting-state functional connectivity; ROI-to-ROI.

Introduction

Extracranial internal carotid artery stenosis (eICA) due to the presence of an atherosclerotic plaque is a common clinical condition associated with increasing risk of transient ischemic attack (TIA) and ischemic stroke [Howard et al., 2021]. Several findings from literature suggest also that eICA stenosis is associated with deficits in neurocognitive function, which in turn can be at least partly caused by the associated impairments in cerebral blood perfusion [Porcu et al., 2020]. Carotid endarterectomy (CEA) and carotid artery stenting (CAS) are revascularization techniques used as therapeutic approach in combination with best medical treatment for the treatment of asymptomatic patients with 70-99% eICA stenosis and symptomatic patients with 50-99% eICA stenosis [Aboyans et al., 2017; Bonati et al., 2021].

There is growing evidence in literature that CEA and CAS are useful not only for the prevention of stroke, but also in improving neurocognitive performances in patients with eICA stenosis eligible for this procedure [Piegza et al., 2021]. For example, the studies by *Carta MG et al.* [Carta et al., 2015] and by *Whooley JL et al.* [Whooley et al., 2020] evidenced how CEA procedure was associated with improvements in neurocognitive performances (especially executive functioning) evaluated with specific neurocognitive tests. According to these evidences, in the last years, few study started to investigate the effects of carotid revascularization on brain activity by using resting-state functional Magnetic Resonance Imaging (rs-fMRI) [Wang et al., 2017; Porcu et al., 2019a; Porcu et al., 2021a; Chinda et al., 2021], demonstrating a rearrangement of brain networking and brain activity following revascularization procedure in patients with eICA stenosis eligible for CEA or CAS. In particular, recent research by Porcu et al. [Porcu et al., 2021a] showed that the regional neural activity of several cerebral areas tends to increase in patients with unilateral eICA stenosis eligible for CEA 12 months after the surgical procedure. Further, a recent electroencephalography (EEG) connectivity study by *Quandt et al.* [Quandt et al., 2019], analysed the oscillatory connectivity changes in 14 patients with unilateral eICA stenosis eligible for revascularization procedure, identifying oscillatory connectivity changes in patients with asymptomatic carotid stenosis correlating with regional hypoperfusion. In particular, the authors observed that in a period between 6-10 weeks following revascularization procedure, the cognitive performances tend to improve, and the EEG connectivity pattern tend to normalize by getting similar to that of healthy subjects (HS) [Quandt et al., 2019].

According to these findings, we hypothesized that this "normalization" or "restoring" process of brain connectivity pattern towards the healthy condition can be observed on Magnetic Resonance Imaging (MRI) as well, more specifically on resting-state functional connectivity Magnetic Resonance Imaging (rs-fc MRI) with the Region of Interest to Region of Interest (ROI-to-ROI) technique [Lv et al., 2018]. In order to verify this hypothesis, we performed an exploratory prospective study that analysed rs-fMRI data and cognitive performances of a population of patients that suffered of asymptomatic unilateral eICA stenosis, and we compared them with the same data of a healthy control population matched for age and sex. Our study analysed the data acquired in a previous study by *Porcu et al.* [Porcu et al., 2021a], integrated with the cognitive and rs-fMRI data of a control population of HS matched for age and sex.

Materials and methods

Study population

The ethical committee gave his approval to this study, in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

The study population consisted of two distinct groups: a patient group (PG) and a control group (CG) of voluntary HS, matched for age and sex.

The PG was the same of the previous study by *Porcu et al.* [Porcu et al., 2021a] where consecutive patients with asymptomatic monolateral eICA stenosis eligible for CEA according to the 2017 ESC guidelines [Aboyans et al., 2017] were recruited at the University Hospital of Cagliari (Italy) in the period between January 2017 and December 2019. Patients who met at least one of the following exclusion criteria were excluded from the recruitment: 1) not right-handed dominant patients; 2) smoker patients; 3) patients with medical history of severe systemic inherited or acquired disease (in particular symptomatic patients with eICA stenosis, i.e. patients with clinical history of amaurosis fugax, TIA and major stroke ipsilateral to the lesion), except cognitive dysfunction; 4) contraindications for MRI examination 5) presence of functional disability (values ≥ 2 according to modified Rankin scale [van Swieten et al., 1988]); 6) patients with significant incidental organic pathology detected during the execution of the MRI scan.

