NeuroImage 88 (2014) 202–211

Contents lists available at ScienceDirect

NeuroImage

journal homepage: www.elsevier.com/locate/ynimg

Broad intrinsic functional connectivity boundaries of the macaque prefrontal cortex☆

R. Matthew Hutchison [a,*], Stefan Everling [a,b,c]

[a] Robarts Research Institute, University of Western Ontario, London, Ontario, Canada
[b] Department of Physiology and Pharmacology, University of Western Ontario, London, Ontario, Canada
[c] Research Group Competition and Priority Control in Mind & Brain, Center for Interdisciplinary Research, Bielefeld University, Germany

ARTICLE INFO

Article history:
Accepted 14 November 2013
Available online 21 November 2013

Keywords:
Connectivity
Macaque
Parcellation
Prefrontal cortex
Resting-state fMRI

ABSTRACT

Based upon cytoarchitectonic properties, the primate prefrontal cortex has been partitioned into different subregions that show unique structural connectivity patterns, with ongoing efforts to provide more fine-grained divisions. While meaningful divisions may be found within the sub-millimeter range, the subdivisions exist within an overall hierarchical architecture and at higher levels likely share similar activity patterns and functionality. Here, we used resting-state fMRI in lightly anesthetized macaque monkeys to measure the intrinsic functional connectivity of the prefrontal cortex. At a gross anatomical level, the data driven approach revealed five broad clusters that showed distinct brain-wide functional connectivity. Although each cluster encompasses several cytoarchitectonic subregions, the clusters overlap with the intrinsic structural connectivity of the prefrontal cortex and each cluster may subserve common functions.

Introduction

The primate prefrontal cortex (PFC) has been implicated in virtually all higher brain functions, including affect, social behavior, and cognitive/executive control (e.g. working memory, attention, decision-making, planning, inhibitory control, reward, anticipation, etc.) (Fuster, 2008; Miller and Cohen, 2001; Passingham and Wise, 2012). While several unifying theories of PFC function have been proposed (Duncan, 2001; Fuster, 2008; Miller and Cohen, 2001; Passingham and Wise, 2012; Wilson et al., 2010), there is general agreement that the PFC is comprised of regions that can be differentiated by both their function and connectivity patterns. However, the functional organization of the PFC still remains poorly understood. A major problem lies in the technical difficulties associated with detecting functional areas and borders between PFC regions. In contrast to sensory areas where single unit recording studies have been helpful in delineating the boundaries between cortical areas based on neurons' response fields and properties (e.g. Kravitz et al., 2012; Rauschecker and Scott, 2009), it has been difficult to detect functional differences between PFC regions (Asaad et al., 1998; Rao et al., 1997; Tsujimoto et al., 2012). Instead, existing parcellations of the macaque PFC are based on stained cell bodies and/or the pattern of myelinated fibers.

In the first mapping of the PFC based on one macaque and three cercopithecus monkeys, Brodmann divided the monkey PFC (regio frontalis) into only five subregions (areas 8, 9, 10, 11, and 12) and stated explicitly that he could not detect homologs of human areas 44, 45, and 46 in monkeys (Brodmann, 1905, 1909). In contrast, Walker (1940) identified twelve PFC areas in macaques, including an area 46 which occupied the cortex in and around the principal sulcus and an area 45 situated in, and anterior to, the lower arm of the arcuate sulcus. Petrides and Pandya (1999, 2002) subdivided the PFC even further into 17 subregions and introduced areas 9/46d and 9/46v which separate areas 8Ad and 8Av, respectively, from area 46. As already acknowledged by Walker in 1940, cytoarchitectonic mapping is a difficult and tedious process, which led Passingham and Wise to point out that "an objective reliable, and faster way of marking the borders has been the holy grail of cortical architectonics for decades" (Passingham and Wise, 2012). Because of the inconsistencies in PFC mapping and unclear functional differences between some of the cytoarchitectonic areas, Passingham and Wise used broader terms and divided the PFC into caudal, dorsal, medial, orbital, and ventral regions (Passingham and Wise, 2012). Different functional roles of these five subdivisions are supported by both lesion and tracer studies (Passingham, 1993; Passingham and Wise, 2012).

