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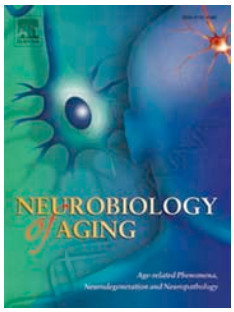

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Functional overlap and divergence between ALS and bvFTD

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Abstract

Amyotrophic lateral sclerosis (ALS) and behavioural variant frontotemporal dementia (bvFTD) lie on a clinical, pathological and genetic continuum. Neuroimaging techniques have proven to be potentially useful to unravel the shared features of these syndromes.

Using resting state functional magnetic resonance imaging (RS-fMRI), we investigated functional connectivity of brain networks in 15 ALS and 15 bvFTD patients in early stages of disease, and 15 healthy controls, looking expressly for connectivity pattern divergence or overlap between the two disorders.

Compared to controls, we found decreased RS-fMRI signals within sensorimotor, right fronto-parietal, salience and executive networks in both patient groups. Within the default mode network, divergent connectivity patterns were observed, with RS-fMRI signals in the posterior cingulate cortex enhanced in bvFTD patients and suppressed in ALS patients.

Our findings confirm that ALS and bvFTD broadly share common RS-fMRI connectivity patterns, probably representing different phenotypical expressions of the same neurodegenerative process, but also differ in the default mode network, probably reflecting a different stage of neurodegeneration.

Keywords: amyotrophic lateral sclerosis; behavioral variant frontotemporal dementia; RS-fMRI; connectivity; pathological continuum

1. Introduction

Amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) are multisystem neurodegenerative disorders, which have been found highly related, occupying two poles of a disease spectrum, with a predominance of motor dysfunction at one end and cognitive symptoms at the other (Clark and Forman, 2006; Neumann et al., 2006). In fact, the two disorders have been recognized as representative of a neuropathological continuum since they share several clinical, genetic and pathogenetic characteristics (Lomen-Hoerth et al., 2002; Murphy et al., 2007; Ling et al., 2013). Genetic and pathological analysis have demonstrated that mutations of TAR DNA binding protein 43 kD (TARDBP) (Benajiba et al., 2009), fused in sarcoma/translocated in liposarcoma (FUS/TLS) (Blair et al., 2010), ubiquilin-2 (UBQLN2) (Deng et al., 2011) and C9ORF72 (Renton et al., 2011; DeJesus-Hernandez et al., 2011) have a key role in the pathogenesis of the ALS-FTD spectrum. Notably, all these genes may share a common link to cellular RNA dynamics (Thomas et al., 2012; Ling et al., 2013). Furthermore, from the phenotypical point of view, up to 50% of sporadic ALS patients display some degree of cognitive impairment, whereas up to 15% of FTD patients display symptoms typical of motor neuron disease (MND) (Lomen-Hoerth et al., 2002; Ringholz et al., 2005), especially in the behavioural variant subtype of FTD (bvFTD) (Lomen-Hoerth et al., 2004).

Despite the broadly described clinical, genetic and pathological overlap between ALS and FTD, structural and functional magnetic resonance imaging (MRI) correlates across this continuum have been poorly explored. Specifically, structural MRI studies, using voxel-based morphometry (VBM), surface-based morphometry (SBM) and diffusion tensor imaging (DTI), have shown in several cohorts of ALS patients a distributed pattern of gray (GM) and white matter (WM) damage in the frontal and temporal lobes (Chang et al., 2005; Ellis et al., 2001; Cirillo et al., 2012; Schuster et al., 2014; d'Ambrosio et al., 2013) revealing significant commonalities with GM and WM abnormalities described in FTD patients (Borroni et al., 2007; Whitwell et al., 2010; Lillo et al., 2012).

With regard to functional MRI (fMRI), only a few studies, exploring the whole-brain functional connectivity of the resting state (RS) [i.e., resting-state fMRI (RS-fMRI) (Biswal et al., 1995; van de Ven et al., 2004)], have been carried out in ALS (Mohammadi et al., 2009; Douaud et al., 2011; Tedeschi et al., 2012; Agosta et al., 2013) and bvFTD patients (Zhou et al., 2010; Whitwell et al., 2011; Filippi et al., 2013; Farb et al., 2013). Specifically, the most consistent RS-fMRI features of the two syndromes were a suppressed connectivity within the sensorimotor network (SMN) (i.e., involving primary and supplementary motor areas) in ALS (Mohammadi et al., 2009; Tedeschi et al., 2012) and a weakening of connectivity within the so-called “salience” network (SLN) (i.e., including the anterior cingulate and orbitofrontal- insular cortices with the respective subcortical structures) in bvFTD (Seeley et al., 2009; Zhou et al., 2010; Farb et al., 2013). By contrast, in both disorders the results about connectivity within other RS networks (RSNs),

such as the default mode (DMN), the lateralized fronto-parietal (FPN) and the executive (EXN) networks, principally relate to cognition and behaviour, have been lacking or proven inconsistent. In particular, for what concerns the DMN (i.e., composed of the posterior cingulate, precuneus, medial prefrontal cortices, and angular gyri), which is putatively associated with internal processing and deactivated during execution of cognitive tasks (Raichle et al., 2001), most authors described an enhanced connectivity within posterior regions of the network in both ALS and bvFTD (Tedeschi et al., 2012; Agosta et al., 2013; Zhou et al., 2010; Whitwell et al., 2011; Farb et al., 2013), while others revealed a reduced connectivity within the whole network in ALS (Mohammadi et al., 2009). However, none of the previous studies expressly investigated whether and to what extent RSNs abnormalities in ALS would differ or overlap with those described in bvFTD. As such, the present study aimed to explore RS-fMRI connectivity changes in a series consecutive patients with ALS and bvFTD compared with healthy controls (HCs). We hypothesized of revealing similar connectivity changes of RSNs across the two syndromes, thus expecting to depict the functional signatures of the broadly described genetic and pathological continuum between ALS and bvFTD.

2. Methods

2.1 Subjects

Thirty right-handed patients, 15 patients (6 M, 9 F; mean age 61.8 ± 11.8) affected by probable or definite ALS according to diagnostic El-Escorial criteria (Brooks et al., 2000) and 15 patients (6 M, 9 F; mean age 61.5 ± 9.8) affected by probable bvFTD according to established criteria (Rascovsky et al., 2011), were recruited consecutively at the Department of Neurology of the Second University of Naples (Naples, Italy).

