NeuroImage: Clinical 7 (2015) 288–296

Contents lists available at ScienceDirect

NeuroImage: Clinical

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

Cerebro-cerebellar connectivity is increased in primary lateral sclerosis

Avner Meoded[a,1], Arthur E. Morrissette[a,2], Rohan Katipally[a,3], Olivia Schanz[a], Stephen J. Gotts[b], Mary Kay Floeter[a,*]

[a] National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD, USA
[b] National Institute of Mental Health, National Institutes of Health, Bethesda, MD, USA

ARTICLE INFO

Article history:
Received 23 September 2014
Received in revised form 2 November 2014
Accepted 5 December 2014
Available online 9 December 2014

Keywords:
Motor neuron disease
Resting state functional MRI
Connectivity
Cerebellum
Primary lateral sclerosis

ABSTRACT

Increased functional connectivity in resting state networks was found in several studies of patients with motor neuron disorders, although diffusion tensor imaging studies consistently show loss of white matter integrity. To understand the relationship between structural connectivity and functional connectivity, we examined the structural connections between regions with altered functional connectivity in patients with primary lateral sclerosis (PLS), a long-lived motor neuron disease. Connectivity matrices were constructed from resting state fMRI in 16 PLS patients to identify areas of differing connectivity between patients and healthy controls. Probabilistic fiber tracking was used to examine structural connections between regions of differing connectivity. PLS patients had 12 regions with increased functional connectivity compared to controls, with a predominance of cerebro-cerebellar connections. Increased functional connectivity was strongest between the cerebellum and cortical motor areas and between the cerebellum and frontal and temporal cortex. Fiber tracking detected no difference in connections between regions with increased functional connectivity. We conclude that functional connectivity changes are not strongly based in structural connectivity. Increased functional connectivity may be caused by common inputs, or by reduced selectivity of cortical activation, which could result from loss of intracortical inhibition when cortical afferents are intact.

1. Introduction

Functional connectivity in resting state networks is decreased in many neurodegenerative and neuropsychiatric disorders (Alexander-Bloch et al., 2013; Chhatwal and Sperling, 2012; Damoiseaux et al., 2012; Gotts et al., 2012). In motor neuron disorders such as amyotrophic lateral sclerosis (ALS), some studies report that functional connectivity is increased compared to healthy controls, particularly in the regional sensorimotor network (Douaud et al., 2011; Jelsone-Swain et al., 2010; Verstraete et al., 2010), whereas others report decreased functional connectivity (Agosta et al., 2011; Mohammadi et al., 2009; Schmidt et al., 2014; Zhou et al., 2014). Increased functional connectivity in the sensorimotor network has also been reported in primary lateral sclerosis (PLS), a rare motor neuron disorder variant with slow progression and long survival periods (Agosta et al., 2014).

In motor neuron disorders, functional connectivity may be affected by the stage of disease and the structural integrity of long-range white matter tracts in the brain. In ALS, the most common motor neuron disorder, the earliest stage of degeneration affects motor cortex neurons, with spread of degeneration to anterior cortical areas over time (Braak et al., 2013). Diffusion tensor imaging (DTI) shows disruption of the corticospinal tract and callosal white matter (Agosta et al., 2010; Ciccarelli et al., 2009; Ellis et al., 1999; Iwata et al., 2008). In ALS, functional connectivity was inversely related to the diffusion tensor imaging measures of the structural integrity of cortical white matter tracts (Douaud et al., 2011; Verstraete et al., 2011).