In humans, several studies have started to use different tasks to parcellate the PFC based on the corresponding fMRI activations (e.g. Hampshire and Owen, 2006; Hampshire et al., 2012). Although possible in principle, a comparable approach in monkeys would be difficult due to the extensive training requirements (Gamlin et al., 2006). A more straightforward and feasible approach is to use a mapping based on resting-state fMRI (Biswal et al., 1995), which uses correlations of low frequency fluctuations of the spontaneous blood oxygenation-level-

☆ Grant sponsor: Canadian Institutes of Health Research (CIHR).
* Corresponding author at: Center for Brain Science, Harvard University Northwest Building — East Wing, 52 Oxford Street, Cambridge, MA 02138, USA.
E-mail address: rhutchison@FAS.Harvard.edu (R.M. Hutchison).

1053-8119/$ – see front matter © 2014 Elsevier Inc. All rights reserved.
http://dx.doi.org/10.1016/j.neuroimage.2013.11.024

dependent (BOLD) signal to measure functional connectivity (FC) between brain regions (for reviews, see Buckner et al., 2013; Fox and Raichle, 2007). This technique has been successfully applied to parcellate cortical areas in humans (e.g. Cohen et al., 2008; Goulas et al., 2013). Here, we used resting-state fMRI in lightly anesthetized macaque monkeys to measure the intrinsic FC of the PFC. This completely data driven approach unveiled five subregions of the PFC. The whole-brain FC of these five PFC subregions shows distinct large-scale functional networks, suggesting strong within region cooperation and shared functional specialization.

Material and methods

Subjects and data acquisition

All surgical and experimental procedures were carried out in accordance with the Canadian Council of Animal Care policy on the use of laboratory animals and approved by the Animal Use Subcommittee of the University of Western Ontario Council on Animal Care. Data was collected from eleven naïve, isoflurane-anesthetized (1%) macaque monkeys (4 *Macaca mulatta* and 7 *Macaca fascicularis*). These data have been published previously as two different datasets: Set 1 ($N = 6$, $TR = 2$ s, EPI resolution $= 1.3 \times 1.3 \times 1.5$ mm^3, 2 scans of 300 volumes) (Hutchison et al., 2011, 2012a, 2012b, 2013; Shen et al., 2012) and Set 2 ($N = 5$, $TR = 2$ s, EPI resolution $= 1 \times 1 \times 1$ mm^3, 10 scans of 150 volumes) (Babapoor-Farrokhran et al., 2013). Animal preparation, imaging parameters, and preprocessing are described therein. One monkey from Set 1 was scanned again with the increased spatial resolution and scan numbers of Set 2, and the original scanning session from Set 1 removed from the analysis leaving a total of 11 naïve animals. Data was normalized to the F99 atlas template (0.5 X 0.5 X 0.5 mm^3; Van Essen, 2004; see http://sumsdb.wustl.edu/sums/macaquemore.do) and to decrease the computational demands, was downsampled by a factor of 2 (resulting in a voxel resolution of $1 \times 1 \times 1$ mm^3).

Cortical parcellation

A cortical gray matter mask was created that encompassed all areas anterior to the genu of the arcuate sulcus. The time signal was extracted from all voxels contained within the defined mask of a given run. A cross correlation matrix between all voxels was then calculated for each run while partialling out the average white matter (WM) and cerebrospinal fluid (CSF) time series in addition to six motion parameters. Following a Fisher z-score transformation, the average across scans of the same animal, and then across all animals, was calculated revealing the average pairwise correlation between all voxels within the PFC mask. The standard Euclidean pairwise distance (the square root of the sum of the squares of the differences) between all voxel pairs of this group-averaged intrinsic connectivity matrix was then calculated to determine the voxel to voxel dissimilarity based upon their temporal correlations and then scaled by the standard deviation. Unweighted average-linkage hierarchical cluster analysis was applied to the distance matrix, forming a hierarchy of clusters, progressively merging clusters from the individual data elements based on the mean distance between elements to reflect the structure present in the dissimilarity matrix until a single cluster emerges. A hierarchical cluster tree (dendrogram) was then visualized by applying the linkage criterion to the distance matrix. The parameters for clustering were selected by evaluating the cophenetic correlation for the resulting cluster tree for both unweighted average distance and complete (based on furthest distance) algorithms applied across Euclidean (average/complete = 0.5494/0.5070), standard Euclidean (0.5625/0.5328), cityblock (0.5384/0.5507), and Minkowsi (0.5494/0.5070) distance metrics. The cophenetic correlation evaluates how faithfully the resulting cluster tree represents the dissimilarities among observations and was found to be highest when applying unweighted average-linkage using standard Euclidean distance and thus this was subsequently used for the analysis.