Probable (9 patients) and definite (6 patients) ALS was diagnosed when combined lower and upper motor neuron dysfunction was identified in bulbar and spinal-innervated regions. All patients had a “classical” phenotype with bulbar signs present in seven subjects. Clinical parameters in all ALS patients we measured by ALS functional rating scale-revised (ALSFRS-R) score, index of disability status (Cedarbaum et al., 1999), and upper motor neuron (UMN) score, measure of pyramidal dysfunction through the evaluation of the number of pathologic reflexes elicited from 15 body sites (i.e., glabellum, masseter, and orbicularis oris, biceps, triceps, finger jerks, knee, ankle, and Babinski responses bilaterally) (Turner et al., 2004) (for more details about clinical characteristics, see Table 1). Respiratory function, measured by forced vital capacity (FVC), was above 70% in all ALS patients and there was no evidence of nocturnal hypoventilation. We excluded from the study patients with dominant lower motor neuron (LMN) impairment, such as progressive muscular atrophy and flail leg syndrome or pseudopolyneuritic form, progressive bulbar palsy, primary lateral sclerosis, post-poliomyelitis ALS and motor neuron disease with major cognitive impairment (e.g., ALS-

Dementia, ALS-FTD).

Probable bvFTD was diagnosed by a comprehensive evaluation including neurological history and examination, neuropsychological testing, structural routine MRI, and functional neuroimaging [i.e., 18F-2-fluoro-2-deoxy-D-glucose (FDG) positron emission tomography (PET) or single photon emission computed tomography (SPECT) with Technetium-99m-bicisate ethyl cysteinate dimer]. Clinical assessment was performed by an experienced neurologist blinded to MRI. History was taken with a structured interview from patients' relatives. All bvFTD patients showed progressive deterioration of behaviour and/or cognition, including disinhibition, apathy, loss of empathy, perseveration, dietary changes, executive dysfunction and frontal and/or anterior temporal atrophy on MRI. Clinical staging of bvFTD was performed using the newly developed Frontotemporal Dementia Rating Scale, a caregiver questionnaire, which assesses patient function and behaviour (Mioshi et al., 2010). None of the bvFTD patients manifested symptoms of ALS. However, UMN score was also estimated in bvFTD patients. We did not include patients with non-progressive syndromes mimicking bvFTD (Kipps et al., 2010).

None of the patients recruited had additional neurological disease or previous mental illness. Age, sex, education, and disease duration were matched for the patient groups (Table 1).

Genetic analysis was available in all patients. Specifically, nobody had familial ALS or bvFTD or was positive for mutations of most genes associated with ALS and/or FTD (i.e. superoxide dismutase 1 or SOD1; TARDBP; FUS/TLS; C9ORF72; microtubule associated protein or MAPT; and progranulin or GRN).

Right-handed HCs were enrolled among the non consanguineous caregivers of patients and by word of mouth. They underwent a multidimensional assessment, including neurological examination and a brief neuropsychological evaluation. Fifteen neurologically and cognitively normal subjects (6 M, 9 F; mean age 62.7 ± 7.3) were included in the study.

Approval was received from the Ethics Committee of the Second University of Naples and informed consent was obtained from all participants or their legal caregivers according to the Declaration of Helsinki.

2.2 Cognitive and Behavioral Screening

All 45 subjects included in the study underwent Addenbroke's Cognitive Examination Revised (ACE-R), a sensitive and specific battery to detect early cognitive impairment and dementia (Mioshi et al., 2006). Changes in behaviour of all patients were measured by the Frontal Systems Behavior (FrSBe) Scale (Grace et al., 1999), a 46-item behaviour rating scale for both patients and caregivers, designed to provide a total frontal disturbance score (T score) and three subscale scores (or subscores) which allow to assess apathy, disinhibition, and executive dysfunction. It quantifies behavioral

changes over time by combining retrospective and current assessments of frontal dysfunction (T > 65 is defined as impaired behaviour and executive functions). In our population we considered T scores and subscores derived from caregivers and referring to the present time (Table 1). Moreover, Frontal Assessment Battery (FAB) (Dubois et al., 2000) and phonemic and semantic fluency tests (Novelli et al., 1986) were administered to all patients to assess executive functions.

BvFTD patients underwent further neuropsychological tests performed by experienced neuropsychologists (M.d.S., D.B.) blinded to the MRI results to evaluate: long and short term verbal memory with memory prose (Novelli et al., 1986) and digit span (Orsini et al., 1987); long and short term spatial memory with Rey's figure delayed recall test (Caffarra et al., 2002) and spatial span (Orsini et al., 1987); visuo-spatial abilities with the Rey's Figure Copy Test (Caffarra et al., 2002); and attention with attentive matrices (Spinnler and Tognoni, 1987).

2.3 Magnetic resonance imaging

Magnetic-resonance images were acquired on a 3T scanner equipped with an 8-channel parallel head coil (General Electric Healthcare, Milwaukee, Wisconsin). Functional MRI data consisted of 240 volumes of a repeated gradient-echo echo planar imaging T2*-weighted sequence (TR = 1,508 ms, axial slices = 29, matrix = 64 x 64, field of view = 256 mm, thickness = 4 mm, interslice gap = 0 mm). During the functional scan, subjects were asked to simply stay motionless, awake, and relax, and to keep their eyes closed. No visual or auditory stimuli were presented at any time during functional scanning. Three-dimensional T1-weighted sagittal images (GE sequence IR-FSPGR, TR = 6,988 ms, TI = 1,100 ms, TE = 3.9 ms, flip angle = 10, voxel size = 1 x 1 x 1.2 mm3) were acquired in the same session to have high-resolution spatial references for registration and normalization of the functional images.

2.4 Resting-state fMRI analysis

2.4.1 fMRI data preparation and preprocessing

Standard functional image data preparation and preprocessing, statistical analysis and visualization were performed with the software BrainVoyager QX (Brain Innovation BV, Maastricht, the Netherlands). Data preprocessing included the correction for slice scan timing acquisition, a 3D rigid body motion correction based on a 6-parameter rigid-body alignment to correct for minor head movements and the application of a temporal high-pass filter with cut-off set to three cycles per time course. Translational motion parameters were verified to be always less than one functional voxel for all included participants. Structural and functional data were co-registered and spatially normalized to the Talairach standard space using a 12-parameter affine transformation. During this procedure, the functional images were resampled

to an isometric 3 mm grid covering the entire Talairach box. Finally, the resulting image time series were spatially smoothed with a 6 mm full-width-half-maximum (FWHM) isotropic Gaussian kernel.

2.4.2 Resting-state network functional connectivity analysis

To extract RSN maps, single-subject and group-level independent component analysis (ICA) (Hyvärinen et al., 2001) were carried out on the preprocessed functional time series using two plug in extensions of BrainVoyager QX respectively implementing the fast ICA algorithm (Hyvärinen et al., 2001) and the self-organizing group ICA algorithm (sog-ICA) (Esposito et al., 2005).

