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Pallidal functional connectivity changes are associated with disgust recognition in pure motor amyotrophic lateral sclerosis

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ABSTRACT

In the present study, we aimed to investigate the resting-state functional connectivity (RS-FC) of the globus pallidus (GP) in patients with amyotrophic lateral sclerosis (ALS) compared to healthy controls, and the relationship between RS-FC changes and disgust recognition. Twenty-six pure-motor ALS patients and 52 healthy controls underwent RS functional MRI and a neuropsychological assessment including the Comprehensive Affect Testing System. A seed-based RS-FC analysis was performed between the left and right GP and the rest of the brain and compared between groups. Correlations between RS-FC significant changes and subjects' performance in recognizing disgust were tested. Compared to controls, patients were significantly less able to recognize disgust. In ALS compared to controls, the seed-based analysis showed: reduced RS-FC between bilateral GP and bilateral middle and superior frontal and middle cingulate gyri, and increased RS-FC between bilateral GP and bilateral postcentral, supramarginal and superior temporal gyri and Rolandic operculum. Decreased RS-FC was further observed between left GP and left middle and inferior temporal gyri and bilateral caudate; and increased RS-FC was also shown between right GP and left lingual and fusiform gyri. In patients and controls, lower performance in recognizing disgust correlated with reduced RS-FC between left GP and left middle and inferior temporal gyri. In pure-motor ALS patients, we demonstrated altered RS-FC between GP and the rest of the brain. The reduced left pallidum-temporo-striatal RS-FC may have a role in the lower ability of patients in recognizing disgust.

1. Introduction

Amyotrophic lateral sclerosis (ALS) is a fatal and heterogeneous neurodegenerative disease of the motor system and its wider connections in which the possible presence of cognitive and/or behavioural symptoms is a universally known neuropsychological feature (McKenna et al., 2021). In the past few years, a growing literature focused on the study of social cognition in ALS, from emotional processing to others' intention attribution. Emotional and social deficits in ALS have a great clinical impact, since they may influence the quality of life of patients and increase caregiver burden (Caga et al., 2019). Several studies on emotion perception impairment reported that ALS patients have a diminished psychophysiological arousal to emotional stimuli and difficulties in recognizing and attributing emotions (Andrews et al., 2017; Crespi et al., 2014; Girardi et al., 2011; Oh et al., 2016; Savage et al., 2014; Zimmerman et al., 2007), judging socio-emotional stimuli as more

Abbreviations: RS-FC, resting-state functional connectivity; GP, globus pallidus; CATS, Comprehensive Affect Testing System; RS fMRI, RS functional MRI.

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positive than healthy controls (Lule et al., 2005). In particular, the emotions that ALS patients, with and without cognitive impairment, mostly fail to recognise are disgust and surprise (Bora, 2017), followed by fear (Aho-Ozhan et al., 2016), anger (Oh et al., 2016; Zimmerman et al., 2007), and sadness (Martins et al., 2019).

ALS's failure in both emotion recognition and attribution has been mainly ascribed to the degeneration of fronto-striatal, temporal and occipital brain regions and their white matter (WM) connections. In ALS populations in which nearly the 30 % of patients were cognitively/behaviourally impaired, one voxel-based morphometry study found positive correlations between emotion attribution scores and gray matter (GM) density of right fronto-insular and anterior cingulate cortices (Cerami et al., 2014), and two diffusion tensor imaging studies (Crespi et al., 2014; Crespi et al., 2016) reported an association between emotion recognition difficulties and microstructural changes in ventral associative WM bundles connecting occipital, temporo-limbic and orbito-frontal areas of the right hemisphere. In a recent study in which the 40 % of ALS patients were cognitively impaired, positive correlations were observed between emotion recognition and the cortical thickness of the left caudal middle frontal, bilateral precentral, right middle and inferior temporal, lateral occipital and inferior parietal regions (Benbrika et al., 2019). Using a task-based functional magnetic resonance imaging (fMRI) paradigm with emotional pictures as stimuli, it emerged that ALS patients without major cognitive deficits, compared to controls, showed decreased activation of extra-striate visual areas and increased activation of the right supramarginal gyrus (Lule et al., 2007). The reduced activity of extra-striate visual regions could be an indicator of lower subjective excitement at neural and behavioral level in ALS patients, while the increased response of supramarginal gyrus may be interpreted as a compensatory recruitment of somatosensory regions (Lule et al., 2007). Interestingly, some authors observed a decreased resting-state functional connectivity (RS-FC) in the default mode, fronto-parietal, and salience networks at baseline, and the occurrence of affective theory of mind deficits after 6 months in cognitively unimpaired ALS patients (Trojsi et al., 2017). Alterations of resting-state fMRI have been considered a possible biomarker of social cognition impairment in ALS patients (Trojsi et al., 2017).

In pure-motor ALS patients, we recently observed that an altered processing of disgust was related with smaller volume of the left pallidum (Castelnovo et al., 2021a) in the absence of significant volume differences relative to controls. For this reason, in the present study, we aimed to investigate the RS-FC of the pallidum with the rest of the brain in ALS patients compared to healthy controls, in order to explore the presence of early functional connectivity alterations which could anticipate structural damage. We further investigated to which pallidal RS-FC changes were related the difficulties in recognising disgust.

