

The Functional Connectome of Cognitive Reserve

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Abstract: Cognitive Reserve (CR) designates the brain's capacity to actively cope with insults through a more efficient use of its resources/networks. It was proposed in order to explain the discrepancies between the observed cognitive ability and the expected capacity for an individual. Typical proxies of CR include education and Intelligence Quotient but none totally account for the variability of CR and no study has shown if the brain's greater efficiency associated with CR can be measured. We used a validated model to estimate CR from the residual variance in memory and general executive functioning, accounting for both brain anatomical (i.e., gray matter and white matter signal abnormalities volume) and demographic variables (i.e., years of formal education and sex). Functional connectivity (FC) networks and topological properties were explored for associations with CR. Demographic characteristics, mainly accounted by years of formal education, were associated with higher FC, clustering, local efficiency and strength in parietal and occipital regions and greater network transitivity. Higher CR was associated with a greater FC, local efficiency and clustering of occipital regions, strength and centrality of the inferior temporal gyrus and higher global efficiency. Altogether, these findings suggest that education may facilitate the brain's ability to form segregated functional groups, reinforcing the view that higher education level triggers more specialized use of neural processing. Additionally, this study demonstrated for the first time that CR is associated with more efficient processing of information in the human brain and reinforces the existence of a fine balance between segregation and integration. *Hum Brain Mapp* 37:3310–3322, 2016. © 2016 The Authors Human Brain Mapping Published by Wiley Periodicals, Inc.

Key words: cognitive reserve; connectome; brain reserve



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INTRODUCTION

The concept of “cognitive reserve” (CR) emerged to explain why some aged individuals displaying neuropathologic hallmarks of Alzheimer’s disease (AD) in post-mortem brain analysis, never presented any clinical manifestation of the disease in life [Katzman et al., 1988]. Given that these subjects presented larger brain volumes than the average, the associated notion of “brain reserve” (BR) arose [Katzman, 1993]; with brain size and neuronal numbers constituting standard proxies for BR [Katzman et al., 1988; Mortimer et al., 1982, 2003; Satz et al., 2011]. According to the model, brain size determines the amount of damage that can be sustained before it leads to some clinical expression. Specifically, it hypothesizes that bigger brains can sustain more damage, since enough neural substrate remains to support normal functioning. This is as a passive model of reserve, since there is a fixed threshold below which functional impairment will be observed [Stern, 2009b].

In contrast, several models have been proposed to explain how the brain can actively cope with brain damage through compensatory mechanisms [Barulli and Stern, 2013; Stern, 2002]. In this context, the CR model was proposed to account for the variability that cannot be explained by pathological indices. This model states that individuals with higher CR capacity make a more efficient or flexible use of the brain’s resources in order to perform better at a given task [Stern, 2002, 2009a]. The rationale for the CR model is that individuals with a richer environment in terms of cognitive activities/demands through the lifespan accumulate strategies that help them to overcome more easily the challenges of everyday life. CR has been postulated as the reason why higher levels of intelligence [Alexander et al., 1997], education [Stern et al., 1992], and occupational status [Richards and Sacker, 2003], make individuals more capable of sustaining greater brain damage without clinical manifestations. Two distinct mechanisms were proposed to explain the underlying features of CR: neural reserve and neural compensation [Stern et al., 2005]. Neural reserve is thought to be related to brain networks that are less susceptible to deterioration, due to their increased efficiency [Neubauer and Fink, 2009]. These networks may be recruited when individuals are faced with brain pathology. Conversely, the neural compensation mechanism relies on the hypothesis that individuals affected by some brain damage recruit networks not usually activated, as a means to compensate the impairments caused by damage.

Altogether, research provides support for both the abovementioned types of reserve. It was demonstrated that several anatomic measures such as brain volume, head circumference, synaptic count, and dendritic branching are susceptible to alterations during the lifetime. In fact, it is thought that the underlying mechanisms conducting to these changes are also involved in the development of CR [Stern, 2006]. Despite this, there is no direct

way to quantify CR and the most common proxies of CR present several limitations. In order to overcome this, a latent model was recently developed as a strategy to obtain a quantitative measure of CR that was closer to the definition of the discrepancy between observed and expected cognitive abilities [Reed et al., 2010]. Briefly, the authors isolated the variance of episodic memory explained by (1) demographic characteristics and (2) brain pathology, measured by structural MRI. This residual variance (i.e., the variance in episodic memory not explained by any of the abovementioned variables) was defined as CR. The authors observed that this measure correlated with longitudinal cognitive decline and with the degree of brain atrophy attenuation, such that individuals with low CR had an augmented cognitive decline as a result of brain atrophy. Since then, this model has been replicated and extended [Zahodne et al., 2013]. Despite these findings, few researchers have explored the brain networks associated with CR using neuroimaging techniques [Stern et al., 2005, 2008] and, none have explored such properties when individuals are at rest (i.e., independently of a task-performance).

Herein, we used the abovementioned latent model in order to isolate CR and then to assess the brain correlates of CR, by exploring its relationship with FC networks and network topological properties, using graph theory measures [Bullmore and Sporns, 2009]. With this strategy, we tested whether CR is associated with higher FC and/or higher global efficiency of neuronal networks.

METHODS

Ethics Statement

The present study was conducted in accordance with the principles expressed in the Declaration of Helsinki and was approved by the local and national ethics committees. The study goals and tests were explained to the participants and all gave informed written consent.