For each single subject, 40 independent components were extracted (corresponding to one-sixth of the number of time points) (Greicius et al., 2007) and scaled to spatial z-scores (i.e. the number of standard deviations of their whole-brain spatial distribution). To generate group components and allow for population-level inferences in each RSN, all individual component maps from all subjects were "clustered" in the subject space according to the mutual similarities of their whole-brain distributions using the sog-ICA algorithm. Thereby, all 40 individual independent components were uniquely assigned to one out of 40 "clusters" of independent components. Once the components belonging to a cluster were retrieved, the corresponding maps were averaged and the resulting map was assumed as the representative of the cluster. The 40 single-group average maps were visually inspected to recognize the spatial patterns associated with the main RSNs (Smith et al., 2009; Mantini et al., 2007) and to remove components clearly related to artifacts. To this purpose, single-group one-sample t-tests were used to analyze the whole-brain distribution of the components in each group separately and the resulting t-maps were thresholded at p=0.05 (Bonferroni corrected over the entire brain). From this analysis, an inclusive mask was also created from the union of the three single-group maps and used to define a new search volume for within-network two-group comparisons. To correct the resulting t-maps for multiple comparisons, regional effects within the search volume were only considered significant for compact clusters after the joint application of a voxel- and a cluster-level threshold. The cluster-level threshold was estimated non-parametrically with a randomization approach: starting from an initial (uncorrected) threshold of p=0.005 applied to all voxels, a minimum cluster size was calculated that protected against false positive clusters at 5% after 500 Montecarlo simulations (Forman et al., 1995). We also computed the false discovery rate (FDR, Genovese et al., 2002) for the entire distribution of voxel values and, in all cases, the used initial voxel-level threshold resulted in an FDR level below 5%. However, we did not use the FDR as general thresholding mechanism, because this would have led to different voxel-level significance across different RSNs.

In both groups of patients, the correlations between the RS-fMRI abnormalities, expressed as mean z-scores of the

voxel clusters showing abnormal RS-fMRI signal fluctuations, and the disability (i.e., ALSFRS-R; UMN score) and neuropsychological variables (i.e., executive test performances; FrSBe scale T-score and subscores for apathy, dysinhibition and executive dysfunction) were assessed by calculating Pearson's correlation coefficients. P values < 0.05, after correction for multiple comparisons with the Bonferroni method, were considered statistically significant for these correlation analyses.

2.5 Regional atrophy measurements: voxel-based morphometry

To control for the confounding influences of GM atrophy on RS signal, we performed a VBM analysis using the VBM8 toolbox (http://dbm.neuro.uni-jena.de/vbm.html) of SPM8 software package (http://www.fil.ion.ucl.ac.uk/spm/) with default parameters incorporating the DARTEL toolbox in order to obtain a high-dimensional normalization protocol (Ashburner, 2007). Data processing was carried out according to a previously described protocol (Farb et al., 2013). In particular, anatomical images were created using the non-linear components of the model, thereby controlling for the linear transformations of global brain size and orientation while displaying local, nonlinear differences in GM volume. Moreover, modulated images were smoothed with a 6 mm full-width half maximum Gaussian kernel to create the final probability maps. GM atrophy results of comparisons between bvFTD and ALS patients and HCs were familywise error (FWE) corrected at a level of p FWE<.05 and covaried for age and total intracranial volume (TIV).

3. Results

Neither of the patients groups differed significantly from the control group or one from each other in demographic variables such as sex, age or education (Table 1). Importantly, disease duration did not differ between the ALS and bvFTD groups. Both cohorts of patients showed a mild stage of disease disability: with regard to ALS patients, mean score of ALSFRS-R was > 22, while 13 of bvFTD patients show a mild grade of disability and 2 a moderate grade of disability according to Frontotemporal Dementia Rating Scale Stage.

The patients included in the study were able to perform all the scheduled tests, except for two ALS patients, who had difficulties in performing the FAB and were not able to copy overlapping pentagons. Cognitive assessment by ACE-R showed lower scores for bvFTD patients compared with ALS ($p<0.001$) and HCs ($p<0.001$). Moreover, FAB scores resulted significantly reduced in bvFTD patients compared with ALS patients ($p=0.01$). By contrast, FrSBe scale T scores were significantly higher than normal values in all patients, indicating behavioral dysfunction in both groups, without significant differences between ALS and bvFTD patients in both total scores and subscores (Table 1).

The main RSN components were uniquely identified within the set of estimated independent components from the

single-group maps with similar (Damoiseaux et al., 2006) or identical (Mantini et al., 2007) methodologies used in previous studies. Among these, SMN, DMN, SLN, right and left FPNs (R- and L-FPNs) and EXN components exhibited statistically significant regional group effects in their spatial distribution (Figures 1-5). In particular, the FPNs, including regions subserving attention, executive processing, planning, and working memory, were found as lateralized in left and right one (Fig. 3 a-f), according to previous evidence (Damoisieaux et al., 2006, Seeley et al., 2007; Agosta et al., 2012), whereas two more bilateral networks, including dorsal anterior cingulate and dorsolateral prefrontal, anterior insular, and inferior parietal cortices, were labeled as EXN (Fig. 4 a-c) and SLN (Fig. 5 a-c) (Cole et al., 2012; Filippi et al., 2013).

The SMN showed significant group differences in bilateral primary motor cortex (PMC) and in supplementary motor cortex (SMC), where the network-level RS-fMRI fluctuations resulted suppressed in both ALS and bvFTD patients compared with HCs. However, while in ALS patients the suppression was strongly confined to the precentral gyri, in bvFTD patients the effect started from the precentral gyri and extended towards the temporal cortex (Fig. 1 d, e).

The DMN showed significant group differences in posterior cingulate cortex (PCC) in both ALS and bvFTD patients. However, while ALS patients exhibited reduced RS-fMRI fluctuations, bvFTD patients exhibited increased RS-fMRI in the PCC. In addition, bvFTD patients, but not ALS patients, exhibited decreased connectivity in the frontal part of the DMN (Fig. 2 d, e).

The R-FPN exhibited two clusters of decreased RS connectivity in the right superior frontal gyrus (SFG) in bvFTD patients and in the right supramarginal gyrus (SMG) in both bvFTD patients and ALS compared with HCs, although with a more marked decrease of network-specific RS-fMRI fluctuations in bvFTD patients (Fig. 3 g, h).

For what concerns EXN and SLN, network-specific RS-fMRI fluctuations were significantly reduced in both bvFTD and ALS patients compared with HCs, respectively in the middle frontal cortex (albeit with different lateralization) (Fig. 4 d, e) and in the medial prefrontal cortex and in the insula (Fig. 5 d, e).

With regard to correlation analyses between RS-fMRI abnormalities and disability and neuropsychological variables in both groups of patients, we found that in ALS patients phonemic fluency scores were inversely related to RS-fMRI z-scores in the SMG within the R-FPN ($r=-0.55$; $p=0.03$) and in the medial prefrontal cortex within the SLN ($r=-0.56$; $p=0.02$), and semantic fluency scores were inversely related ($r=-0.53$; $p=0.03$) to z-scores in the right middle frontal cortex within the EXN. In bvFTD patients we revealed that the apathy subscore of the FrSBe scale was inversely related ($r=-0.57$; $p=0.02$) to RS-fMRI z-scores in the medial frontal cortex (MFC) within the DMN, while FAB scores were positively related to z-scores in this frontal area of the DMN. No significant correlations were observed between the clinical disability scores evaluated (i.e., UMN and ALSFRS-R scores) and RS-fMRI z-scores within the SMN in the

two groups of patients.