In the period between June 2017 and December 2018, at the same institution, a CG of voluntary HS, matched for age and sex to the subjects of the patient group [Raina, 2015], was recruited. Prior to the study inclusion, all the HS performed a screening carotid ultrasound (US) in order to evaluate the status of the ICA arteries. HC with mono or bilateral carotid artery plaques visualized at US, as well as those with an intima-media thickness (IMT) > 0.9 mm [Mancia et al., 2013] were excluded from the recruitment. In the same day, all the HS performed the Italian version of the Mini-Mental State Examination (MMSE) corrected for age and schooling [Folstein et al., 1975; Magni et al., 1996] in order to evaluate the cognitive integrity, and all the HS with a MMSE score < 30 were excluded from the inclusion. Further, as well as for the patients population, the HS that met at least one of the following exclusion criteria were excluded from the study: 1) not right-handed dominant patients; 2) smoker patients; 3) patients with medical history of systemic inherited or acquired disease (in particular patients with known diagnosis of severe neurological or psychiatric illness); 4) contraindications for MRI examination; 5) patients with significant incidental organic pathology detected during the execution of the MRI scan.

All the patients and the voluntary HS gave their written informed consent before enrollment.

MRI examination

As indicated in the previous paper by *Porcu et al.* [Porcu et al., 2021a], all the subjects of the patient population performed a Magnetic Resonance Imaging (MRI) examination for analyzing the rs-fcMRI one week before the CEA procedure (pre-CEA) and twelve months after the CEA procedure (post-CEA). The subjects of the control group underwent an MRI examination with the same protocol used for the patient group; the MRI was performed on the same day of the recruitment after the US and the MMSE examination.

In analogy to previous studies [Porcu et al., 2019a; Porcu et al., 2020b] , the MRI scan was made with a 1.5 Tesla Philips "Achieva dStream" scanner (Philips, Best, Netherland), with a 16 channels head coil. The MRI scan protocol included the following two sequences: a) structural isotropic 3D T1-weighted Turbo Field Echo (TFE) sequence (TR= 7.5 ms, TE = 3.43 ms, flip angle = 8°, slice thickness = 1 mm, spacing between slices = 1 mm); and b) resting state functional T2 weighted Echo-Planar Imaging (EPI) sequence (TE = 50 ms; TR = 3000 ms; flip angle = 90°; slice thickness = 5 mm; matrix: 80 x 80; volumes acquired: 326). Prior the examination, all the subjects were carefully instructed by the radiologist to keep the eyes closed without thinking of anything while in a fully relaxed state during the execution of the functional T2 weighted EPI sequence.

Rs-fc MRI analysis

As indicated in the previous paper by *Porcu et al.* [Porcu et al., 2021a], the rs-fc MRI analysis was performed on the Matlab platform vR2020b (Mathworks, Inc., California, USA) with the CONN-fMRI fc toolbox v20b [Whitfield-Gabrieli & Nieto-Castanon, 2012] based on the SPM 12 package (Wellcome Department of Imaging Neuroscience, London, UK; http://www.fil.ion.ucl.ac.uk/spm/).

The pre-CEA and post-CEA MRI of the patient group (pre-CEA PG MRI and post-CEA PG MRI, respectively), as well as the MRI of the HS of the control group (CG MRI), were processed by following the same pipeline. Similarly to previous studies [Porcu et al., 2019a; Porcu et al., 2020b], structural 3D T1-weighted (T1-W) TFE and functional T2-weighted (T2-W) EPI sequences were pre-processed according to the CONN's default pipeline for volume-based analysis with the following consecutive steps: 1) functional realignment and unwarping; 2) slice-timing correction; 3) functional outlier detection with intermediate settings (97th percentile in normative sample in functional outlier detection system: global-signal z-value threshold = 5 standard deviations; subject-motion threshold = 0.9 mm); 4) functional and structural direct segmentation of grey matter (GM), white matter (WM) and cerebrospinal fluid (CSF), and subsequent normalization to Montreal Neurological Institute (MNI) [Collins et al, 1994] exploiting the default tissue probability maps (structural target resolution = 1 mm; functionals target resolution = 2 mm); 5) functional smoothing with 8 mm full width half maximum Gaussian kernel filter.