2. Materials and methods

2.1. Participants

From a sample of 87 patients with motor neuron disease, we selected 26 cognitively and behaviourally unimpaired sporadic patients with a clinical diagnosis of probable or definite ALS (Brooks et al., 2000), without significant respiratory failure and without a known genetic mutation (such as the presence of GGGGCC hexanucleotide expansion in the first intron of *C9orf72*, as well as known pathogenic mutations in *GRN*, *MAPT*, *TARDBP*, *SOD1*, *FUS*, *TBK1*, *TREM2*, *OPTN* and *VCP* genes), from those attending three ALS centers in Milan, Italy. We included only patients who underwent a comprehensive standard neuropsychological assessment including the Comprehensive Affect Testing System (CATS), a clinical evaluation, and a 3 T brain MRI. Fifty-two healthy controls, age-, sex-, and education-matched with patients, were recruited among non-consanguineous relatives. Inclusion criteria for healthy controls were: a normal neurological exam, a mini mental state examination (MMSE) score $\geq$ 28, and no familiarity with neurodegenerative disorders. Healthy controls underwent the same standard neuropsychological assessment and MRI scan of ALS patients. Exclusion criteria for all participants were the following: significant medical illnesses or substance abuse which could interfere with cognitive functioning; any (other) major systemic, psychiatric, or neurological illnesses; and (other) causes of focal or diffuse brain damage, including cerebrovascular disease at conventional MRI scans. No participants were excluded for motion-related artifacts in the MR images. The 92 % of participants described in the present study overlap with a recently published sample (Castelnovo et al., 2021a).

The study protocol was approved by local ethical standards committee on human experimentation and all participants provided written informed consent.

2.2. Clinical assessment

Disease severity was scored using the revised ALS Functional Rating Scale (ALSFRS-R) (Cedarbaum et al., 1999). Disease duration was calculated from symptom onset to MRI date in months. The disease progression rate was calculated with the following formula: (48–ALSFRS-R score)/disease duration.

2.3. Cognitive and behavioral assessment

Cognitive evaluation were performed by experienced neuropsychologists, who were unaware of the MRI results, and consisted in the administration of a comprehensive standard neuropsychological battery, which was necessary to detect the presence of cognitive and/or behavioural deficits according to Strong's revised criteria (Strong et al., 2017). The following cognitive functions were explored as previously described (Castelnovo et al., 2021b): global cognitive functioning with the MMSE and the Edinburgh Cognitive and Behavioural ALS Screen (ECAS); long- and short-term verbal memory with the Rey Auditory Verbal Learning Test and the digit span forward, respectively; executive functions with the digit span backward, the Cognitive Estimation Task (CET), and the Modified Card Sorting Test; fluency with the phonemic and semantic fluency tests and the relative fluency indices (controlling for individual motor disabilities); language with the confrontation naming subtests of Italian battery for the assessment of aphasic disorders (BADA); emotion processing with the CATS; mood with the Hamilton Depression Rating Scale (HDRS); and the presence of behavioral disturbances with the Amyotrophic Lateral Sclerosis-Frontotemporal Dementia-Questionnaire (ALS-FTD-Q) administered to patients' caregivers. Healthy controls underwent the entire assessment except for the ECAS, CET and BADA subtests; moreover, in healthy controls the Beck Depression Inventory (BDI) was used to assess mood.

CATS is a battery measuring different aspects of the emotion processing by using the famous Ekman's faces expressing the six basic emotions (disgust, surprise, happiness, anger, fear and sadness) (Froming et al., 2006). From this battery, we selected and administered six subtests: identity discrimination (which is the control condition that investigates the correct discrimination of faces with no emotion involvement), affect discrimination, affect naming, affect matching, affect selection, affect confrontation. Summing up the number of correct answers at the CATS subtests, we calculated a total score for each of the six basic emotions.

2.4. MRI acquisition

Using a 3.0 T scanner (Ingenia CX, Philips), the following brain MRI sequences were obtained from all participants: 3D T1-weighted (TFE) (TR = 7 ms; TE = 3.2 ms; flip angle = 9 [degrees]; 204 contiguous sagittal slices with voxel size = 1 × 1 × 1 mm, matrix size = 256 × 240, FOV = 256x240 mm^2); 3D FLAIR (TR = 4800 ms; TE = 267 ms; TI = 1650 ms; ETL = 167; NEX = 2; 192 contiguous sagittal slices with voxel size = 0.89 × 0.89 × 1 mm, matrix size = 256 × 256, FOV = 256x256

mm^2); 3D T2 (TR = 2500 ms; TE = 330 ms; ETL = 117; NEX = 1; 192 contiguous sagittal slices with voxel size = 0.89 × 0.89 × 1 mm, matrix size = 256 × 258, FOV = 256x256 mm^2); and T2* weighted (GE-*EPI*) sequence for RS fMRI (TR = 1567 ms; TE = 35 ms; flip angle = 70; MB = 2; SENSE = 2; FOV = 240x240; pixel size = 2.5x2.5 mm; slice thickness = 3 mm; 320 sets of 48 contiguous axial slices; acquisition time = 8′ and 32′′). Before starting the RS fMRI scanning, the technician talked with the participants through their earphones instructing them to remain motionless, to keep their eyes closed, not to fall asleep, and not to think about anything in particular.

2.5. MRI analysis

MRI analysis was performed at the Neuroimaging Research Unit, IRCCS Scientific Institute San Raffaele, Milan, Italy. The presence of WM hyperintensities was evaluated on 3D FLAIR and 3D T2-weighted images.