Participants

The participants included in the present study are part of the sample recruited for the SWITCHBOX Consortium project (www.switchbox-online.eu/). Details regarding participants’ selection, neurocognitive assessment and inclusion/exclusion criteria were previously described [Marques et al., 2015b]. Briefly the recruitment was performed in two-phases. In the first phase, a larger sample, representative of the general Portuguese older population in terms of age, gender, and education [$n = 1,051$, after inclusion/exclusion criteria; subjects were randomly selected from the Guimarães and Vizela local area health authority registries] underwent the neuropsychological assessment [Costa et al., 2013; Santos et al., 2013, 2014]. In the second recruitment phase, 120 subjects were selected

from the previous sample in order to provide cognitive profiles of overall good cognitive performance ($n = 60$) and overall poor performance ($n = 60$), based on the neuropsychological testing, and underwent the MRI scanning protocol [Marques et al., 2015b]. Primary exclusion criteria included inability to understand the informed consent, participant choice to withdraw from the study, incapacity and/or inability to attend the MRI session, dementia and/or diagnosed neuropsychiatric and/or neurodegenerative disorder (medical records). Adjusted thresholds for cognitive impairment were calculated depending on factors such as age and/or education [Busch and Chapin, 2008; Grigoletto et al., 1999]. Thus, the applied Mini Mental State Examination (MMSE) test score thresholds were the following: MMSE score < 17 if individual with ≤ 4 years of formal school education and/or ≥ 72 years of age, and MMSE score < 23 otherwise (follows the MMSE validation study for the Portuguese population) [Guerreiro et al., 1994].

From the 120 subjects originally recruited for the main project, nine refused to undergo the MRI acquisition protocol, four had brain lesions/pathology detected at the time of the acquisition and seven presented excessive motion/artifacts, leaving a final sample of 100 older adults that were considered in the present study.

Neuropsychological Assessment

A team of certified psychologists performed the neuropsychological assessments. The neuropsychological test battery included the following tests: Digit-span Forward (DB) and Backward (DB) test, Stroop Words (SW), Stroop Colors (SC), Stroop Words/Colors (SWC), Controlled Oral Word Association Test (COWAT-FAS; admissible words), Selective Reminding Test (SRT), Digit Symbol Substitution Test (DSST), Mini-Mental State examination (MMSE), Geriatric Depression Scale (GDS, long-version) [Santos et al. 2014].

A Principal Component Analysis (PCA) was performed using the sample of 1,051 participants in order to reduce the dimensionality of the data with the least possible loss of information. PCA resulted in the identification of two significant factors, "MEM" (memory) and "GENEXEC" (general and executive function), and this factor structure has been confirmed [Santos et al., 2015]. Briefly, the MEM factor was composed of the long-term storage (LTS), consistent long-term retrieval (CLTR) and delayed-recall (DR) variables evaluated with SRT; while, the GENEXEC factor was composed of the variables MMSE, DF, DB, SW, SC, SWC, COWAT-FAS admissible words. For further details regarding the exploratory and confirmatory factor analysis please consult [Santos et al., 2015].

Missing values in cognitive scores were imputed using multiple imputation (linear regression, 10 imputation datasets) as implemented in SPSS 23 using all the data points available in the dataset. The total amount of missing values

was 1.5% of the total of data points available. Four subjects were identified as multivariate outliers according to a 95% confidence interval ($P < 0.05$) criterion for the Mahalanobis distance, which corresponds to a threshold value of 3.84. Thus, 96 subjects composed the final sample for the Structural Equation Model (SEM) and resting-state fMRI analysis.

Magnetic Resonance Imaging Acquisition

The imaging session was performed at Hospital de Braga (Braga, Portugal) on a clinical approved Siemens Magnetom Avanto 1.5 T MRI scanner (Siemens Medical Solutions, Erlangen, Germany) and using a 12-channel receive-only head-coil. The imaging protocol included several different acquisitions. For the present study, only one structural and one rs-fMRI acquisition were considered. For the structural acquisition, a T1-weighted magnetization prepared rapid gradient echo (MPRAGE) sequence with the following parameters was used: 176 sagittal slices, TR/TE = 2,730/3.48 ms, FA = 7°, slice thickness = 1 mm, slice gap = 0 mm, voxel size = 1 × 1 mm², FoV = 256 mm. For the rs-fMRI acquisition, a blood oxygen level dependent (BOLD) sensitive echo-planar imaging (EPI) sequence was used: 30 axial slices, TR/TE = 2,000/30 ms, FA = 90°, slice thickness = 3.5, slice gap = 0.48 mm, voxel size = 3.5 × 3.5 mm², FoV = 1,344 mm and 180 volumes. During the resting state scan, the subjects were instructed to remain still, awake, with their eyes closed, as motionless as possible and trying to think of nothing in particular. After the scan, all participants confirmed that they had not fallen asleep.

Structural Segmentation

The structural scans of each subject were segmented with in FreeSurfer toolkit version 5.1 (<http://surfer.nmr.mgh.harvard.edu>), which implements a semi-automated segmentation workflow. FreeSurfer enables the segmentation of both GM and WM as well as subcortical segmentation. The stages of processing implemented in this pipeline are fully described elsewhere [Desikan et al., 2006; Destrieux et al., 2010; Fischl et al., 2002, 2004]. Validation against manual segmentations has also been described [Fischl et al., 2002] and its results are considered robust across sessions, scanner platforms, updates, and field strengths [Jovicich et al., 2009].

For the present study, only the intracranial volume (ICV), total GM volume (GMV) and white matter hypointensities volume (i.e., WMSA) were considered. This T1-based WMSA volume estimate has been successfully used as a measure of WM lesion volume [Salat et al., 2012], and showed sensitivity in measuring WM lesions in Alzheimer's disease [Salat et al., 2012], as well as to correlate with estimates based on FLAIR acquisitions and to