The whole-brain VBM analysis produced some clusters of locally reduced GM volume in the medial frontal gyrus, bilaterally, and in the right middle temporal gyrus comparing bvFTD patients to HCs ($p < 0.001$, cluster size>100 voxels, uncorrected level of significance). No significant clusters of regional GM atrophy were found in ALS patients compared to HCs.

4. Discussion

The present study offers several important contributions about functional connectivity changes across the ALS-FTD continuum, identifying for the first time both similar and distinctive features of the connectivity patterns in early stages of ALS and bvFTD. In fact, in the sensorimotor domain, we found a strong functional signature of neuronal degeneration, not only in ALS, but also in bvFTD. In the cognitive domain, we described similar patterns of decreased connectivity in R-FPN, EXN and SLN, but also divergent connectivity patterns in the DMN, with decreased connectivity in the posterior part of the network in ALS and in the frontal part of the network in bvFTD. Thus, our findings reinforce the concept of a continuum between ALS and bvFTD not only on a pathological and genetic, but also on a functional connectivity level.

To our knowledge, this is the first study reporting a significant reduction of RS-fMRI connectivity in SMN, especially in PMCs, in both ALS and bvFTD. While these findings are not surprising for what concerns ALS patients, being consistent with previous RS-fMRI data (Mohammadi et al., 2009; Verstraete et al., 2010; Tedeschi et al., 2012), of particular interest is our RS-fMRI evidence of motor system dysfunction in a bvFTD population with mild degree of disability and in early stages of disease. To date, there is only another functional evidence of motor system degeneration in FTD that derives from a recent neurophysiological analysis by Burrell et al. (2011). Therefore, in our bvFTD population we may hypothesize that the reduced amount of network-specific RS-fMRI fluctuations in SMN, involving but not limited to PMC (as, conversely, has been shown in ALS patients), may reflect an asymptomatic upper motor neuron dysfunction, unapparent in early stages of disease in comparison to behavioural disturbs. Interestingly, our results concerning SMN confirm the increasing immunohistochemical (Wilson et al., 2001; Kawashima et al., 2001; Geser et al., 2008, 2009) and neuroimaging (Lillo et al., 2012; Schuster et al., 2014) evidence in favor of degeneration of similar neural networks across the whole ALS-FTD spectrum, involving both motor and temporal areas and their afferents and efferents in both syndromes, with a more pronounced and extensive impairment of extra-motor areas in presence of cognitive or behavioural symptoms. In fact, these findings may be interpreted as effects of the different pathophysiological evolution of the same neurodegenerative process probably underlying both diseases, which might

exhibit differentiated mechanisms of spreading in each syndrome, causing various phenotypical presentations in relationship to the cerebral areas involved.

In the present study other relevant commonalities of RS-fMRI connectivity patterns between ALS and bvFTD patients were detected in most RSNs concerning cognitive functions. Specifically, the reduced connectivity in R-FPN, EXN and SLN found in ALS patients compared with HCs mirrored the RS-fMRI connectivity patterns seen in the same RSNs in bvFTD patients, although the alterations were more marked in the bvFTD group. In particular, with regard to the reduced connectivity within the R-FPN reported in our ALS group, this finding resembles what has been previously described by Tedeschi et al. (2012) in another cohort of more disable ALS patients with executive dysfunction. However, we did not found a pattern of increased RS-fMRI signal of inferior parietal cortices and middle cingulum within the L- and R-FPNs, as reported by Agosta et al. (2013). Probably, this discordance may be due to the different clinical characteristics of the ALS populations enrolled. Specifically, our ALS population differed from the ALS group studied by Agosta et al. (2013) for the evidence of behavioural dysfunctions, albeit the two samples of patients were similar for global disability status, disease duration and dysexecutive symptoms.

On the other hand, the biological significance of an increased connectivity between brain regions is still poorly understood, although some hypotheses have been formulated. First, it is conceivable that an enhanced functional recruitment of regions that are relatively unaffected by the ALS pathology, such as parietal cortices, may be attributable to an attempt to maintain an efficient cognitive performance overcoming loss of function in primary motor and/or motor imagery-specific networks (Lulè et al., 2007; Agosta et al., 2013; Tedeschi et al., 2012). Alternatively, an enhanced functional connectivity may be secondary to a loss of cortical inhibitory neurons, probably involved in ALS pathogenesis (Douaud et al., 2011; Turner et al., 2005). Speculatively, our group of ALS patients might have been examined in an early stage of ALS pathology, when network rearrangements and/or loss of cortical inhibitory modulation, leading to FPNs hyperactivity, were probably still unapparent.

With regard to RSN connectivity in the cognitive domain of our bvFTD group, the findings of a reduced amount of RS-fMRI fluctuations within SLN, EXN and R-FPN are in keeping with several previous studies and contribute to identify a peculiar functional pattern for bvFTD, mainly characterized by a selective impairment of the frontoinsular connectivity (Seeley et al., 2007, 2008, 2009; Zhou et al., 2010; Whitwell et al., 2011; Filippi et al., 2013; Farb et al., 2013). It is worth noting that in our ALS patients we found altered RS-fMRI signals within both EXN and SLN, similarly to what revealed about EXN and SLN in bvFTD patients. However, the significant correlations which we found in both ALS and bvFTD patients between executive and behavioural scores (i.e., FAB, FrSBe scale subscore for apathy and phonemic and semantic fluency scores) and RS-fMRI z-scores within the cognitive domain suggest a

dissimilar modulation of brain connectivity changes in the two groups of patients in response to a different degree of cognitive and behavioural dysfunctions. Specifically, the negative correlations observed in our ALS patients between executive scores and RS-fMRI z-scores within R-FPN, EXN and SLN induce to hypothesize an attempt to compensate the mild impairment of executive performances found in these patients with an enhancement of RS-fMRI connectivity within extra-motor networks. Conversely, in our bvFTD patients, who showed a positive correlation between FAB scores and z-scores in MFC within DMN, we speculated that this mechanism of functional compensation was already disappeared in presence of a more pronounced impairment of cognitive networks. Notably, the small number of the examined patients may have represented a significant limitation for our correlation analyses and, thus, further studies on larger and well-characterized populations of patients (i.e., considering many more parameters, such as clinical, genetic and neuropsychological data) could be more effective for identifying radiological markers of extra-motor degeneration in the ALS-FTD continuum.