2.5.1. Resting-state fMRI preprocessing

RS fMRI data processing was performed using the FMRIB software library (FSLv5.0) as outlined previously (Canu et al., 2020). The first four volumes of the RS fMRI data were removed to reach complete magnet signal stabilization. The following FSL-standard preprocessing pipeline was implemented: (1) motion correction using MCFLIRT; (2) high-pass temporal filtering (lower frequency: 0.01 Hz); (3) spatial smoothing (Gaussian Kernel of FWHM 6 mm); (4) single-session independent component analysis-based automatic removal of motion artifacts (ICA_AROMA) (Pruim et al., 2015) in order to identify those independent components (ICs) representing motion-related artifacts.

2.5.2. Seed-based resting-state functional connectivity (for a brief overview of the method see (Castelnovo et al., 2020))

Left and right pallidum were selected as seeds and were defined in the MNI space using the automated anatomical labelling atlas (AAL) in WFU PickAtlas (toolbox of SPM12), moved to each subject native T1-weighted space trough non-linear and affine registrations, and visually inspected in the individual brains by neuroimaging experts. Seed-based RS-FC was then performed using a 2-step regression analysis as implemented in the FMRIB software library (FSLv5). First, time series of WM, cerebrospinal fluid, and whole brain volumes in RS fMRI native space were extracted from the preprocessed and denoised data and their effects were regressed out using the FMRI Expert Analysis Tool. Seed mean time-series were then calculated. The output of this step is represented by subject-level maps of all positively and negatively predicted voxels for each regressor. Subject-level maps were registered to the MNI standard template to enter the statistical analysis.

2.6. Statistical analysis

2.6.1. Demographic, clinical and cognitive data

Demographic characteristics and cognitive scores between groups were compared using Kruskal-Wallis one-way ANOVA models (using *U* test of Mann–Whitney) and Fisher's exact test, for continuous and categorical variables, respectively.

2.6.2. Seed-based resting-state functional connectivity

Seed-based RS-FC was compared between ALS patients and healthy controls using GLM, which included RS-FC maps as dependent variables. Corrections for multiple comparisons were carried out at a cluster level using Gaussian random field theory, $z > 2.3$; cluster significance: $p < 0.05$, corrected for multiple comparisons.

For the correlation analysis between significant RS-FC changes and cognitive scores, we firstly extracted the mean RS-FC value for each subject from each significant cluster emerging from the seed-based RS-FC analysis. In all subjects, CATS disgust score was correlated with mean RS-FC values of the significant clusters using Pearson's correlation analysis. Analyses were thresholded at $p < 0.05$ adjusted for multiple comparisons using Bonferroni's correction. We finally investigated a potential relationship between significant CATS disgust score and patients' mood as assessed with the HDRS (Hamilton, 1960). The statistical analyses were performed with SPSS software (version 24.0; IBM Corp., Armonk, NY, USA).

Table 1
Sociodemographic, clinical and neuropsychological features of healthy controls and ALS patients.

	Healthy controls	ALS patients	P value
N	52	26	–
Sex [women]	33 (63 %)	12 (46 %)	0.16
Age at MRI [years]	60.36 ± 8.49	56.90 ± 12.30	0.25
Education [years]	12.40 ± 3.79	13.27 ± 4.68	0.54
Disease duration [months]	–	30.19 ± 22.89	–
ALSFRS-R, 0–48	–	37.45 ± 7.55	–
Disease progression rate	–	0.50 ± 0.47	–
Site of onset, limb	–	87 %	–
Global cognition			
MMSE	29.36 ± 0.80	29.17 ± 1.37	0.95
ECAS, Language	–	25.34 ± 2.06	–
ECAS, Fluency	–	20.08 ± 2.15	–
ECAS, Executive Functions	–	38.80 ± 5.00	–
ECAS, ALS-specific functions	–	84.72 ± 6.47	–
ECAS, Memory	–	18.92 ± 3.45	–
ECAS, Visuospatial	–	11.68 ± 0.56	–
ECAS, ALS non-specific functions	–	30.40 ± 3.45	–
ECAS, total score	–	115.64 ± 9.65	–
Memory			
Digit span, forward	5.96 ± 1.07	6.13 ± 1.39	0.56
Digit span, backward	4.82 ± 1.20	5.27 ± 1.83	0.38
RAVLT, immediate	49.62 ± 7.08	49.84 ± 11.04	0.88
RAVLT, delayed	10.92 ± 2.42	10.12 ± 2.73	0.18
Executive function			
CET	–	12.24 ± 3.83	–
MCST, perseverative responses	3.52 ± 3.63	1.91 ± 2.74	0.03*
Language			
BADA (nouns)	–	28.75 ± 0.96	–
BADA (actions)	–	27.50 ± 1.00	–
Fluency			
Phonemic fluency, Index	5.10 ± 1.84	4.35 ± 2.47	0.01*
Semantic fluency, Index	3.81 ± 1.58	3.84 ± 1.44	0.73
Mood & Behaviour			
BDI	7.04 ± 4.81	–	–
HDRS	–	6.87 ± 4.38	–
ECAS, behaviour score	–	0.50 ± 0.70	–
ALS-FTD-Q	–	5.63 ± 7.46	–

Values denote mean ± standard deviations or numbers (percentages). Neuropsychological values are reported as raw scores. P-values refer to Kruskal-Wallis one-way ANOVA models (using *U* test of Mann–Whitney) and Fisher's exact test, for continuous and categorical variables, respectively. *=significant differences between groups at $p < 0.05$. Abbreviations: ALS = amyotrophic lateral sclerosis; ALS-FTD-Q = amyotrophic lateral sclerosis-frontotemporal dementia-questionnaire; ALSFRS-R = ALS Functional Rating Scale Revised; BADA = battery for the analysis of aphasic deficits; CET = cognitive estimation test; CPM = coloured progressive matrices; HC = healthy controls; HDRS; Hamilton Depression Rating Scale; MMSE = mini-mental state examination; MRI = Magnetic Resonance Imaging; RAVLT = Rey auditory verbal learning test; WCST = Wisconsin card sorting test. Disease Progression Rate has been obtained as following: (48–ALSFRS-R score)/time between symptom onset and first visit. Fluency indices have been obtained as following: time for generation condition - time for control condition (reading or writing generated words)/total number of items generated.