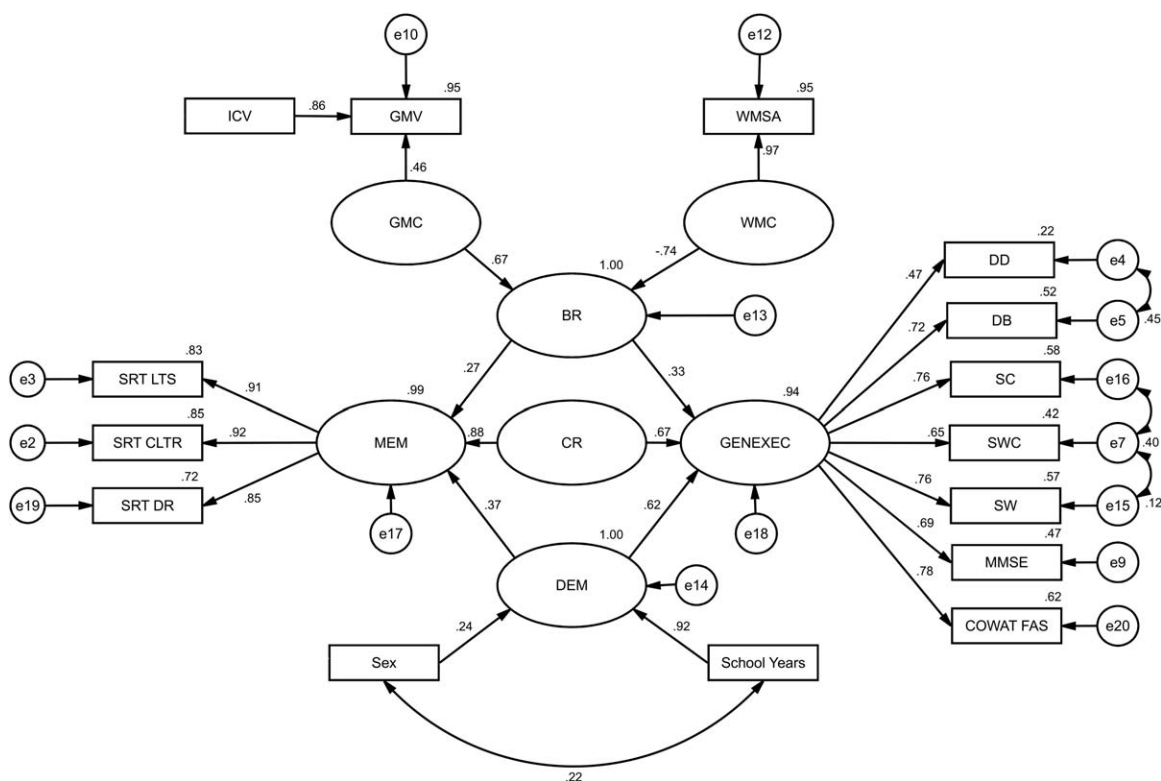


Figure 1.

Structural equation model used in order to decompose memory (MEM) and general executive functioning (GENEXEC) factors into independent variables of demographic characteristics influence (DEM), brain reserve capacity measures (BR) and cognitive reserve (CR). DEM was mainly accounted for school years ($r = 0.92$) but also by sex ($r = 0.24$). Sex and school years were also correlated ($r = 0.22$). Intracranial Volume (ICV) correlated strongly ($r = 0.86$) with gray matter volume (GMV). As expected, the latent gray matter component (GMC) was signifi-

cantly modeled by GMV ($r = 0.46$) and the latent white matter component (WMC) was almost entirely ($r = 0.97$) modeled by the volume of white matter signal abnormalities (WMSA). GMC positively accounted for BR ($r = 0.67$) and WMC negatively accounted for BR ($r = -0.74$) due to the inverse relationship between white matter lesioning and BR. CR was the greatest predictor of GENEXEC ($r = 0.67$), followed by DEM ($r = 0.62$) and then BR ($r = 0.33$). MEM was mostly predicted by CR ($r = 0.88$), then by DEM ($r = 0.37$) and BR ($r = 0.27$).

correlate better with clinical symptoms in MS [Bagnato et al., 2010].

Specification of the Structural Equation Model

A structural equation model was defined in order to estimate the variance of the performance in cognitive dimensions explained by CR. For that, three main latent variables were created: one expressing the variance of cognitive performance uniquely dependent on demographic characteristics (Demographic, DEM); one uniquely associated with structural MRI variables (Brain Reserve, BR); and, one accounting for the variance not attributed to the other two dimensions (Cognitive Reserve, CR). This model constitutes a replication of a previously validated and replicated model [Reed et al., 2010]. Nevertheless, some

modifications were applied to the model proposed by Reed and colleagues in order to address the goals of the current study and these differences will be detailed in the following paragraphs.

Similarly to the original model, structural MRI variables were regressed on the BR latent variable. Namely, GMV was regressed on ICV and a latent variable was specified in reflecting gray matter component (GMC), accounting for ICV. WMSA were included as an observed variable (log-transformed in order to reduce skewness) and was modeled through a latent variable (WMC – White Matter Component). GMC and WMC were finally regressed on BR. Differing from the model proposed by Reed and colleagues; hippocampal volume was not included as a measure of BR capacity. Indeed, in the present study we did not assess individuals with any diagnosed temporal dementias; furthermore, despite being relevant for

TABLE I. Demographic and cognitive characterization of the sample assessed in the study

| | Mean (SD) | Range | Skewness | Kurtosis |
|-----------------------------|---------------------------|----------------------------|--------------|--------------|
| <i>Demographic measures</i> | | | | |
| Gender (0=Male) | 0.48 (0.5) | [0; 1] | | |
| Age | 64.77 (8.11) | [51; 82] | 0.09 | -1.00 |
| School Years | 5.40 (3.80) | [0; 17] | 1.35 | 1.09 |
| <i>MRI measures</i> | | | | |
| WMSA (log) | 3.42 (0.29) | [2.98; 4.27] | 1.04 | 0.89 |
| GMV | 5.63 E + 05 (4.99 E + 04) | [4.52 E + 05; 6.88 E + 05] | 0.12 | -0.71 |
| ICV | 1.47 E + 06 (1.61 E + 05) | [1.13 E + 06; 1.90 E + 06] | 0.41 | -0.20 |
| <i>Cognitive Measures</i> | | | | |
| SRT LTS | 27.43 (13.18) | [4; 58] | -0.07 | -0.76 |
| SRT CLTR | 16.48 (12.27) | [0; 46] | 0.27 | -0.77 |
| SRT DR | 5.87 (2.66) | [0; 12] | -0.10 | -0.14 |
| DD | 7.63 (2.20) | [3; 14] | 0.54 | 0.03 |
| DB | 4.32 (2.45) | [0; 10] | 0.54 | -0.27 |
| SW | 63.75 (20.82) | [22; 103] | 0.40 | -0.42 |
| SC | 48.88 (14.80) | [18; 81] | 0.18 | -0.48 |
| SWC | 28.83 (12.02) | [5; 58] | 0.40 | -0.42 |
| FAS | 18.22 (11.60) | [0; 49] | 0.58 | -0.05 |
| MMSE | 26.78 (3.23) | [17; 30] | -1.27 | 1.37 |