For what concerns our data about connectivity changes within the DMN, ALS and bvFTD groups resulted different in the posterior part of the network (i.e., PCC), where only bvFTD patients showed an enhanced RS-fMRI signal, and in its frontal part, where only bvFTD patients exhibited a reduced connectivity, in line with previous findings on other cohorts of bvFTD patients (Whitwell et al., 2011; Zhou et al., 2010). By contrast, in ALS RS-fMRI findings about altered connectivity within DMN are poorly consistent (Mohammadi et al., 2009; Tedeschi et al., 2012; Agosta et al., 2013). Undoubtedly, as discussed above about the lacking detection of parietal hyperactivity within R-FPN in our ALS population, the early stage of disease which characterized the ALS and bvFTD samples examined may have determined a more marked divergence of DMN pathways between the two groups of patients. Probably, a convergence between the two patterns might become manifest in more advanced stages of ALS pathology, when hypothetical RSNs rearrangements might be triggered by activation of neural plasticity mechanisms (Lulè et al., 2007; Tedeschi et al., 2012; Agosta et al., 2013), thereby inducing an up-regulation of networks connectivity, similarly to what observed in the posterior part of DMN in bvFTD from early stages of disease (Zhou et al., 2010; Whitwell et al., 2011).

In terms of cortical atrophy, our GM findings in both ALS and bvFTD patients resemble previous VBM results, since we revealed some clusters of GM atrophy, bilaterally, in medial frontal cortex of bvFTD subjects compared with HCs, and no significant differences of GM regional volumes comparing ALS patients with HCs. The lack of significant results in ALS patients are not surprising when taking into account the early stage of disease considered. In fact, anatomical changes in the frontal cortex have been mostly reported in moderate and advanced stages of pathology (Lillo et al., 2012; Yang et al., 2011). Furthermore, Tedeschi et al. (2012) revealed that in advanced stages of ALS significant clusters of reduced RS-fMRI connectivity within SMN and R-FPN resulted strictly adjacent to regions of reduced GM

volume, while in the posterior part of DMN, where RS-fMRI signals were found increased in older patients, no cluster of GM atrophy was detected by VBM. Therefore, ours, as well as previous, structural and functional evidence may suggest that alterations of functional connectivity in neurodegenerative diseases, in terms of both enhanced and reduced connectivity within RSNs, develop before cortical atrophy or clinical symptoms become manifest.

This study has a major limitation related to sample size and characteristics of patients studied. In fact, the recruitment of bvFTD and ALS patients in early stages of disease, although useful for depicting RS-fMRI connectivity divergence or overlap between the two syndromes at clinical onset, unavoidably impeded to investigate the whole ALS-FTD spectrum of pathologies which would have required the inclusion of patients at later stage of disease or other phenotypes. It is to take into account that, although both groups of patients studied did not significantly differ for disease duration, for a better comparison between the two disease models, the common early clinical stage of disease, as defined by the disability evaluation performed in both ALS and bvFTD, and not merely the disease duration has been considered. However, we did not directly compare the RS-fMRI patterns revealed in ALS with those identified in bvFTD patients. In fact, a direct comparison between the two diseases may probably be affected by their divergent pathophysiological and clinical features, which principally determine dissimilar clinical courses between ALS and bvFTD, thereby making difficult to identify cohorts of patients with these two syndromes exhibiting common stages of pathology.

In conclusion, the present work confirms the importance and the potential impact of RS-fMRI, as a noninvasive technique, to explore whole-brain functional connectivity in degenerative diseases like ALS and FTD whose neurobiological mechanisms are only partially known and, for some aspects, probably converging. Overall, our findings suggest that ALS and bvFTD share common connectivity patterns, corroborating the theory that they probably constitute different phenotypical expressions of the same neurodegenerative process.

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Figure legends

Fig. 1 Sensorimotor network (SMN) in bvFTD and ALS patients compared to HCs. (a-c) Representative images in the three planes of the spatial maps of the SMN in HCs (a), ALS (b) and bvFTD (c) patients. (d, e) Z-maps of statistically significant disease effects within the SMN ($p<0.005$, cluster-level corrected) overlaid on the average Talairach-transformed T1 image (sagittal, coronal and axial cuts) in bvFTD (d) and ALS (e) patients compared to HCs. In ALS patients (e) RS-fMRI signal suppression is principally confined to the precentral gyri and supplementary motor areas, while in bvFTD patients (d) this effect start from motor areas and extend towards the temporal cortex.

Fig. 2 Default mode network (DMN) in bvFTD and ALS patients compared to HCs. (a-c) Representative images in the three planes of the spatial maps of the DMN in HCs (a), ALS (b) and bvFTD (c) patients. (d, e) Z-maps of statistically significant disease effects within the DMN ($p<0.005$, cluster-level corrected) overlaid on the average Talairach-transformed T1 image (sagittal, coronal and axial cuts) in bvFTD (d) and ALS (e) patients compared to HCs. Compared with HCs, ALS patients (e) exhibit reduced RS-fMRI fluctuations in posterior cingulate cortex (PCC), while bvFTD patients (d) exhibit increased connectivity in the PCC with decreased RS-fMRI signals in the frontal part of the network.

Fig. 3 Right and left frontoparietal networks (R-, L-FPNs) in bvFTD and ALS patients compared to HCs. (a-f) Representative images in the three planes of the spatial maps of the R- and L-FPNs in HCs (a, d), ALS (b, e) and bvFTD (c, f) patients. (g, h) Z-maps of statistically significant disease effects within R-FPN ($p<0.005$, cluster-level corrected) overlaid on the average Talairach-transformed T1 image (sagittal, coronal and axial cuts) in bvFTD (g) and ALS (h) patients compared to HCs. Both groups of patients exhibit decreased RS connectivity in the right supramarginal gyrus (SMG) compared with HCs.

Fig. 4 Executive network **(**EXN) in bvFTD and ALS patients compared to HCs. (a-c) Representative images in the three planes of the spatial maps of the EXN in HCs (a), ALS (b) and bvFTD (c) patients. (d, e) Z-maps of statistically significant disease effects within EXN ($p<0.005$, cluster-level corrected) overlaid on the average Talairach-transformed T1 image (sagittal, coronal and axial cuts) in bvFTD (d) and ALS (e) patients compared to HCs. Both groups of patients exhibit significantly reduced network-specific RS-fMRI fluctuations in the middle frontal cortex, although with different lateralization, compared with HCs.

Fig. 5 Salience network (SLN) in bvFTD and ALS patients compared to HCs. (a-c) Representative images in the three planes of the spatial maps of the SLN in HCs (a), ALS (b) and bvFTD (c) patients . (d, e) Z-maps of statistically significant disease effects within SLN ($p<0.005$, cluster-level corrected) overlaid on the average Talairach-transformed

T1 image (sagittal, coronal and axial cuts) in bvFTD (d) and ALS (e) patients compared to HCs. Both groups of patients exhibit significantly reduced network-specific RS-fMRI fluctuations in the medial prefrontal cortex and insula, although with a more marked decrease connectivity in bvFTD patients compared with HCs.

Table 1 Detailed patients and controls characteristics.