3. Results

ALS patients and controls did not differ in age, sex and education (Table 1). All patients were cognitively and behaviourally normal according to Strong's criteria (Strong et al., 2017). The standard neuropsychological battery did not reveal differences between groups (Table 1), except for better performances of ALS patients compared to

Table 2
Cognitive performance of healthy controls and ALS patients at the Comprehensive Affect Testing System (CATS).

	HC	ALS	P-value
CATS Total score	56.79 ± 4.77	53.92 ± 5.75	0.02*
CATS Identity Discrimination	11.81 ± 0.40	11.46 ± 0.99	0.16
CATS Affect Discrimination	11.29 ± 0.98	10.92 ± 0.98	0.07
CATS Affect Naming	4.63 ± 0.97	4.15 ± 1.19	0.07
CATS Affect Selection	5.52 ± 0.67	5.08 ± 0.89	0.03*
CATS Affect Matching	9.31 ± 1.92	8.46 ± 1.53	0.03*
CATS Affect Confrontation	14.23 ± 3.04	13.85 ± 3.33	0.52
Disgust, correct answers (0–9)	5.85 ± 1.59	5.04 ± 1.80	0.048*
Surprise, correct answers (0–8)	6.56 ± 1.34	6.46 ± 1.21	0.54
Happiness, correct answers (0–9)	8.65 ± 0.52	8.62 ± 0.57	0.83
Anger, correct answers (0–10)	5.69 ± 2.11	4.77 ± 1.58	0.06
Fear, correct answers (0–8)	5.15 ± 1.29	5.35 ± 1.26	0.57
Sadness, correct answers (0–9)	6.90 ± 1.54	6.58 ± 1.60	0.38

Values denote mean ± standard deviations. P values refer to Kruskal-Wallis one-way ANOVA models (using *U* test of Mann–Whitney). *=significant differences between groups at $p < 0.05$. Abbreviations: ALS = Amyotrophic Lateral Sclerosis; CATS = Comprehensive Affect Testing System.

controls in the perseverative responses of MCST and in the phonemic fluency index. No patient showed clinical depression according to HDRS neither to clinical interview.

At the CATS, compared to healthy controls, patients obtained significantly lower scores in the total score, affect selection and affect matching subtests and they were significantly less able to recognize disgust (Table 2). No significant associations were observed between patients' mood and CATS disgust score.

3.1. Seed-based resting-state functional connectivity

For each group, Fig. 1 reports the RS-FC mean connectivity between left and right pallidum and the rest of the brain.

In ALS patients compared to controls, the seed-based analysis showed reduced RS-FC between bilateral pallidum and bilateral middle and superior frontal gyri and right middle cingulate gyri, and increased RS-FC between bilateral pallidum and bilateral postcentral gyrus and Rolandic operculum, left superior temporal and right supramarginal gyrus (Figs. 2 and 3). Furthermore, decreased RS-FC was observed between left pallidum and right superior frontal, left middle and inferior temporal gyri and bilateral caudate, and between right pallidum and left anterior cingulate gyrus (Fig. 2). RS-FC was increased between left pallidum and right superior temporal, left middle frontal and precentral and left supramarginal gyri, and between right pallidum and left lingual, supramarginal and fusiform gyri (Fig. 3).

In ALS patients and healthy controls, a lower performance in recognizing disgust was related with a reduced RS-FC between the left pallidum and the left middle and inferior temporal gyrus ($r = 0.282$; $p = 0.048$, corrected for multiple comparisons; Fig. 4).

4. Discussion

In a sample of cognitively unimpaired ALS patients, we confirmed our and previous findings (Bora, 2017) of initial difficulties in emotion recognition limited to the facial identification of disgust. In line with our findings, a recent work (Palumbo et al., 2022) showed lower performance in emotion recognition assessed with the Ekman 60-Faces (EK-