For what concern the data processing, in analogy to *Chiacchierata et al.* [Chiacchierata et al., 2015], the first 5 volumes of T2-W EPI sequence were excluded from analysis for limiting the potential bias derived by the attainment of the steady state magnetization. Subsequently, the following denoising procedure was applied in order to minimize the residual non-neural variability of functional data: 1) linear regression of potential confounding effects, including BOLD signals of the CSF and WM [Porcu et al., 2019a; Porcu et al., 2020b; Porcu et al., 2020c], estimated subject-motion specifications and identified outlier scans for the "scrubbing" procedure [Powder et al., 2014]; and 2) temporal band-pass filtering (0.008 to 0.09 Hz) in order to decrease noise effects and low frequency drift [Porcu et al., 2019a; Porcu et al., 2020b].

ROI-to-ROI connectivity maps were generated by computing for each individual voxel the the root mean square of BOLD signal in the low frequency range (0.008 - 0.09 Hz) [Porcu et al., 2019a; Nieto-Castanon, 2020]. Individual correlation maps of the whole brain were made using the mean resting-state BOLD time course of each ROI, followed by the computation of the correlation coefficients of the BOLD time-course among predefined ROI [Whitfield-Gabrieli & Nieto-Castanon, 2012; Porcu et al., 2019a; Porcu et al., 2020b; Porcu et al., 2021b]. The CONN's default atlas was used for the "a priori" identification of the ROI; in particular, the Harvard-Oxford atlas [Desikan et al., 2006] was adopted for ROI of the cortical and subcortical regions, and the Automated Anatomical Labelling (AAL) atlas [Tzourio-Mazoyer et al., 2002] for the ROI of the cerebellar regions (Supplementary table 1).

The General Linear Model (GLM) was used in the second level group analysis for identifying statistically significant changes of BOLD signal between pre-CEA and post-CEA scans in the PG, between pre-CEA and HS, and between post-CEA and HS. The GLM was exploited by applying a bivariate correlation analysis weighted for Hemodynamic Response Function (HRF) [Whitfield-Gabrieli & Nieto-Castanon, 2012; Porcu et al., 2019a; Porcu et al., 2020b; Porcu et al., 2021b]: higher Z-scores indicated positive correlations as a reflection of the increased synchronicity between ROIs, and vice-versa [Whitfield-Gabrieli & Nieto-Castanon, 2012; Porcu et al., 2019a; Porcu et al., 2020b; Porcu et al., 2021b]. Fisher's transformation was applied to all Z-scores, and the correlation coefficients were converted into standard scores [Whitfield-Gabrieli & Nieto-Castanon, 2012; Porcu et al., 2019a; Porcu et al., 2020b; Porcu et al., 2021b]. The following three comparative analyses were made in order to evidence statistically significant differences in ROI-to-ROI connectivity:

- Analysis 1 (pre-CEA PG - CG): comparison between pre-CEA PG MRI and CG MRI connectivity patterns, by exploiting a two-samples T-test.
- Analysis 2 (pre-CEA PG - post-CEA PG): comparison between pre-CEA PG MRI and post-CEA PG MRI connectivity patterns, by exploiting a paired T-test.
- Analysis 3 (post-CEA – CG): comparison between post-CEA PG MRI and CG MRI connectivity patterns, by exploiting a two-samples T-test.

For every one of the above mentioned analyses we used the Functional Network Connectivity (FNC) multivariate parametric statistics, adopting a p-uncorrected (p-unc) < 0.05 as connection threshold, and a cluster level False Discovery Rate corrected p (p-FDR) < 0.05 as cluster threshold [Jafri et al., 2008; Nieto-Castanon, 2020].