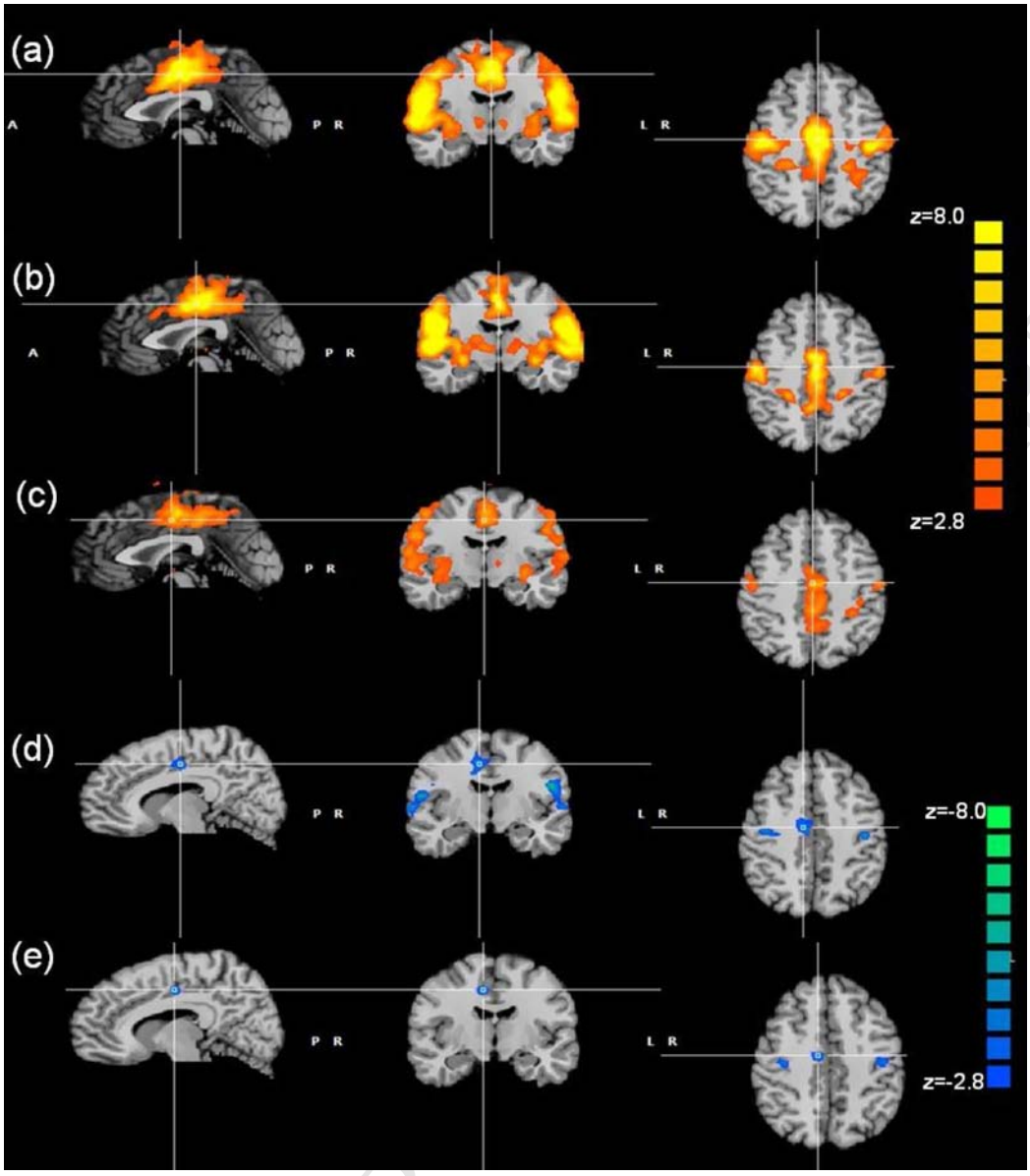
Clinical and neuropsychological features	ALS patients	bvFTD patients	Healthy controls
Mean age (years ± SD) (range)	61.8 ± 11.8 (36-80)	61.5 ± 9.8 (47-76)	62.7 ± 7.3 (52-78)
Gender (male: female)	6:9	6:9	6:9
Education	8.6 ± 4.2 (3-18)	8 ± 2.9 (5-13)	8.9 ± 4.6 (5-18)
Mean disease duration (months ± SD) (range)	24 ± 10.8 (6-36)	31.2 ± 10.8 (12-48)	-
El Escorial criteria (ALS patients) (probable: definite)	9:6	-	-
Clinical onset (bulbar: upper limbs: lower limbs)	0:5:10		
ALS FRS-R (mean ± SD) (range)	35.6 ± 7.4 (18-44)	-	-
UMN score (mean ± SD) (range)	9.7 ± 3.8 (2-15)**	6.7 ± 3 (2-14)**	-
ACE-r (cut off 88) (mean ± SD) (range)	83.06 ± 12.3 (64-99)**	66.8 ± 9 (56-84)**	90.2 ± 5.4 (88-98)°
FAB (raw) (cut off 13.5) (mean ± SD) (range)	8.8 ± 2.6 (6-14)**	6.5 ± 2.1 (3-11)**	-
Phonemic verbal fluency (cut off 17) (mean ± SD) (range)	23.4 ± 6.3 (14-38)**	12.2 ± 3 (8-16)**	-
Semantic verbal fluency (cut off 25) (mean ± SD) (range)	26.9 ± 5.8 (12-34)**	16 ± 3.6 (11-23)**	-
FrSBe scale			
Total score* (mean ± SD) (range)	116.6 ± 19.6 (81-148)	133.3 ± 30.6 (95-184)	-
Apathy subscore (mean± SD) (range)	37.2 ± 7 (28-47)	45.5 ± 12.8 (25-65)	-
Disinhibition subscore (mean± SD) (range)	32 ± 9.8 (21-38)	34.7 ± 8.9 (21-49)	-
Executive dysfunctions subscore (mean± SD) (range)	47.3 ± 11.7 (25-70)	54.4 ± 12.7 (34-75)	-