Hierarchical clustering does not require the number of clusters to be specified a priori and can generate a complete cluster tree showing, in this case, every voxel included in the mask. However, for display purposes the tree was truncated at 20 clusters. Based upon the clustering patterns observed, a Euclidean distance value of 180 was selected resulting in a total of five individual clusters. Further, evaluation of the average silhouette index of clusters sizes (2-25) revealed peaks for 2, 4, and 5 clusters solutions. All voxels contained within each cluster assignment were then projected from volume data to the F99 cortical surface with the CARET (http://www.nitrc.org/projects/caret) enclosed-voxel method (Van Essen et al., 2001). The silhouette index of each voxel was calculated using a correlation metric to evaluate the fit of each voxel's connectivity pattern with that of its assigned cluster and displayed on the cortical surface. The measure allows for the visualization of voxels and/or regions that very closely resemble the characteristic intrinsic connectivity pattern of the cluster and also those that because of the nature of forcing the elements into a certain number of clusters diverge.

The same cortical parcellation analysis used at the group level was also implemented using each individual's average pairwise correlation matrix to examine single-subject PFC divisions.

Seed-based analysis

The five group-level clusters were used as regions-of-interest (ROIs) in a seed-based analysis implemented in the FMRIB Software Library toolbox (FSL; http://www.fmrib.ox.ac.uk) as outlined previously (Hutchison et al., 2012a, 2012b). The mean time course of all voxels within each cluster ROI was extracted for every scan of each animal and were then used as predictors in a model for multiple regression at the individual voxel level for each scan in which nuisance covariates for WM, CSF, and six motion parameters were included. Following this, a second level fixed-effects analysis was used to determine the voxels of the functional connectivity maps, that is, the regression maps of predicted voxels for each regressor from the first level, that were significant across the scans collected for each animal (two scans in Set 1, 10 scans in Set 2). A third-level group analysis was then conducted by implementing a fixed-effects analysis on the second level outputs, producing thresholded z-statistic maps showing brain regions significantly correlated with each seed region across all subjects. Corrections for multiple comparisons were implemented at the cluster level with Gaussian random field theory ($z > 2.3$; cluster significance: $p < 0.05$, corrected). The group z-scores were projected from volume data to the F99 cortical surface with the CARET (http://www.nitrc.org/projects/caret) enclosed-voxel method (Van Essen et al., 2001).

To compare the group level connectivity maps derived for each seed regions spatial correlations were calculated in the volume space using the positively thresholded z-scores ($z \geq 2.3$) quantifying both the shared spatial extent and strength of connectivity in each voxel across maps. Spatial correlations were also calculated using thresholded and binarized ($z \geq 2.3 = 1, z < 2.3 = 0$) values to only quantify the shared spatial extent of connectivity patterns. A conjunction map was computed by adding the 5 individual binarized maps to show, at each voxel, the number of ROIs significantly connected to that voxel. Statistical comparisons between the maps were also calculated using an additional level of analysis in which the group level z-scores for each map from the third level of analysis were contrasted against those of the other four maps at a voxel level ($z > 2.3$; cluster significance: $p < 0.05$, corrected).

Results

We performed an analysis on the intrinsic connectivity of the cortical voxels anterior to the genu of the arcuate sulcus in macaque