To date, most rs-fMRI studies in motor neuron disorders have examined functional connectivity changes within regional rs-fMRI networks that have been defined in healthy controls. That approach may miss new patterns of connectivity emerging as a consequence of the disease. New functional connectivity patterns may be particularly important in PLS patients because patients' long survival allows an extended period

Abbreviations: AFNI, analysis of functional neuroimages; ALS, amyotrophic lateral sclerosis; ALSFRS-R, amyotrophic lateral sclerosis rating scale; ANCOVA, analysis of covariance; BOLD, blood oxygen-level dependent; DTI, diffusion tensor imaging; Epi, echo planar imaging; FA, fractional anisotropy; FWE, family-wise error; fMRI, functional magnetic resonance imaging; FSL, FMRIB Software Library; MNI, Montreal Neurological Institute; PLS, primary lateral sclerosis; ROI, region of interest; TFCE, threshold-free cluster enhancement; TORTOISE, tolerably obsessive registration and tensor optimization indolent software ensemble; TBSS, tract based spatial statistics

* Corresponding author at: Room 7-5680, 10 Center Drive, Bld 10, Bethesda, MD 20892 1404, USA. Tel: +1 301 496 7428; fax: +1 301 402 8796.
E-mail address: floeterm@ninds.nih.gov (M.K. Floeter).
[1] Present address: Dept. of Radiology, Johns Hopkins Medical School, Baltimore, MD, USA.
[2] Present address: Emory University Neuroscience Graduate Program, Atlanta, GA USA.
[3] Present address: Brown University Medical School, Providence, RI, USA.

http://dx.doi.org/10.1016/j.nicl.2014.12.009

in which neuroplasticity could occur. Some evidence indicates that the relationship between functional connectivity and structural integrity is affected by the pace of disease progression, with less alteration of functional connectivity in ALS patients who have slower rates of disease progression (Douaud et al., 2011). In this study we combined functional and structural approaches to look for changes in connectivity in PLS patients. In this study we used a data-driven approach to allow visualization of differing functional connectivity patterns in PLS patients (Gotts et al., 2012). Diffusion tensor imaging was used to assess the integrity of white matter tracts and structural connections between regions of differing functional connectivity.

2. Methods

2.1. Subjects

All subjects gave written informed consent for protocols approved by the NIH Combined Neuroscience Institutional Review Board (NCT00015444; NCT01517087). All subjects had examinations by a neurologist, and all healthy controls had normal neurological examinations. The PLS group consisted of successive clinic patients seen during 2012 and 2013 who had no contraindications to MRI scanning. PLS patients were diagnosed by clinical criteria (Pringle et al., 1992), with testing to exclude alternative diagnoses. Evaluations included neurological examinations and interviews with caregivers. Clinical scales used for correlation analyses included the ALSFRS-R (Cedarbaum et al., 1999), mini-mental state examination (Folstein et al., 1975), measures of timed gait, and foot- and finger-tapping speed. None of the patients met criteria for frontotemporal dementia (Rascovsky et al., 2011) nor had a family history of PLS, ALS or frontotemporal dementia.

2.2. Imaging acquisition

A 3 T MRI scanner was used with a receive-only, eight-channel head coil (GE Medical Systems, Milwaukee, WI). The sequences acquired in each subject included:

1. A high-resolution T1-weighted sequence (slice thickness 1 mm); 2) resting-state fMRI, gradient EPI sequence (3.8 mm slice thickness, TR 2 s/TE 30 ms, FOV 24 cm, 64 × 64 matrix, 7:08 min scan time). Cardiac and respiratory waveforms were collected independently during the EPI scans for later removal; 3) multi-slice diffusion weighted imaging was acquired using a single-shot spin-echo echo-planar sequence with 64 contiguous axial slices (slice thickness = 2.5 mm, FOV = 240 × 240 mm). Diffusion weighting was performed with 80 non-collinear directions with multiple b values: b = 0 s/mm^2, b = 300 s/mm^2 and b = 1100 s/mm^2; 4) axial T2-weighted images were also acquired for EPI distortion correction purpose, with a fast spin echo sequence with the same FOV and 1.7 mm slice thickness.