Abbreviations: WMSA – White Matter Signal Abnormalities; GMV – Gray Matter Volume; ICV – Intra-Cranial Volume; SRT – Selective Reminding Test; LTS – Long term Storage; CLTR – Consistent Long-Term Retrieval; DR – Delayed Recall; DD – Digits Direct; DB – Digits Backward; SW – Stroop Words; SC – Stroop Colors; SWC – Stroop Words Colors; FAS – Controlled Oral Word Association Test (admissible words: FAS); MMSE – Mini-Mental State Examination.

memory processing, hippocampal volume does not account for executive functioning, which was also relevant for the purpose of the current study.

Regarding the DEM latent variable, sex (female as reference) and years of formal education were regressed on this variable. The specification of the DEM variable differs from the one used by Reed and colleagues as they have included variables accounting for the different ethnicities in their sample. Since all of the participants from our study were Caucasians, our model does not include any ethnicity factors. Also, major difference between our model and the one presented by Reed et al. [2010], concerns the fact that we study CR as a function of the variance, not only from episodic memory but rather from cognition in general. As described in the neuropsychological assessment section, two factors comprising memory (MEM) and general executive functioning (GENEXEC) test variables were already validated for this sample [Santos et al., 2015]. Thus, these two dimensions were used in the model instead of measures of episodic memory. Since both MEM and GENEXEC are composite measures of several neuropsychological test scores they were modeled through two latent variables regressed on the corresponding test scores.

Finally, the model was set so that the MEM and GENEXEC dimensions were linear combinations of the DEM, BR and CR latent variables. Therefore, they represent the variance in MEM and GENEXEC that can be attributed to demographic characteristics (DEM), MRI-derived structural variables (BR) and the variance in those dimensions

unrelated to both demographic and structural MRI variables (CR). In other words, CR was quantified as the residual variance in cognitive factors that is not accounted for by the DEM variables (sex and years of formal education) and by the BR capacity variables (brain size and WM lesioning). In order to achieve the model identification, variances for cognitive dimensions (MEM and GENEXEC) and MRI variables (GMC and WMC) were fixed to account for measurement error. Furthermore, variances for main latent variables were constrained, by fixing residual variances of BR and DEM to 0, and CR to 1. Using this strategy, main latent variables (BR, DEM and CR) are assumed to have an independent contribution to cognitive dimensions (MEM and GENEXEC). Thus, with this model, we were able to estimate the amount of variance attributed to BR, DEM and CR for each subject. In order to achieve this, multiple imputation was performed with Bayesian estimation, resulting in 10 complete databases. Values for the variables of interest were then obtained for each subject, by creating a new database, in which the average of imputed databases for each variable was calculated.

The analytical model is represented in Figure 1. Observed variables are expressed in rectangles and circles represent the latent variables. The specification of the model was conducted using IBM AMOS v22. The necessary assumptions were verified and met. Regarding the assumption of univariate normality of the endogenous variables, only WMSA and MMSE scores were considered to present moderate nonnormal distribution (i.e., skewness and kurtosis between 1.0 and 2.3) while all the other

variables presented only slightly non-normal distributions (i.e., skewness and kurtosis lower than 1.0). Values of skewness and kurtosis are presented in Table I. These values were considered acceptable since SEM models were shown to be robust to moderate nonnormality [Lei and Lomax, 2005]. Multivariate normality was tested with the Mardia's test for multivariate kurtosis [Mardia, 1970] that yielded a value of -0.976 .

Rs-fMRI Data Preprocessing

RS-fMRI data preprocessing was performed using FMRIB Software Library (FSL v5.07; <http://fsl.fmrib.ox.ac.uk/fsl/>) tools. The first five volumes of the rs-fMRI acquisition were removed in order to exclude possible magnetic field inhomogeneities at the beginning of the acquisition. The remaining data was corrected for slice timing followed by head motion correction. In order to reduce potential contamination of motion on functional connectivity, motion scrubbing [Power et al., 2012] was also performed in order to identify and further exclude time-points where head motion could be critical. Seven subjects were excluded for having more than 10 motion-contaminated time-points. Each subject functional dataset was then normalized to Montreal Neurological Institute (MNI) standard space through an indirect procedure that included: (i) skull stripping of the mean image of the functional acquisition; (ii) rigid-body registration of the mean functional image to the skull stripped structural scan; (iii) affine registration of the structural scan to the MNI T1 template; (iv) nonlinear registration of the structural scan to the MNI T1 template using the affine transformation previously estimated as the initial alignment; (v) nonlinear transformation of the functional acquisition to MNI standard space through the sequential application of the rigid-body transformation and the nonlinear warp followed by resampling to 2 mm isotropic voxel size. Linear regression of motion parameters, mean WM and cerebrospinal fluid (CSF) signal and motion outliers was performed and the residuals of the regression were band-pass temporal filtered (0.01–0.08Hz) and used for the subsequent analysis.

Network Construction

The network nodes were defined as the Anatomical Automatic Labeling (AAL) atlas regions. The mean time-series of the 116 cortical, subcortical and cerebellar regions were extracted and correlations between each possible pair of regions were calculated. This originated a symmetric adjacency matrix R where each entry r_{ij} represents the Pearson correlation coefficient between the time series of region i and j . These matrices were then transformed to Z-score matrices by the application of Fisher's r -to- Z transform to the correlation coefficients. In the present study, only weighted matrices were considered. For the analysis of local and global network metrics, the matrices were

thresholded at different sparsity thresholds s (from 0.025 to 0.45 in steps of 0.025) and thus the metrics were calculated for the networks composed by different proportions of the strongest edges.