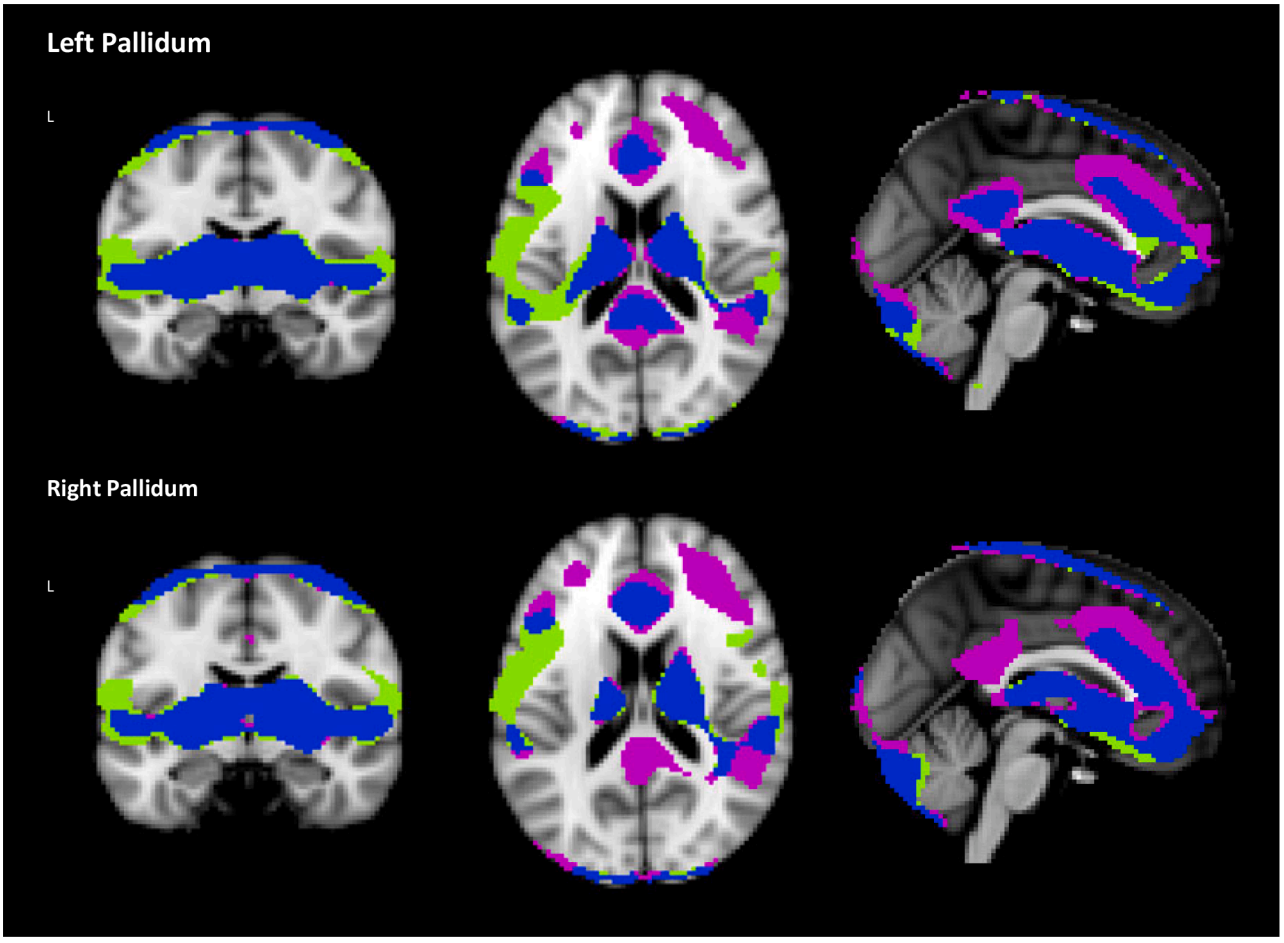

Fig. 1. Seed-based functional connectivity analysis. RS-FC mean connectivity between left and right pallidum and the rest of the brain for healthy controls (pink colour), ALS patients (green colour) and their overlap (blue colour).