2.3. Functional MRI

Preprocessing was done with the AFNI software package (Cox, 1996) using the basic approach described by Gotts and colleagues (Gotts et al., 2012; Gotts et al., 2013). Briefly, the first 4 EPI volumes were removed AFNI 3dDespike was used to remove large transients (due to factors such as head movement (Jo et al., 2013)). Time series were corrected for slice-time acquisition, and all EPI volumes were co-registered with the T1-weighted scan to the first volume in the truncated set, then spatially blurred by a 6-mm (full width at half maximum) Gaussian kernel, with each voxel's time series normalized by its temporal mean to yield units of percent signal change. Linear regression was then used to remove motion (6 motion parameters), cardiac and respiratory cycles (8 regressors for slice time 0;(Glover et al., 2000)), and slower effects of respiration (5 respiration volume per time regressors;(Birn et al., 2008)), as well as average signal from the ventricles and a local average of white matter signal (within a radius of 20 mm centered on each voxel) calculated prior to the spatial blurring step. Ventricle and white matter time series were derived for each subject by segmenting the T1 weighted scan into gray, white, and CSF compartments using FreeSurfer (Fischl et al., 2002), and these masks were resampled to EPI resolution and eroded by 1 voxel to prevent partial volume effects with gray matter. With the exception of the cardiac/respiration regressors (which already incorporate time delays), delayed versions (1 TR) of all regressors were included to allow for delayed effects of noise sources. The cleaned, blurred residual time series were then spatially normalized to the MNI anatomical template (http://www.bic.mni.mcgill.ca/ServicesAtlases/Colin27, as implemented in AFNI's MNI_caez_N27 template) for the purposes of group analyses using each subject's anatomical scan. An index of transient head motion (AFNI's @1dDiffMag) was calculated from each subject's motion parameters for use as a nuisance covariate in the group-level analyses.

2.3.1. Functional connectivity maps

Functional connectivity was assessed for each participant in a whole-brain fashion (Gotts et al., 2012). The average Pearson correlation of each voxel's time series with all voxels in a whole-brain mask was first calculated using AFNI's 3dTcorrMap function. These average correlations were then transformed using Fisher's z to yield normally distributed values and then compared in MNI coordinates between PLS patients and controls using a basic ANCOVA approach (AFNI's 3dttest++), covarying the level of transient head motion for each participant (see Saad et al., 2013 for discussion). Four clusters of voxels survived cluster-size correction for whole-brain comparisons at $P < 0.05$ (voxel-wise P-value threshold of $P < 0.005$) and served as seeds in the next step of the analyses (Fig. 1A). The four clusters were tested individually in more standard seed-based correlation analyses between PLS and control groups using 3dttest++ (covarying transient head motion), correcting for whole-brain comparisons using both cluster size and the number of seeds tested (corrected to $P < 0.05/4 = 0.0125$, with an initial voxel-wise P-value threshold of $P < 0.005$). Following the seed tests, additional voxels were included in subsequent ROI analyses if they were present in the corrected group comparisons of all 4 seeds, yielding an additional 8 voxel clusters for a total of 12 ROIs (see Fig. 1B for details).

All-to-all ROI correlation matrices were calculated for each participant using the average voxel time series from each of the 12 ROIs, transforming the resulting correlations using Fisher's z. The structure of the group-average correlation matrix (pooling both groups) was then analyzed using K-means cluster analyses, principal components analysis (PCA, viewing 1st two PCs), and multi-dimensional scaling (MDS), with an "elbow" criterion on the tradeoff of variance explained versus model complexity yielding 3 clusters and good agreement across the different analysis methods (Fig. 2). Results for single-group correlation matrices, group comparisons, and behavioral correlations for the PLS group were then viewed after sorting ROIs by cluster membership (Fig. 3). As with the other group comparisons of correlation magnitude, values were corrected for the number of comparisons to $P < 0.05$ (Bonferroni) after covarying age and transient head motion values.

Correlations of functional connectivity measures with clinical symptoms for the PLS patients were carried out for ROI-level and whole-brain data using partial correlation to remove the effects of transient motion and age. For the whole-brain analyses, correlation maps for each PLS patient were calculated from each of the four seeds detected in the group comparisons and transformed using Fisher's z. Partial correlations with ALSFRS-R score were then carried out across patients, partialing transient motion and age and correcting for whole-brain comparisons using both cluster size and number of seeds tested (to $P < .05/4$) over a range of voxel-wise P-value thresholds ($P < 0.05$, $P < 0.01$, and $P < 0.005$; see Fig. 4). Correlation of functional connectivity measures