Network-Based Statistics Analyses

In order to assess if BR and CR measures were associated with functional connectivity at the edge level (i.e., at each individual connection) a General Linear Model (GLM) was applied with each Z-transformed correlation coefficient as the dependent variable and the linear and quadratic effects of age as well as the DEM, BR and CR latent measures as independent variables. Sex and school years were not included in the model since they presented high collinearity with the DEM measure. Assessing these effects at the edge level poses a multiple comparisons problem since the networks obtained as described in the previous sections of the manuscript encompassed a total of $116 \times 115 / 2 = 6,670$ edges. In order to increase the statistical power of the analysis, the network-based statistic (NBS) procedure implemented in the NBS toolbox (<https://sites.google.com/site/bctnet/comparison/nbs>) was used [Zalesky et al., 2010]. This approach is similar to the cluster-based thresholding approach used in voxel-wise analyses of fMRI data. Instead of considering the null hypothesis at the single edge level, NBS evaluates the null hypothesis at the level of interconnected edges (i.e., subnetworks) surviving a predefined primary threshold. The null hypothesis assumes that a subnetwork with similar number of edges, surpassing the primary threshold, occurs by chance. The authors of this procedure recommend the use of different primary thresholds in order to capture different effects. In the present study, three different primary thresholds were used ($P < 0.01$, $P < 0.005$, $P < 0.001$) in order to capture less pronounced but more extent effects (less stringent primary threshold— $P < 0.01$) as well as localized and pronounced effects (most stringent threshold— $P < 0.001$). Five thousand permutations were performed and networks were considered significant at a corrected level of $P < 0.05$ family-wise error (FWE) corrected. BrainNet viewer (<http://www.nitrc.org/projects/bnv/>) was used for visualization purposes.

Graph Theory Analysis

Graph theoretic analyses were performed at the node and the global network metrics level. The metrics were computed with weighted undirected networks, using the Brain Connectivity Toolbox (BCT, <http://www.brain-connectivity-toolbox.net>) [Rubinov and Sporns, 2010]. Some studies involving graph theoretic measures of FC networks discard edges displaying negative FC since such measures cannot be calculated in the presence of negative weights [Achard and Bullmore, 2007; van den Heuvel et al., 2008]. In the present study, we decided to use absolute values of

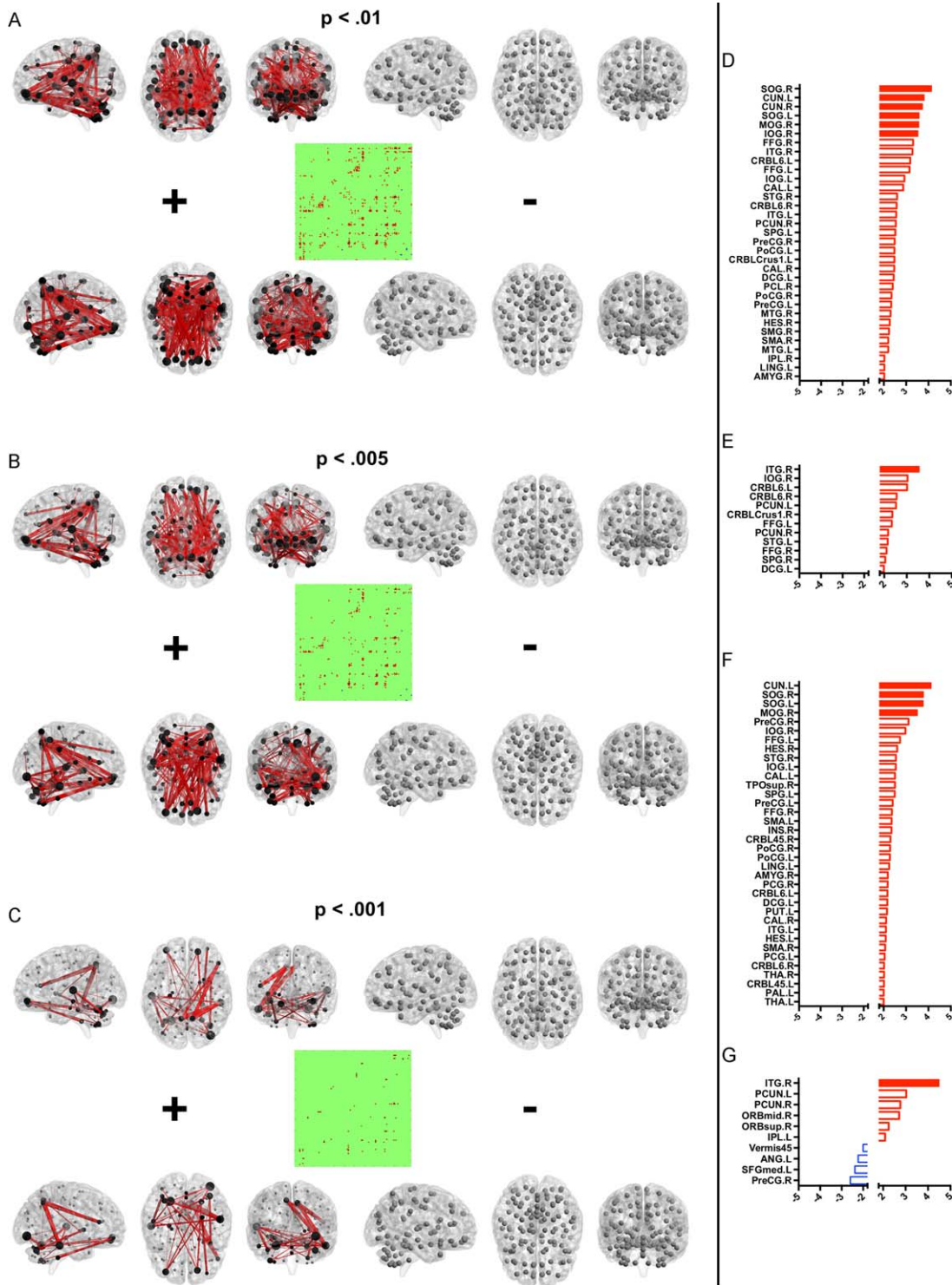


Figure 2.