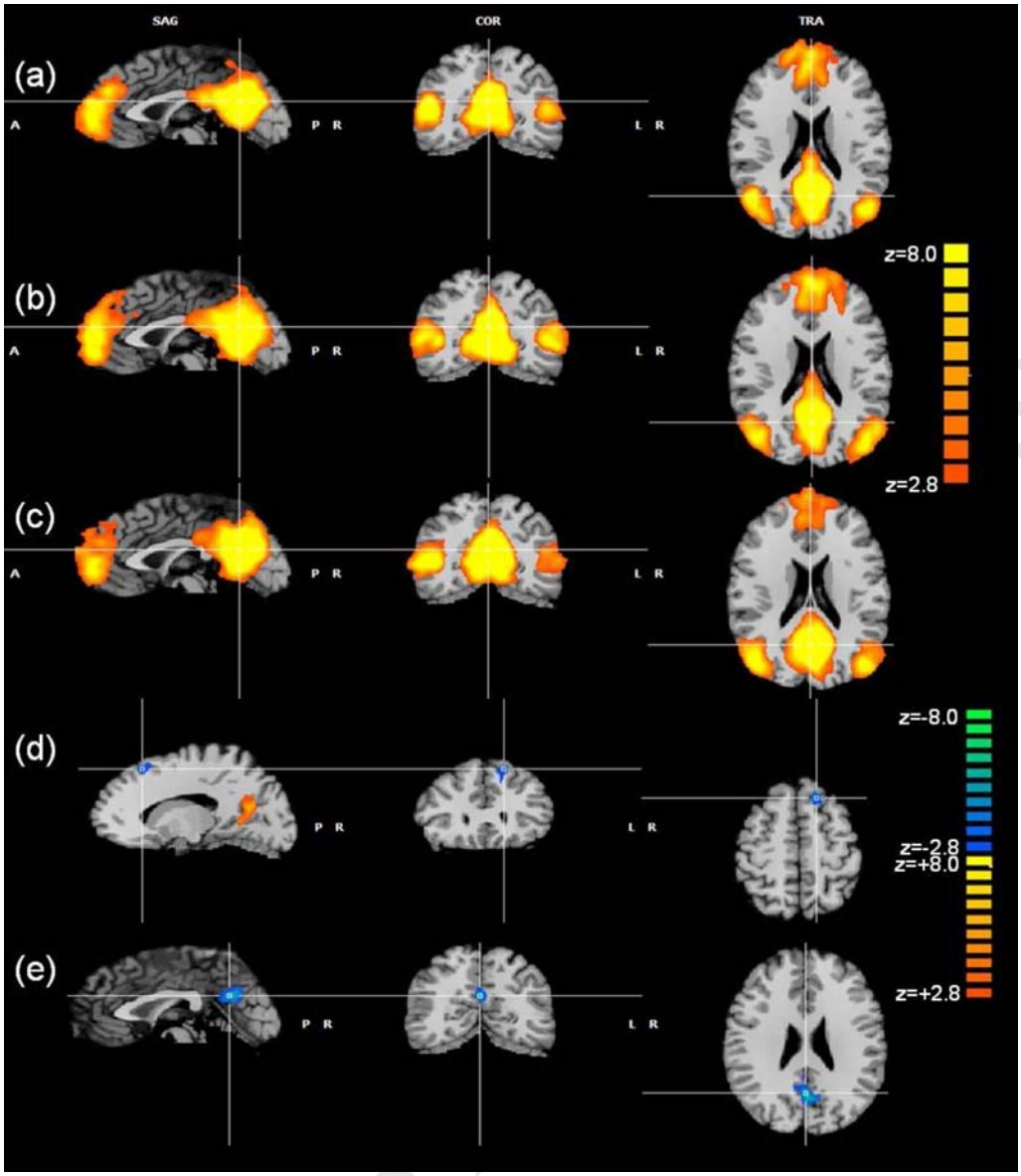
ACE-r = Addenbrooke's cognitive examination revised; ALS FRS-R = amyotrophic lateral sclerosis functional rating scale-revised; bvFTD = behavioural variant fronto-temporal dementia; FAB = frontal assessment battery; FrSBe = frontal systems behaviour; UMN = upper motor neuron

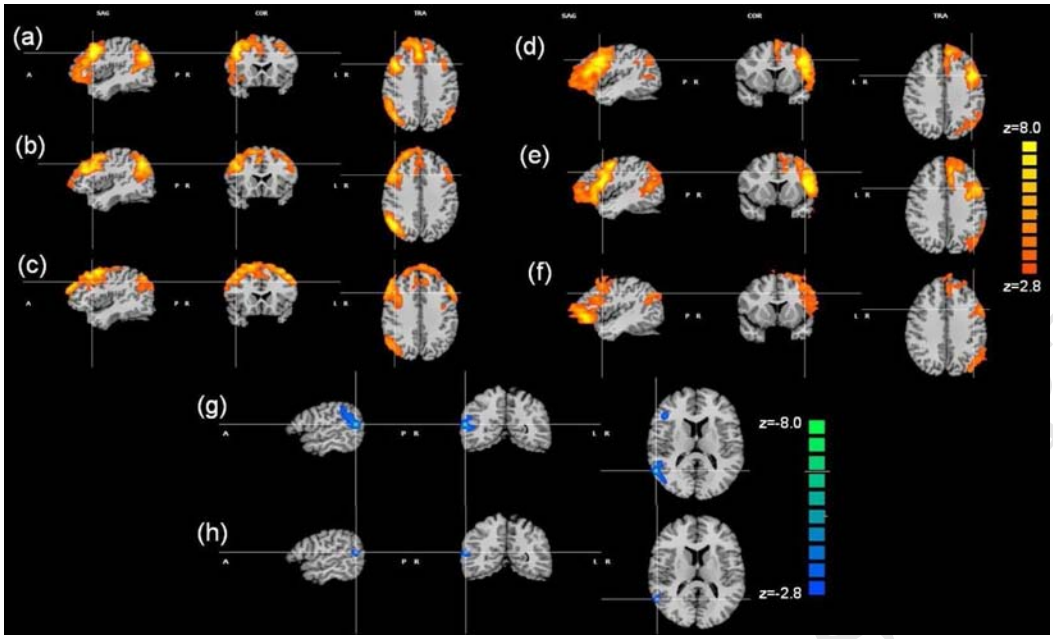
*Total score > 65 is defined as impaired behavior and executive functions (Grace *et al.*, 1999).

