NeuroImage: Clinical 20 (2018) 564–571

Contents lists available at ScienceDirect

NeuroImage: Clinical

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

Brain functional networks become more connected as amyotrophic lateral sclerosis progresses: a source level magnetoencephalographic study

Sorrentino Pierpaolo[a,b,*,1], Rucco Rosaria[c,1], Jacini Francesca[c,d], Trojsi Francesca[e], Lardone Anna[c,d], Baselice Fabio[a], Femiano Cinzia[e], Santangelo Gabriella[f], Granata Carmine[g], Vettoliere Antonio[g], Monsurrò Maria Rosaria[e], Tedeschi Gioacchino[e], Sorrentino Giuseppe[c,d]

[a] Department of Engineering - University of Naples "Parthenope", Centro Direzionale Isola C4, 80133 Naples, Italy
[b] Institute for High Performance Computing and Networking, CNR, via Pietro Castellino 111, 80131 Naples, Italy
[c] Department of Motor Sciences and Wellness - University of Naples "Parthenope", via Medina 40, 80133 Naples, Italy
[d] Hermitage Capodimonte Hospital, via Cupa delle Tozzole 2, 80131 Naples, Italy
[e] Department of Medical, Surgical, Neurological, Metabolic and Aging Sciences - MRI Research Center SUN-FISM, University of Campania "Luigi Vanvitelli", P.zza Miraglia 2, 80138 Naples, Italy
[f] Department of Psychology, University of Campania "Luigi Vanvitelli", viale Ellittico 31, 80100 Caserta, Italy
[g] Institute of Applied Sciences and Intelligent Systems, CNR, via Campi Flegrei 34, 80078 Pozzuoli, NA, Italy

ARTICLE INFO

Keywords:
Motor neuron disease
Connectivity
Magnetic source imaging
Neuroimaging biomarker

ABSTRACT

This study hypothesizes that the brain shows hyper connectedness as amyotrophic lateral sclerosis (ALS) progresses. 54 patients (classified as "early stage" or "advanced stage") and 25 controls underwent magnetoencephalography and MRI recordings. The activity of the brain areas was reconstructed, and the synchronization between them was estimated in the classical frequency bands using the phase lag index. Brain topological metrics such as the leaf fraction (number of nodes with degree of 1), the degree divergence (a measure of the scale-freeness) and the degree correlation (a measure of disassortativity) were estimated. Betweenness centrality was used to estimate the centrality of the brain areas.

In all frequency bands, it was evident that, the more advanced the disease, the more connected, scale-free and disassortative the brain networks. No differences were evident in specific brain areas. Such modified brain topology is sub-optimal as compared to controls. Within this framework, our study shows that brain networks become more connected according to disease staging in ALS patients.

1. Introduction

Recently, autoptic, neuropsychological, genetic and neuroimaging evidence has suggested that amyotrophic lateral sclerosis (ALS) involves regions beyond the primary motor cortex (Turner and Swash, 2015).

According to this evidence, fMRI has identified wide areas of both increased and decreased connectivity across the whole brain (Agosta et al., 2011, 2013; Chiò et al., 2014). Several hypotheses have been taken into account to explain such evidence, including the interpretation of hyper-connectivity as a compensation mechanism or as a by-product of the loss of inhibitory circuitry (Turner and Kiernan, 2012). Furthermore, it was proposed that damage to peripheral areas of the brain might lead more central areas to process information that can no longer be handled locally, resulting in the overload of such areas (Stam, 2014). In primary lateral sclerosis, it was reported that patients with the greatest clinical disability also displayed the highest functional connectivity (Agosta et al., 2014). Interestingly, such increased functional connectivity was widespread, since it was present in the sensorimotor, premotor, prefrontal and talamic regions (Agosta et al., 2014). Furthermore, it was shown in ALS, with combined structural and functional MRI, that regions with lower structural connectivity displayed increased functional connectivity (Douaud et al., 2011). Moreover, patients with a slow rate of progression displayed lower connectivity, thus being more similar to the controls (Douaud et al., 2011). Recently, in a small, MRI based study, cortical excitability (evaluated by the threshold-tracking transcranial magnetic stimulation paradigm) negatively correlated with functional connectivity (Geevasinga et al., 2017).