Associations between CR and FC networks using primary thresholds of $P < 0.01$ (A), $P < 0.005$ (B) and $P < 0.001$ (C). Adjacency matrices after the application of the primary thresholds are presented in the middle of each panel. Edges from the networks revealing positive association are presented in red and from negative associations are presented

in blue. Associations between CR and local efficiency (D), strength (E), clustering (F) and betweenness centrality (G). Statistically significant associations ($P < 0.05$, FDR corrected) are presented in filled bars and associations surviving an uncorrected threshold of $P < 0.05$ are reported for completeness using unfilled bars.

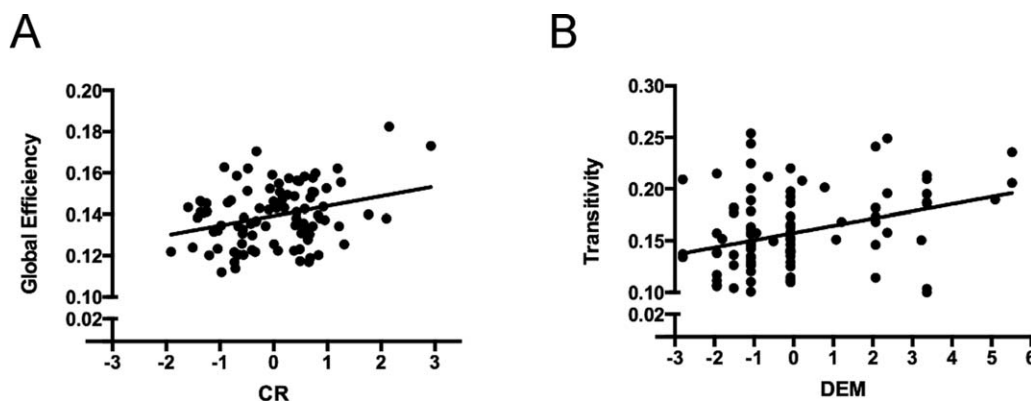


Figure 3.

Scatter plots highlighting significant positive associations between CR and network global efficiency (A) and between DEM (B) and network transitivity.

the network weights for the computation of graph theoretic measures; while we recognize that negative FC might not reflect the same level/kind of meaning as positive FC, we consider this to be less critical than simply neglecting the effect of such edges in network topology.

At the node level, four different metrics were calculated for each region: (1) local efficiency, which is defined as the average of the inverse shortest path length in the neighborhood of the node and, in a weighted network, distinguishes the influence of different paths based on the connection weights of the corresponding neighbors; (2) betweenness centrality that corresponds to the proportion of shortest paths that pass through the node; (3) strength that is the sum of weights of the edges connected to each node; and (4) clustering coefficient, defined as the average intensity of triangles around a node. At the global network level the following measures were calculated: (1) global efficiency, similar to the local efficiency but for the entire network and (2) transitivity which represents a normalized version of the mean of the clustering coefficients of all nodes in the network.

Statistical analysis of graph theoretic metrics computed for different sparsity thresholds were performed with the integrated measures [Tian et al., 2011] of such metrics across the range of sparsities. This methodology has the advantage of reducing the complexity of the analyses and the number of comparisons. The analyses were performed with a similar GLM to the one described for the NBS analyses, with the dependent variable being replaced by the global or local metrics. For the local measures, results were considered significant at $P < 0.05$ corrected for multiple comparisons using the False Discovery Rate (FDR) criterion across all metrics and nodes. Global measures were considered significant at $P < 0.0083$, which corresponds to family wise error (FWE) correction for multiple comparisons among six tests (two global network properties \times three latent variables).

RESULTS

Sample Characteristics

Table I presents the overall demographic and neuropsychological/cognitive characteristics of the participants.

Latent Variable Model

According to the modification indices obtained for the basal model, the covariance between gender and ICV was specified in order to improve model fit. Afterwards, although a significant chi-square statistic was obtained ($\chi^2(83)=129.043, P=0.001$), based on the remaining fit indices, the specified model was assumed to have a satisfactory fit (CFI = 0.952 | TLI = 0.930 | RMSEA = 0.076). The variance of both cognitive dimensions was significantly predicted by the main latent variables. Of notice, it is relevant to observe that CR revealed to be the most relevant predictor of both cognitive dimensions (MEM: $\beta = 0.881, P < 0.001$; GENEXEC: $\beta = 0.669, P < 0.001$).

Effects of the Cognitive Reserve Latent Variable

Regarding the CR variable, a positive association between CR and FC in a cortical network was found (Fig. 2A–C). This association was significant for the three primary thresholds used ($P < 0.01, P < 0.005$ and $P < 0.001$).

Additionally, at the local metrics level, CR was positively associated with local efficiency in the bilateral superior occipital, bilateral cuneus, right middle occipital and right inferior occipital regions (Fig. 2D) and positively associated with the strength of the inferior temporal gyrus (Fig. 2E). CR was also positively correlated with the clustering coefficient of the left cuneus, bilateral superior occipital and right middle occipital (Fig. 2F) and positively associated with the betweenness centrality of the left