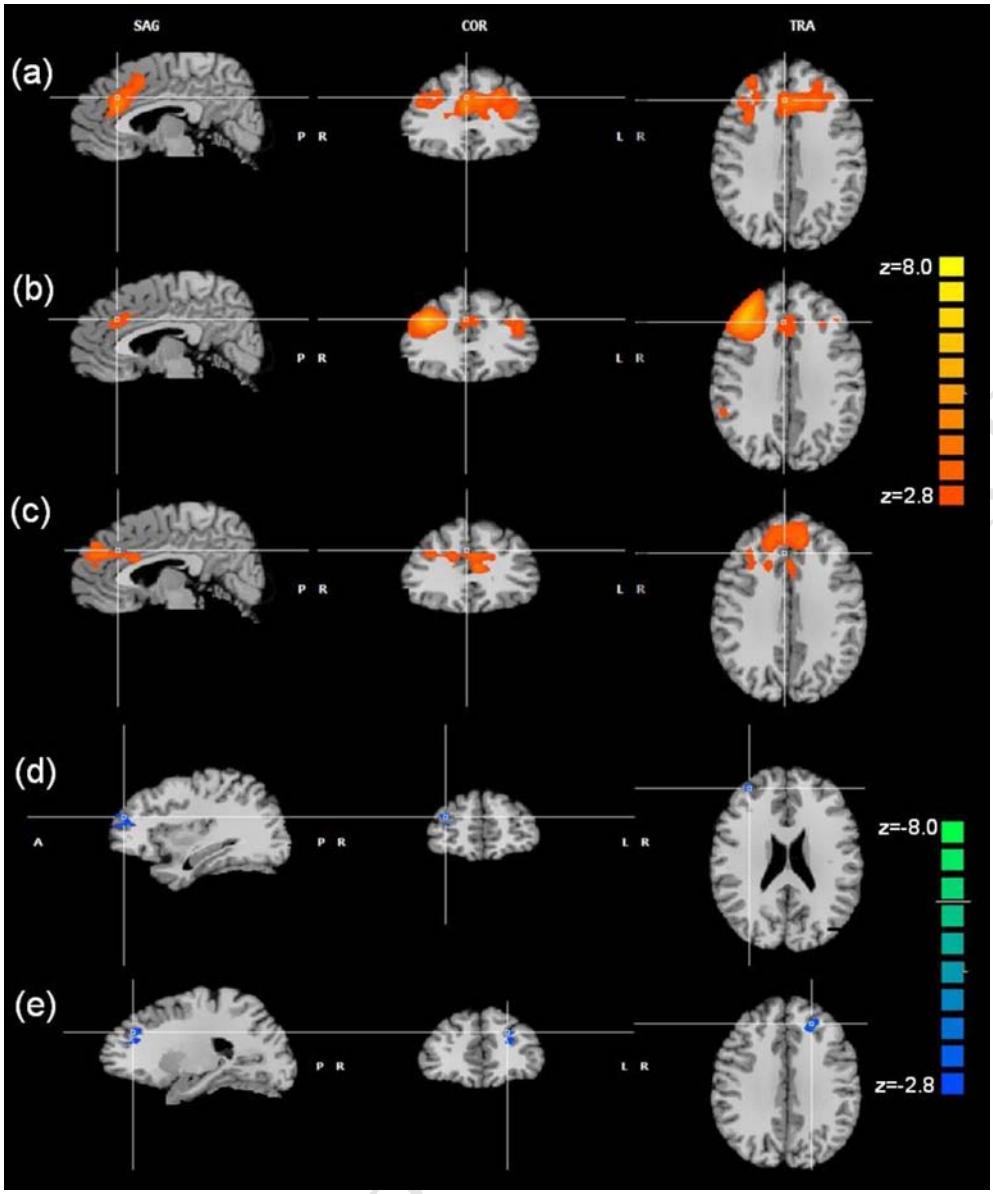
**Significant between-group differences comparing clinical and neuropsychological scores in ALS versus bvFTD patients ($p < .05$) (two sample t-test).

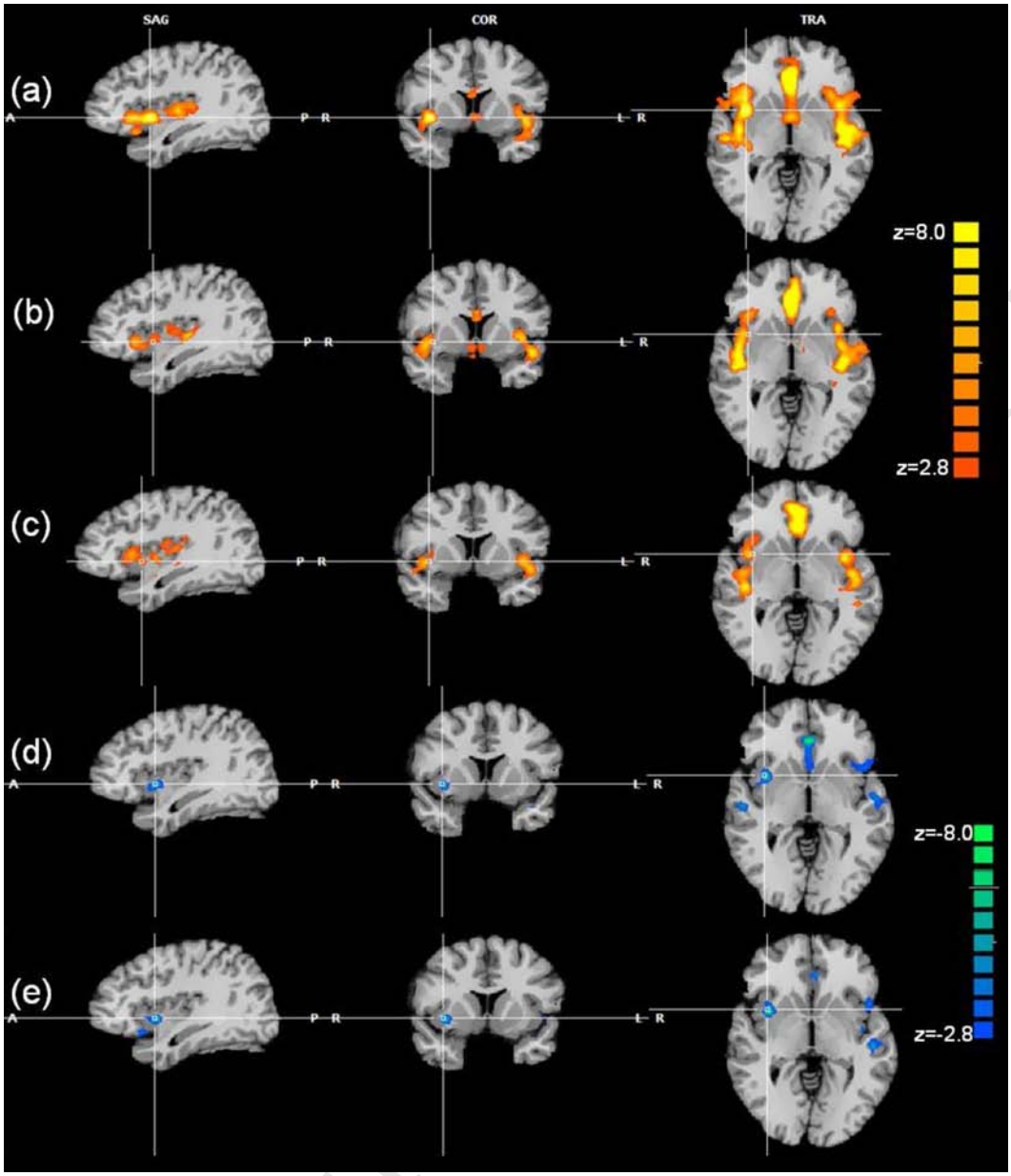
° Significant between-group difference comparing ACE-r scores in bvFTD patients versus healthy controls ($p=4.1*10^{-10}$) (two sample t-test).











Highlights

- we investigated brain connectivity networks in both ALS and bvFTD patients;
- we examined brain connectivity patterns in patients compared to healthy controls;
- we found decreased RS-fMRI signals within several networks in both patient groups;
- we identified both diverging and overlapping aspects between the two syndromes;
- we argued that the two diseases belong to a common functional continuum.