Results

Study population

The final study population consisted of 40 subjects, 28 females and 12 females. The PG, which is the same of the previous study by *Porcu et al.* [Porcu et al., 2021] consisted of 20 asymptomatic subjects, 14 males and 6 females (overall mean age = 75.09; mean age female group = 73.33; mean age male group = 74.45). CEA was performed in the right side in 11 patients, and in the left side in 9 patients. No procedural or peri-procedural complications were observed following CEA, and the clinical course between the baseline and the follow-up assessment was uneventful. The CG consisted of 20 subjects, matched for age and sex (gender composition: 14 males and 6 females; overall mean age = 75.09; mean age female group = 73.33; mean age male group = 74.45). No subjects of the whole study population were excluded according to the above mentioned exclusion criteria; in particular, no incidental pathological findings were found following MRI scan were detected. The resume demographic data are reported in Table 1.

Rs-fc MRI analysis

The quality control data of the study population following rs-fMRI preprocessing are reported in Supplementary table 2 and Supplementary table 3.

Of the three analyses conducted, only Analysis 1 (comparison between pre-CEA PG and CG) revealed statistically significant results, whereas no statistically significant findings were found for Analysis 2 (comparison between pre-CEA PG and post-CEA PG) and 3 (comparison between post-CEA PG and CG).

Analysis 1 evidenced the presence of two clusters (Cluster 1 and 2) of statistically significant reduced connectivity in pre-CEA PG patients when compared to the CG (Figure 1). Cluster 1 (F-value = 13.28; p-unc = 0.000042; p-FDR = 0.006494) consisted of 18 connections (T-score range: between -4.95 and -2.13; p-unc range: between 0.000015 and 0.039706; p-FDR range: between 0.006494 and 0.696324) between several frontal and temporal areas of the opposite sides of the brain (Table 2). Cluster 2 (F-value = 9.16; p-unc = 0.000056; p-FDR = 0.007273) consisted of 49 connections (T-score range: between -4.52 and -2.25; p-unc range: between 0.000056 and

0.029891; p-FDR range: between 0.007273 and 0.48946) between several temporal and parietal areas of the opposite sides of the brain (Table 3).

The connectograms of the three analyses are reported in Figure 1, and a three-dimensional representation of Analysis 1 is reported in Figure 2.

Discussion

Even if there is ongoing debate in literature, several evidences from literature have demonstrated that eICA stenosis appears to be an independent risk factor for cognitive decline [Piegza et al., 2021]. The cognitive decline observed in these patients could at least partly attributed by to the dysfunction of brain networking following a complex series of vascular rearrangements and structural changes of the brain [Porcu et al., 2020a]. Even if there is still debate about the impact of CEA and CAS on cognitive spheres, the evidences in literature tend to confirm that revascularization procedures improve neurocognitive performances in patients with eICA stenosis eligible for CAS and CEA [Piegza et al., 2021].

Both rs-fMRI and rs-EEG allow to study the brain networking, even if with significant differences in terms of temporal and spatial resolution [Deligianni et al., 2014]; more in details, the rs-fMRI technique measures the changes in allows to study the low-frequency fluctuations of blood oxygen level dependent (BOLD) signal of the brain, with a high spatial resolution due to the intrinsic imaging nature of the method, whereas the EEG has a higher temporal resolution due to its intrinsic ability to measure the spontaneous high-frequency electrical activity of the brain, but suffer of a lower spatial resolution due to the absence of a imaging brain map [Deligianni et al., 2014].

Starting from the above mentioned evidences, in this exploratory observational study we speculated that CEA procedure in patients with unilateral eICA stenosis eligible for carotid revascularization procedure induces a series of brain networking rearrangements measurable on rs-fMRI that tend to "restore" the brain activity and to normalize it when compared to the brain activity of healthy subjects, similarly to what observed in a recent rs-EEG study by *Quandt et al.*

[Quandt et al., 2019]. even if with some differences. In particular, the study by *Quandt et al.* [Quandt et al., 2019] evaluated a different population (12 patients and 23 HC); further, 9 patients out of 12 performed CEA and the other 3 underwent CAS, and the post-revascularization EEG evaluation was conducted on a different follow-up timing (6-10 weeks).