* Corresponding author at: Department of Engineering, Centro Direzionale, Isola C4, 80143 Naples, Italy.
E-mail address: pierpaolo.sorrentino@uniparthenope.it (S. Pierpaolo).
[1] These authors contributed equally to this paper.

https://doi.org/10.1016/j.nicl.2018.08.001
Received 1 February 2018; Received in revised form 12 July 2018; Accepted 2 August 2018
Available online 04 August 2018

Furthermore, Verstraete et al., by exploring abnormalities of MRI structural and functional connectivity in a cohort of ALS patients compared to healthy controls, noted that the connectedness of the network related directly with the progression rate (Verstraete et al., 2010). In a large, fMRI based study, Schulthess et al. revealed increased functional connectivity, with a topography linked to the spreading of the pTDP-43 pathology (Schulthess et al., 2016). The reported evidence might be compatible with the idea that hyper connectedness might relate to neuronal damage in ALS (Trojsi et al., 2017).

Beside fMRI, relevant information on the functioning of brain networks can be obtained using neurophysiological techniques, such as electroencephalography (EEG) and magnetoencephalography (MEG), since they directly capture the electrical/magnetic activity of the neuronal ensembles (Lopes Da Silva, 2013). In ALS, increased EEG coherence in the theta and gamma band was shown to correlate with structural MRI changes (Nasseroleslami et al., 2017) and EEG based network topological metrics related to disease burden (Fraschini et al., 2016b).

MEG systems measure magnetic fields produced by neuronal activity. Such fields are minimally distorted by the layers surrounding the brain, allowing for a temporally and spatially accurate reconstruction of the neural signals within the brain *(source space)* (Baillet, 2017).

Recently, a source level MEG study based on power confirmed that hyperactivation might be a relevant feature in ALS during eyes opened resting state (Proudfoot et al., 2018). Such evidence would confirm further that the hyperactivation would relate to neuronal damage.

However, MEG can be further exploited extracting the phases of the signals in order to evaluate the amount of information exchanged between brain areas. More precisely, the estimation of the phases allows the quantification of true synchrony, defined as a constant phase difference between time-series in isofrequency (Tass et al., 1998). We chose the phase lag index (PLI) (Stam et al., 2007) to estimate functional connectivity between brain areas, since the PLI quantifies synchronization between time series while being entirely unaffected by the amplitude of the signal. The PLI was also chosen since it is insensitive to volume conduction (at the cost of discarding true zero-lag interactions). Interestingly, the fact that the PLI estimates synchronization rather than simultaneous activations of brain areas, makes the information provided in this paper complementary to the fMRI based evidence and power-based MEG analyses.

Some of the properties of the interactions among brain areas can be captured using Graph Theory (Bullmore and Sporns, 2009). In fact, the human brain can be modelled as a network, with the brain areas as nodes and their interactions as edges. However, comparing such metrics is non-trivial, as they are influenced by network size, thresholding or edge density, not allowing for a purely topological interpretation of the results (van Wijk et al., 2010). The minimum spanning tree (MST) algorithm allows the computation of statistically comparable metrics, while retaining most of the information about the original network (Tewarie et al., 2015). The combination of the PLI and the MST, while it is on the one hand costly in the sense that it might discard some information, on the other hand reduces to a minimum the detection of either artefactual or uninterpretable differences between groups.