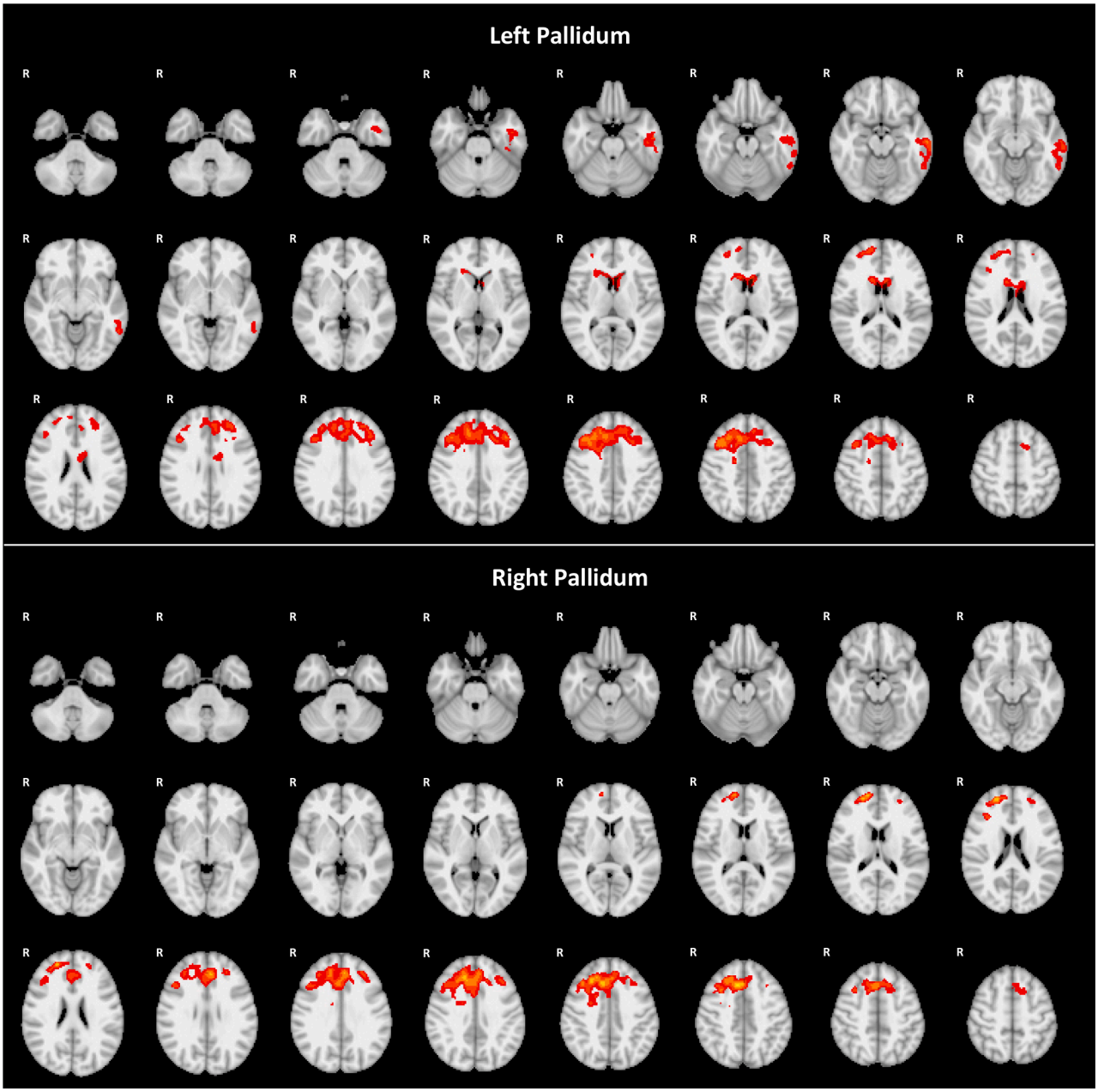

Fig. 2. Seed-based functional connectivity analysis. Regions where ALS patients showed decreased resting state functional connectivity with left pallidum and right pallidum compared to healthy controls.

60F) Test (Dodich et al., 2014) in both cognitively impaired and cognitively normal ALS. Differently from our work, in cognitively normal ALS, they observed first an impairment in recognizing sadness and fear, followed only later by disgust. Although the EK-60F Test uses the same stimuli as the CATS (i.e., the Ekman and Friesen series of Picture of Facial Affect) (Ekman, 1976), it only reflects the CATS affect naming subtest, which requires the patient to select the label corresponding to the emotion face expression. The discrepancy between our and other study (Palumbo et al., 2022) could be due to the fact that here we combined emotion recognition accuracy among different tasks beyond the affect naming (i.e., affect discrimination, matching, selection, confrontation). Thus, our combined measure assessed with CATS could reflect a more general (and perhaps more reliable) emotion recognition failure able to detect disgust as the first emotion affected in ALS. However, since this is a very recent field of research, further studies in larger samples are needed to clarify this point.

In our previous work we observed an association between disgust recognition and pallidum volume although no significant volume differences were found between ALS and healthy controls (Castelnovo et al., 2021a). Here, on the basis of our initial hypothesis, we demonstrated an altered RS-FC of the bilateral pallidum both in terms of increased and decreased RS-FC in ALS patients compared to healthy controls. Furthermore, in ALS patients and healthy controls, we observed that a reduced RS-FC of the left pallidum with the left middle and inferior temporal gyri was associated with a lower ability in recognizing disgust. Taken together, our results suggest that emotional processing in ALS is associated with pallidum functioning and that RS functional changes of this region and related circuits may precede structural alterations.

In ALS patients compared to controls, we observed a reduced RS-FC between pallidum, medial frontal and temporal cortices, and caudate. According to the phosphorylated 43 kDa TAR DNA-binding protein