For doing this, we recruited a study population of 40 subjects, divided in two groups: a PG of 20 subjects (analyzed on rs-fMRI before and 12 months after the CEA procedure), and a CG of 20 HS, matched for age and sex. The patients and the rs-fMRI data of the PG were the same of a previous paper by *Porcu et al.* [Porcu et al., 2021a]; in that paper, the authors analyzed the changes in brain neural activity on rs-fMRI with the Amplitude of Low Frequency Fluctuations (ALFF) technique, evidencing not only a statistically significant improvement of the cognitive scores measured with the italian version of the MMSE corrected for and schooling, but also a general increase of ALFF signal in several supratentorial regions of the brain, included the right precentral gyrus, the right middle frontal gyrus and the anterior division of the cingulate gyrus.

In our research we conducted three different rs-fc MRI analyses: analysis 1, for evaluating differences in connectivity patterns between pre-CEA PG and HC, analysis 2 for comparing pre-CEA and post-CEA connectivity patterns changes in the PG, and analysis 3 for comparing differences in brain connectivity between post-CEA PG and HC. Analysis 1 revealed reduced connectivity between several supratentorial areas of the brain: looking more deeply at the results, it is possible to observe a reduced interhemispheric connectivity between the frontal, temporal and parietal areas, whereas no statistically significant differences were found in Analysis 2 and 3.

By looking globally at the results of our findings, we speculate that the cognitive impairments observed in patients with eICA stenosis when compared to CG can be at least partly due to a sensitive reduction in interhemispheric connectivity in areas of the brain vascularized by intracranial branches of the ICA; from an anatomical point of view, the blood supply of the frontal, temporal and parietal areas is guaranteed by the middle and anterior cerebral arteries, which originates inside of the cranial cavity from the ICA [Agarwal & Carare, 2021]. Following the revascularization procedure, the cognitive performances improve and the interhemispheric connectivity tend to restore to physiological conditions, as evidenced by the fact that no statistical significant differences in brain connectivity were found between PG and HC in Analysis 3. Then, it is reasonable to assume that CEA determines a significant improvement in neurocognitive performances associated with a reassessment of brain networks that lastly bring to a "normalization" or "restoring" of the normal brain connectivity. Some proofs in this sense can be found in literature.

The first proof derives from the previous study by *Porcu et al.* [Porcu et al., 2021b] in which it was already observed a statistically significant improvement of the neurocognitive performances and increase of regional neural activity of several areas of the brain following CEA. Surprisingly, in our research, analysis 2 revealed no statistically significant changes in brain connectivity between pre-CEA and post-CEA. This fact could appear controversial, especially by looking at the results of a previous similar exploratory research by *Porcu et al.* [Porcu et al., 2019a], that analyzed 14 patients with asymptomatic eICA stenosis eligible for CEA before and 3-6 months following CEA, observing a reorganization of brain networking characterized by an increased connectivity throughout the whole brain. The relative low number of patients enrolled in the PG could have masked the presence of reorganization patterns of cerebral networks, and it is important to take into account the fact that in our study the follow-up timing was different. We speculate that the brain networking reorganization observed after CEA is a dynamic process, that results more pronounced in the short term period and tends to assuage in the mid- and long-term period; however, this theory should be verified in future longitudinal studies.

A similar reasoning can be applied to the results of Analysis 3 (comparison between post-CEA PG and CG), according to which, despite no statistically significant patterns of altered connectivity were found, it is reasonable to speculate that the small study population could mask some brain networking differences.

The second proof is given by the fact that other techniques for the analysis of brain networking different from rs-fMRI, evidenced similar pattern of increased interhemispheric activity following carotid revascularization procedure in asymptomatic patients with eICA stenosis eligible for revascularization procedure. For example, we already mentioned the rs-EEG study by *Quandt et al.* [Quandt et al., 2019] that observed a short-term significant improvement of the interhemispheric connectivity following revascularization procedure (CEA or CAS), whereas another study by *Porcu et al.* [Porcu et al., 2019b] observed a short-term (3-6 months) improvement of cognitive performances and an increased interhemispheric local connectivity in the corpus callosum and in both the cerebellar hemispheres following CEA, analyzed on Diffusion Weighted Imaging (DWI) with the connectometry technique [Yeh et al., 2016].