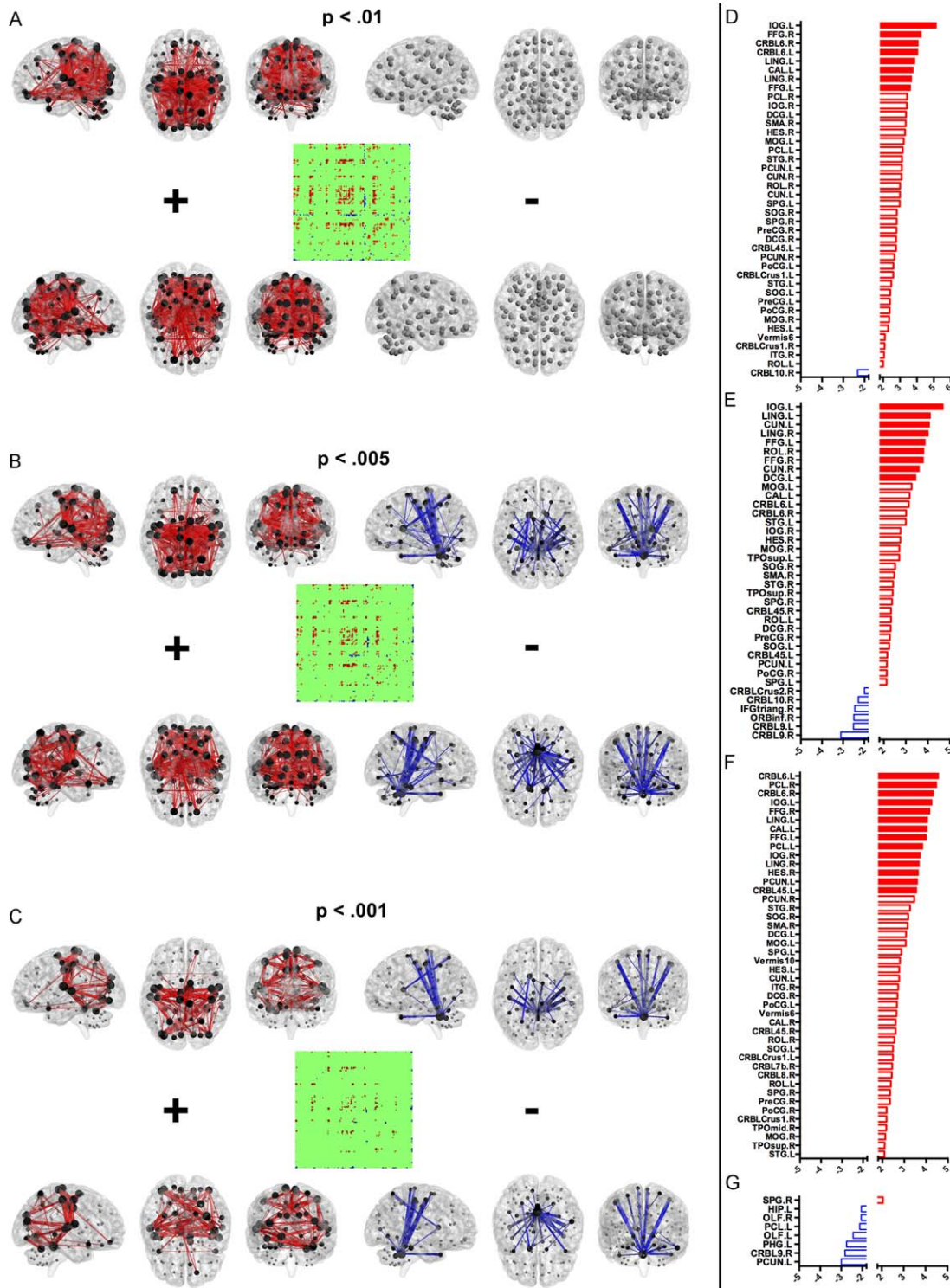


Figure 4.

Associations between DEM and FC networks using primary thresholds of $P < 0.01$ (A), $P < 0.005$ (B) and $P < 0.001$ (C). Adjacency matrices after the application of the primary thresholds are presented in the middle of each panel. Edges from the networks revealing positive association are presented in red and edges from negative associations are presented in blue.

Associations between DEM and local efficiency (D), strength (E), clustering (F) and betweenness centrality (G). Statistically significant associations ($P < 0.05$, FDR corrected) are presented in filled bars and associations surviving an uncorrected threshold of $P < 0.05$ are reported for completeness using unfilled bars.

inferior temporal gyrus (Fig. 2G). At the global network level, CR was positively associated with global efficiency ($t = 3.17$; $P = 0.002$) (Fig. 3A), but not transitivity ($t = 1.76$; $P = 0.082$).

Effects of the Demographic Latent Variable

Regarding the NBS results, the demographic latent variable (DEM) was positively associated with FC in a large network encompassing connections between several cortical regions. These results were significant for all primary thresholds used (Fig. 4A–C). Additionally, using the intermediate and most strict primary thresholds (i.e., $P < 0.005$ and $P < 0.001$), a network mainly involving connections between the cerebellar vermis and cortical regions evidenced a negative association with the DEM variable (Fig. 4B,C).

Regarding the local network metrics, the DEM variable revealed a significant positive association with local efficiency of the left inferior occipital, bilateral fusiform, bilateral cerebellum 6, bilateral lingual and left calcarine (Fig. 4D) and positively associated with local strength in the left inferior occipital, bilateral lingual, bilateral cuneus, bilateral fusiform, right rolandic operculum and left cingulum middle (Fig. 4E). Positive associations between DEM and local clustering were found in bilateral cerebellum 6, bilateral paracentral, bilateral inferior occipital, bilateral fusiform, bilateral lingual, bilateral precuneus, left calcarine, right Heschl and cerebellum 4 and 5 (Fig. 4F). No significant association was found between the DEM variable with betweenness centrality (Fig. 4G).

At the global network level DEM evidenced a positive association with transitivity ($t = 3.05$; $P = 0.003$) (Fig. 3B), but no association with global efficiency ($t = 1.92$; $P = 0.058$).

Effects of the Brain Reserve Latent Variable

No significant associations between the BR variable and FC networks, local network metric or global network metrics were found.