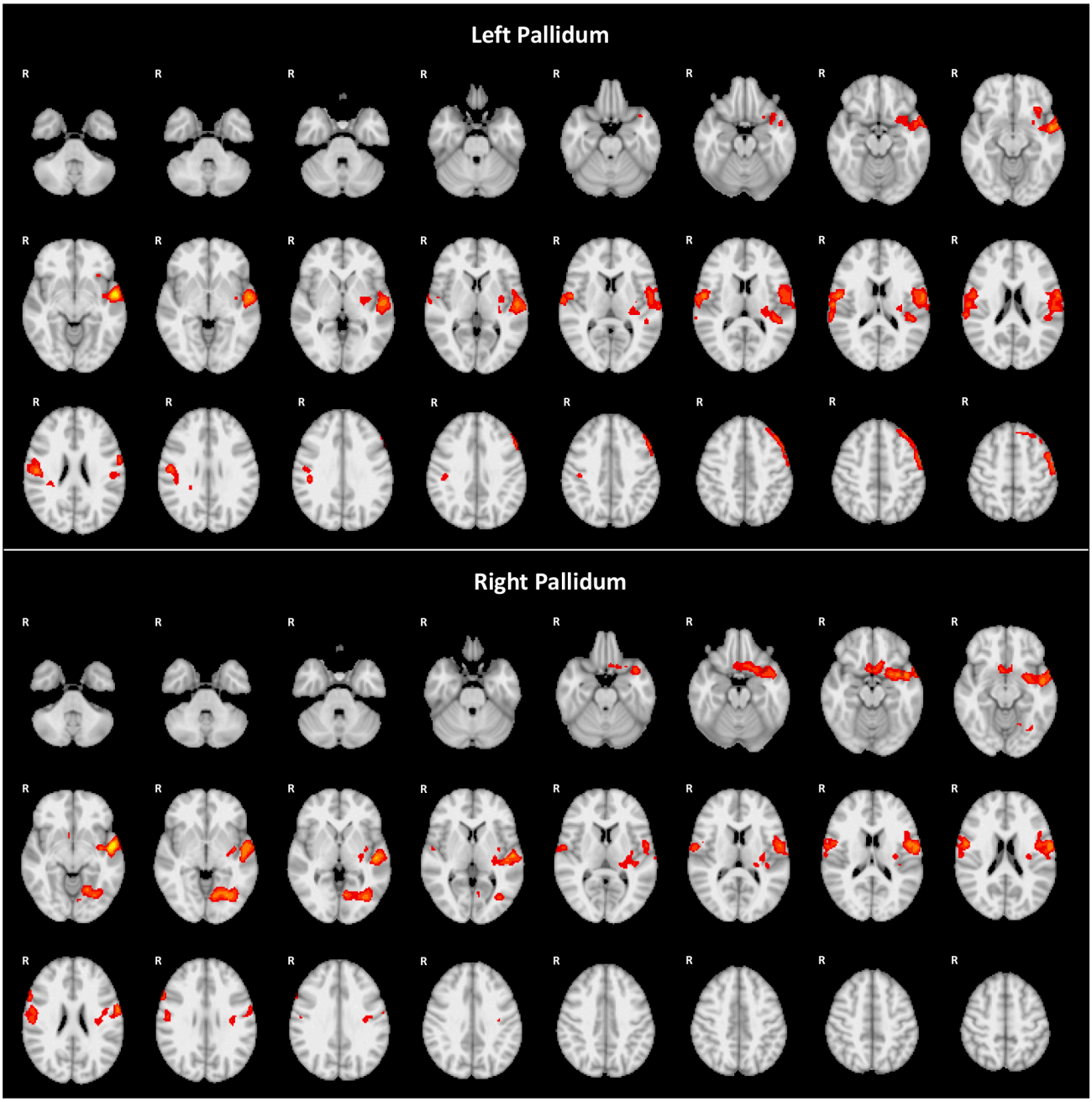

Fig. 3. Seed-based functional connectivity analysis. Regions where ALS patients showed increased resting state functional connectivity with left pallidum and right pallidum compared to healthy controls.

(pTDP-43) pathological staging of ALS (Brettschneider et al., 2013), all these regions, including the pallidum, are affected from stage 3, which probably does not reflect the disease stage of our mild ALS sample. Since we investigated functional (and not structural) connectivity, we can speculate that the alterations that we observed could represent subtle and early disease changes that do not reflect the timing of ALS pathological staging. From a neuropsychological point of view, these brain regions were observed to activate together with pallidum, insula, other basal ganglia, and fusiform gyrus during the processing of disgusted faces in healthy subjects (Calder et al., 2000; Fusar-Poli et al., 2009; Phillips et al., 1998). This is an important circuit, named cortico-basal ganglia-thalamo-cortical loop (Pessoa, 2017), involved in emotional processing, especially in disgust, in which the cortex is directly connected with the striatum, which projects to the pallidum which, in turn, communicates with the thalamus projecting back to the cortical regions and closing the loop (Pessoa, 2017). In this circuit, cortical regions, such as the medial frontal cortex, participate in the conscious experience of emotion (Fusar-Poli et al., 2009), while the temporal cortex and the fusiform gyrus activate during the processing of human emotional faces (Fusar-Poli et al., 2009).

Pallidum is the main target of striatal outflow and its ventral part belongs to the ventro-striatopallidal system. It plays a role in the regulation of emotion, specifically in the sensory perception of disgust and in initiating a movement in reaction to an emotional stimulus to produce an avoidance behavior (Calder et al., 2007; Ho and Berridge, 2014; Holtmann et al., 2020; Singh-Bains et al., 2016; Smith et al., 2009). Therefore, in our patients, a reduced RS-FC between fronto-temporo-striatal regions and the pallidum could lead to an altered processing of the stimulus, performed by cortical regions and, consequently, to an altered ability to recognize disgust.

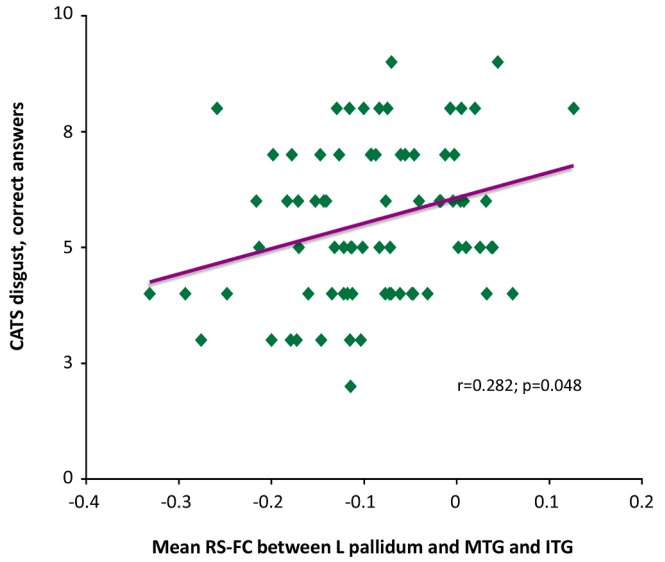

Fig. 4. Cognitive-fMRI positive relationship. In ALS patients and healthy controls, worse performance in recognizing disgust was related with decreased resting state functional connectivity of the left pallidum with left middle and inferior temporal gyrus (r = 0.282; p = 0.048, corrected for multiple comparisons).