We identified several limits in our study. The first one is the relatively small population study, even if the study was designed as explorative and not confirmative. The second one is related to the fact that the cognitive assessment was assessed in the PG only with the Italian version of the MMSE corrected for age and schooling, as reported in the previous study by *Porcu et al.* [Porcu et al., 2021b]; a more in-depth cognitive evaluation would result necessary in future studies in order to better understand the relationships between the effects of CEA on brain networking and cognitive sphere, as well as other neurological aspects such as mood and motor function. The third one relies on the great variability of the rs-fMRI analysis methods that represent an intrinsic limitation of this analysis technique and can reveal different results according to the different analysis method used [Botvinik et al., 2020]; however, in this case we adopted an analysis method already extensively used in literature, and we underlined that our results are not in contradiction with the results obtained with other techniques for the analysis of brain networking different from rs-fMRI. The last one is related to the fact that the side of the intervention, as well as other potential confounding factors such as the white matter status or the configuration of the circle of Willis, have not been taking into account in the analysis; however, these factors could represent interesting targets to be analyzed in future studies for better understanding the processes of brain networking reorganization following carotid revascularization.

Conclusions

This exploratory case-control observational study we analyzed the effects of CEA on brain networking oof patients with unilateral eICA stenosis by rs-fc MRI with a ROI-to-ROI approach. The results of our study evidence that patients with eICA stenosis show a reduced supratentorial interhemispheric connectivity when compared to HC, and that this condition tend to restore to the healthy condition 12 months after CEA. The results of this research could be a useful starting point for investigating the brain networking alterations underlying the cognitive impairment observed in patients with eICA stenosis and the changes following revascularization procedure.

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Author contributions

Conceptualization, M.P., L.C.; Methodology M.P., L.C.; Validation M.P., L.C., L.S.; Data Curation M.P., R.C.; Investigation M.P.; Writing – Original Draft, M.P., L.C.; Writing – Review and Editing, L.S., J.S.S., G.L., M.W., J.P., M.C.; Supervision, L.S., M.C., M.W.

Declaration of Interests

The authors declare no conflict of interests.

Ethical approval

The ethical committee approved the study in accordance with the ethical standards as laid down in the 1964 Declaration of Helsinki and its later amendments or comparable ethical standards.

Figure legends

Figure 1: Connectograms of the ROI-to-ROI analyses. The connectogram of Analysis 1 (pre-CEA PG - CG) is reported on the left side, and it shows how the interhemispheric connectivity result extensively reduced in the pre-CEA PG when compared to the CG; in particular, the connectogram shows two clusters of reduced interhemispheric connectivity between several supratentorial areas of the frontal, parietal and frontal lobes; the color of the connections reflect the T-values according to the color bar reported inside the connectogram. The connectogram of Analysis 2 (pre-CEA PG – post-CEA) and 3 (post-CEA PG - CG), that showed no statistically significant differences in brain connectivity, are reported on the right side. The complete results of analysis 1 is reported in table 2 and 3.

Figure 2: Three-dimensional reconstruction of Analysis 1; it is clearly possible to note the reduced interhemispheric connectivity observed in pre-CEA PG when compared to CG. The ROI description is reported in supplementary table 1, whereas the complete results are reported in table 2 and 3.

Table legends

Table 1: Population study.

Table 2: Results of Analysis 1 – Cluster 1. F = F-vale; T = T-score; p-unc = p-uncorrected (connection threshold); p-FDR = cluster level False Discovery Rate corrected p (cluster threshold).

Table 3: Results of Analysis 1 – Cluster 2. F = F-vale; T = T-score; p-unc = p-uncorrected (connection threshold); p-FDR = cluster level False Discovery Rate corrected p (cluster threshold).

Supplementary tables legends

Supplementary table 1: Atlas – ROIs legends [Desikan et al., 2006; Tzourio-Mazoyer et al., 2002]

Supplementary table 2: Quality control data of the patient group following fMRI preprocessing. BOLD: Blood oxygen level depended; std: standard deviations.

Supplementary table 3: Quality control data of the control group following fMRI preprocessing. BOLD: Blood oxygen level depended; std: standard deviations

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