On the basis of the available evidence, we hypothesized that the topological alterations associated to different stages of ALS may involve vast areas of the brain and not be confined to motor areas. Secondly, given the evidence showing that hyper-connectedness is related to atrophy and disease progression, we hypothesize that, as the patients reach more advanced stages, the brain network will show a more connected topology accordingly. A more integrated topology implies that it is quicker and/or less costly, on average, to move among the nodes of the network. Lastly, we hypothesize that the alterations will be spread across frequency bands. To test our hypothesis, a large cohort of ALS patients and healthy controls underwent clinical evaluation, MRI and MEG scan. The population was classified into “early” and “advanced” stage on the base of the King's disease staging system (Balendra et al., 2015). In particular, considering the current unavailability of validated markers of disease progression, we chose to base our analyses on such clinical staging system since it seems to reproduce the curvilinear course of disease progression typical of ALS (Gordon et al., 2010) especially in the later stages of the disease (Balendra et al., 2015). Based on the PLI and the MST, we computed topological metrics, focusing on features such as scale-freeness, assortativity and connectedness of the brain networks, as well as on the betweenness centrality of each area, in order to test if such parameters would differ among controls and ALS patients at different disease stages.

2. Material and methods

2.1. Participants

Fifty-four right-handed and native Italian speakers patients (39 males, 15 females; mean age ± SD, 58.84 ± 12.14) with probable or definite ALS, according to the revised El-Escorial criteria of ALS (Brooks et al., 2000), were consecutively recruited at the ALS Center of the First Division of Neurology of the University of Campania “Luigi Vanvitelli” (Naples, Italy). The patients were classified according to the King's disease staging system (Balendra et al., 2015) that is based on the appearance of sequential clinical milestones during the ALS course and does not include cognitive information (i.e., stage 1 = impairment of one body site; stage 2 = impairment of two body sites; stage 3 = impairment of three body sites; stage 4 = non-invasive ventilation or percutaneous endoscopic gastrostomy). In order to improve the group sizes, we classified as “early stage” the patients belonging to King's stage 1 and 2 (9 and 17 patients, respectively) and as “advanced stage” the patients in King's stages 3 and 4 (12 patients in each group). None of the patients showed any mutation in any of the following genes: *SOD1*, *TARDBP*, *FUS/TLS* and *C9ORF72*. More clinical details of participants included in the final analysis (4 patients were dropped out of the analysis as they did not have enough high quality MEG data) are given in Table 1. Twenty-five controls (16 males and 9 females; mean age ± SD, 57.00 ± 9.35), enrolled by “word of mouth”, were age-, −gender and education-matched with the ALS patients. To be included in this study, all participants had to satisfy the following criteria: a) to have no major medical illnesses and not to abuse substances or use

Table 1
Detailed characteristic of patients and controls used for the analysis.

Parameters	ALS “advanced” patients mean (SD) (n = 24)	ALS “early” patients mean (SD) (n = 26)	Controls mean (SD) (n = 25)
Demographic and clinical measures			
Age	59.96 (13.89)	57.50 (10.76)	57 (9.35)
Male/Female	19/5	18/8	16/9
Education	10.46 (4.51)	10.19 (4.09)	11 (4)
Disease duration (months)	61.83 (60.23)	28.77 (20.69)	
ALSFRS-R score	30.70 (8.79)	41.15 (4.80)	
UMN score	8.22 (5.13)	6.46 (4.58)	
Site of onset	6 bulbar	5 bulbar	
	6 UL	11 UL	
	9 LL	9 LL	
	2 UL and LL	1 UL and LL	
	1 respiratory	0 respiratory	
Phenotype	9 classic	9 classic	
	6 predominant LMN	12 predominant LMN	
	9 predominant UMN	5 predominant UMN	
Neuropsychological parameters			
ECAS test (total score)	83.13 (28.08)	91.50 (21.09)	

ALSFRS-R = Amyotrophic Lateral Sclerosis Functional Rating Scale-Revised; ECAS = Edinburgh Cognitive and Behavioural ALS Screen; LL = Lower Limb; LMN = Lower Motor Neuron; UL = Upper Limb; UMN = Upper Motor Neuron.