We further observed that a reduced RS-FC between the left pallidum and the left middle and inferior temporal gyri was associated with lower performances in recognizing disgust in ALS and controls. This association confirms once again the left lateralization of the disgust circuit, as proposed by a prominent set of neuroimaging studies (Guo et al., 2016; Holtmann et al., 2020). These works withstand a primary left activation of insular cortex to disgust stimuli (Royet et al., 2003; Small et al., 2003; Sprengelmeyer et al., 1998; Wicker et al., 2003) and observed that left-lesioned patients in the insular cortex-basal ganglia complex performed significantly worse in disgust recognition compared to right-lesioned patients or healthy controls (Holtmann et al., 2020). The middle and inferior temporal gyri are involved in multiple high-cognitive functions including social cognition and, specifically, emotion regulation (Lin et al., 2020; Xu et al., 2019).

On the other hand, in ALS patients compared to controls, we observed an increased RS-FC of the Rolandic operculum (i.e., part of the insular cortex), postcentral gyrus and supramarginal gyrus with bilateral pallidum. These brain regions were observed to activate during emotion recognition fMRI-tasks in pure motor ALS (Lule et al., 2007; Lule et al., 2005; Yoshimura et al., 2005). According to some authors (Lule et al., 2007; Yoshimura et al., 2005), the activation of these somatosensory regions in ALS could reflect a compensatory mechanism: they would create internal somatosensory representations of facial and emotional cues in order to remedy the initial emotional difficulties. In line with these findings, the increased connectivity of these regions with bilateral pallidum in our sample could be in part explained by compensatory attempts. On the other hand, the increased RS-FC of brain regions directly involved in the circuit of disgust, such as the insular cortex, could reflect a pathophysiological consequence of the functional and, likely, structural disconnection between the pallidum and frontal and temporal regions. According to the disconnection hypothesis, increased functional connectivity can reflect a loss of local inhibitory neuronal circuits and can be due to cortical excitability (Douaud et al., 2011). This has been demonstrated by several imaging studies which observed increased functional connectivity occurring when structural connectivity with distant brain regions of a network is disrupted (Agosta et al., 2018; Basaia et al., 2020; Douaud et al., 2011).

Our study has some limitations, mainly related to the relatively small sample size and the cross-sectional nature of the design, which did not allow us to investigate the evolving trajectory of emotion recognition deficits in these patients. Furthermore, it was not possible to detect which emotions were more frequently confused with disgust, because the CATS has not been realised with this aim.

In conclusion, in a sample of cognitively unimpaired ALS patients, we confirmed our previous findings of early difficulties in recognizing disgust and we demonstrated an altered RS-FC between pallidum and the rest of the brain corroborating the role of left pallidum in the altered processing of disgust. Reduced left pallidum-frontotemporal-striatal RS-FC may be linked to future frontotemporal dementia-like manifestations in ALS.

5. Standard protocol Approvals, Registrations, and patient consents

Local ethical standards committee on human experimentation approved the study protocol and all participants provided written informed consent (Ethical committee numbers: GR-2013–02357415, StG-2016_714388_NeuroTRACK and Foundation Research on Alzheimer Disease).

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CRediT authorship contribution statement

Veronica Castelnovo: Conceptualization, Methodology, Resources, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization. **Elisa Canu:** Conceptualization, Methodology, Resources, Formal analysis, Investigation, Data curation, Writing – original draft, Writing – review & editing, Visualization, Project administration. **Maria Antonietta Magno:** Methodology, Formal analysis, Writing – review & editing. **Elena Gatti:** Formal analysis, Writing – review & editing. **Nilo Riva:** Resources, Writing – review & editing. **Debora Pain:** Resources, Writing – review & editing. **Gabriele Mora:** Resources, Writing – review & editing. **Barbara Poletti:** Conceptualization, Resources, Writing – review & editing. **Vincenzo Silani:** Resources, Writing – review & editing, Funding acquisition. **Massimo Filippi:** Conceptualization, Resources, Methodology, Writing – review & editing, Funding acquisition, Supervision. **Federica Agosta:** Conceptualization, Methodology, Supervision, Writing – original draft, Writing – review & editing, Funding acquisition, Project administration.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: V. Castelnovo, M.A. Magno, S. Basaia, N. Riva, D. Pain, G. Mora, B. Poletti report no disclosures. E. Canu has received research supports from the Italian Ministry of Health. V. Silani received compensation for consulting services and/or speaking activities from AveXis, Cytokinetics and Italfarmaco; and receives or has received research supports form the Italian Ministry of Health, AriSLA (Fondazione Italiana di Ricerca per la SLA), and E- Rare Joint Transnational Call. M. Filippi is Editor-in-Chief of the *Journal of Neurology* and Associate Editor of *Human Brain Mapping*; received compensation for consulting services and/or speaking activities from Almiral, Alexion, Bayer, Biogen, Celgene, Eli Lilly, Genzyme, Merck- Serono, Novartis, Roche, Sanofi, Takeda, and Teva Pharmaceutical Industries; and receives research support from Biogen Idec, Merck-Serono, Novartis, Roche, Teva Pharmaceutical Industries, Italian Ministry of Health, Fondazione Italiana Sclerosi Multipla, and ARiSLA (Fondazione Italiana di Ricerca per la SLA). F. Agosta is Section Editor of

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Data availability

Data will be made available on request.

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