DISCUSSION

In the present study, we investigated how CR impacts functional connectivity networks, independently of education level. For this purpose, CR was quantified as the residual variance in cognitive factors that is not accounted for by the DEM variables (sex and years of formal education) and by the BR capacity variables (brain size and WM lesioning). This type of model was shown to be in line with the CR theory [Reed et al., 2010] and has been, thereafter, validated and extended on [Zahodne et al., 2013]. The replication of the latent variable model revealed to be valid for the present study, as proved by the satisfactory fit indices obtained for the model. With this, it was

possible to dissect and quantify the effects of CR based on the variance of cognitive functioning not explained either from brain structural variables or by the demographic characteristics. Ultimately, this strategy enabled us to study how CR is manifested at the FC level at rest.

Regarding the demographics variable, the present results closely mimic those obtained in a previous study from our group in which the effects of years of formal education in FC networks were investigated [Marques et al., 2015a]. Briefly, it was shown that education level was associated with higher FC in a large cortical network and with decreased (i.e., more negative) FC between the cerebellum and cerebrum. This was interpreted as more efficient brain network in individuals with higher number of years of education and a more effective differential involvement of the cerebrum and cerebellum. This finding is not surprising since the DEM variable is mainly accounted for by the number of years of formal education. However, in the present study it is further demonstrated that this demographic variable (DEM) is also associated with particular topological characteristics of the brain FC network. At the local metrics level, DEM is also associated with higher local efficiency, nodal strength and clustering coefficient in several brain regions spanning most of the occipital and parietal lobes. Associations in some sensorimotor regions and the cerebellum were also found. This suggests that these regions are more tightly connected between themselves and more segregated from the rest of the brain, facilitating the communication within these networks and revealing the positive effects, mainly of years of formal education, in posterior brain regions. Additionally, at the global network level, DEM was associated with the network transitivity but not global efficiency. Transitivity is a well-known measure of functional segregation [Rubinov and Sporns, 2010; Watts and Strogatz, 1998]. This suggests that demographic variables, especially education level, are associated with the increased ability of the brain to form segregated groups of brain regions (clusters or modules), reinforcing the view that higher education level triggers more specialized use of neural processing.

Furthermore, the present results provide evidence that CR impacts on the brain FC network architecture, even when education level is accounted for. As expected, CR was associated with a large and sparse network displaying higher FC in individuals with higher CR. Similarly to the associations found for education level, this is likely to reflect the positive effect in brain functioning facilitating communication and integration. Higher CR was also associated with higher local efficiency and local clustering of the cuneus as well as superior and middle occipital regions. This suggests that connections involving these regions display higher FC in subjects with higher CR, highlighting their importance in creating tighter functional connections within the brain network while also contributing for the formation of local clusters of information processing. Additionally, the inferior temporal gyrus stands as

the only region whose nodal strength and betweenness centrality correlated positively with CR, despite not evidencing any association with its local clustering and local efficiency. This suggests that the inferior temporal gyrus is an important hub of the CR network although it does not contribute to a higher local organization or efficiency of the network. This region is known to be involved in word recognition [Dien et al., 2013], visualization of numerals [Grotheer et al., 2016; Shum et al., 2013], processing of faces [Weiner and Grill-Spector, 2013] and complex emotional pictures [Geday et al., 2001]. It has also been associated with working memory dysfunction in depressed individuals [Parra et al., 2010] and is also part of the Default Mode Network (DMN) [Greicius et al., 2003]. This might suggest that individuals with higher CR can better process visual information through pathways involving the inferior temporal gyrus.

Of particular interest is the association between CR and global efficiency. FC networks have weaker connections between modules, when compared to structural networks, and thus global efficiency values are typically low [Rubinov and Sporns, 2010]. Despite this, here we show that individuals with higher CR displayed higher global efficiency values compared to those with lower CR. Global efficiency is a measure of functional integration, where higher values mean that shorter and more efficient paths are available in order to connect every possible pair of nodes [Rubinov and Sporns, 2010]. This means that individuals with higher cognitive performance, controlling for BR and demographic differences, present a more tightly connected network likely to reflect an increase capacity for parallel information transfer and integrated processing [Bullmore and Sporns, 2012]. This is closely related to the concept of a more efficient use of the brain's resources, one of the hallmarks of CR [Stern, 2002]. Previous studies have found similar associations in structural [Li et al., 2009] and functional [van den Heuvel et al., 2009] brain networks with IQ, a proxy of CR. Higher network efficiency would also explain activation of higher cognitive resources in challenging task conditions [Zihl et al., 2014].

Of note, in this study we assessed these effects in a sample of older individuals with a large spectrum of cognitive performance. In fact, some individuals did not go to school at all and others scored zeros in some of the cognitive tests despite not presenting any diagnosed neurological pathology or dementia. Besides being representative of the Portuguese older population [INE, 2012] it also enabled us to assess this in a cohort comprising individuals with high and low proxies of cognitive reserve.

The present study has some limitations. The model used should probably include not only measures of hippocampal, but also other temporal lobe structures, such as the parahippocampal gyrus and perirhinal cortex, which are known to be involved in the encoding of verbal material. It would be also appropriate to include the volume of prefrontal cortex in the model to account for the variance of

executive functioning explained by MRI-related variables. Microstructural measures of white matter integrity such as the ones derived from Diffusion Tensor Imaging (DTI) could be useful for quantifying BR capacity as it might add important information on brain structure. Additionally, since the proposed latent model has the potential to provide longitudinal trajectories of CR, longitudinal studies should be carried out in order to investigate if CR can be a moderator of age related cognitive decline in older individuals as proposed by others [Bozzali et al., 2015; Tucker-Drob et al., 2009]. Finally, it would be of interest to consider for other demographic characteristics (such as primary occupation) and/or cohorts characterized by high education levels to have a broader picture of the impact of FC in brain functional connectivity.

CONCLUSION

The present study reveals that CR is associated with increased FC in a large network and with a better organization of the network topology. It is also shown that demographic characteristics, mainly accounted for by years of formal education, are also associated with brain network reorganizations. More importantly we brought evidence that while years of education are likely to be associated with a higher specialization of the brain network, CR is associated with higher network efficiency.

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The authors declare that they have no conflicts of interest.

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