Neurobiology of Disease

Cognitive Deterioration and Functional Compensation in ALS Measured with fMRI Using an Inhibitory Task

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Amyotrophic lateral sclerosis (ALS) is characterized by degeneration of upper and lower motor neurons, resulting in progressive weakness and muscle atrophy. Recent studies suggest that nondemented ALS patients can show selective cognitive impairments, predominantly executive dysfunction, but little is known about the neural basis of these impairments. Oculomotor studies in ALS have described deficits in antisaccade execution, which requires the implementation of a task set that includes inhibition of automatic responses followed by generation of a voluntary action. It has been suggested that the dorsolateral prefrontal cortex (DLPFC) contributes in this process. Thus, we investigated whether deterioration of executive functions in ALS patients, such as the ability to implement flexible behavior during the antisaccade task, is related to DLPFC dysfunction. While undergoing an fMRI scan, 12 ALS patients and 12 age-matched controls performed an antisaccade task with concurrent eye tracking. We hypothesized that DLPFC deficits would appear during the antisaccade preparation stage, when the task set is being established. ALS patients made more antisaccade direction errors and showed significant reductions in DLPFC activation. In contrast, regions, such as supplementary eye fields and frontal eye fields, showed increased activation that was anticorrelated with the number of errors. The ALS group also showed reduced saccadic latencies that correlated with increased activation across the oculomotor saccade system. These findings suggest that ALS results in deficits in the inhibition of automatic responses that are related to impaired DLPFC activation. However, they also suggest that ALS patients undergo functional changes that partially compensate the neurological impairment.

Key words: **amyotrophic lateral sclerosis; antisaccade; cognitive control; fMRI; prefrontal cortex; task set**

Introduction

Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease affecting motor neurons in the cerebral cortex, brainstem, and spinal cord. The neuropathology of ALS is marked primarily by degeneration of upper motor neurons in the brainstem and motor cortex, and of lower motor neurons in the brainstem and spinal cord. The resulting muscle denervation leads to physical symptoms of muscle weakness, atrophy, and tone reduction, which progresses to the loss of voluntary movement (Kiernan et al., 2011).

Beyond motor impairments, patients with ALS often display behavioral and cognitive deficits, including dysfunctions within the executive system (Gallassi et al., 1985; Strong et al., 1996; Abrahams et al., 2000; Phukan et al., 2007; Raaphorst et al., 2012). These deficits are commonly associated with pathologies in prefrontal circuits and can also be seen in patients with frontotemporal dementia (Abrahams et al., 1996; Murphy et al., 2007). Most brain imaging activation studies that investigated the neural basis of cognitive deficits in ALS have used tests that depend on verbal, written, or hand movement responses, which can be confounded by the ALS motor impairment. Nevertheless, these studies have found a general correlation between performance on different neuropsychological tests and reduced frontal lobe activity, including the dorsolateral prefrontal cortex (DLPFC) (Tsermentseli et al., 2012).

However, it is unknown how reduction in activity within a specific area would result in a specific executive dysfunction in ALS. A task that has been valuable to probe the processes involved in executive dysfunctions is the antisaccade task, which relies on the implementation of flexible behaviors (Hallett, 1978; Hallett and Adams, 1980). This flexibility has been attributed to variations in readiness to make a response or in the intention to perform a particular task and has been referred to as "preparatory set" (Everling and Munoz, 2000). Correct antisaccade execution requires a preparatory set that includes inhibition of automatic

Received March 10, 2014; revised Aug. 27, 2014; accepted Sept. 5, 2014.

Author contributions: R.M., B.C.C., N.A., M.M., and D.P.M. designed research; K.W., J.F.-R., R.M., B.C.C., and M.M. performed research; K.W. and J.F.-R. analyzed data; K.W., J.F.-R., and D.P.M. wrote the paper.

This work was supported by ALS Society of Canada Bernice Ramsay Discovery Grant to D.P.M. and M.M. and Canadian Institutes of Health Research Operating Grant MOP-97741 to D.P.M. who was also supported by the Canada Research Chair Program. We thank members of the D.P.M. laboratory for comments on an earlier version of the manuscript.

The authors declare no competing financial interests.

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DOI:10.1523/JNEUROSCI.1111-14.2014

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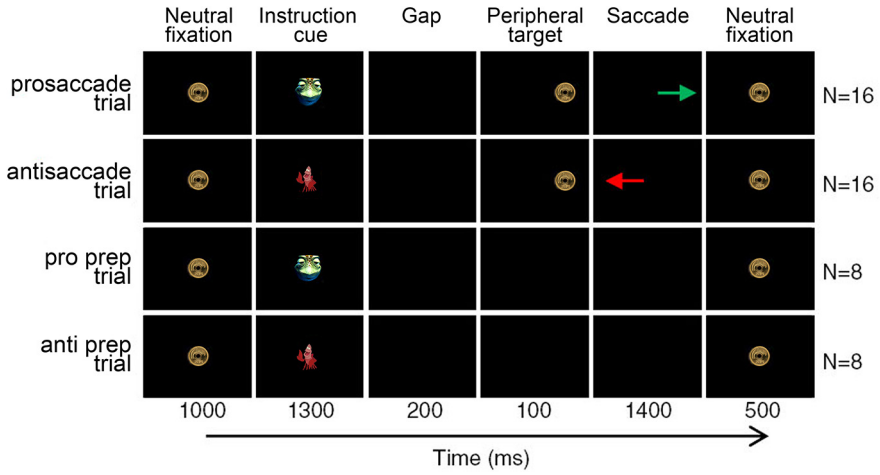

Figure 1. Behavioral paradigm. Representation of stimuli and timing of events for the four trial types. Trials were pseudo-randomly presented and intermixed with periods of fixation on the neutral fixation stimulus that lasted 1.5, 3, and 4.5 s. Arrows indicate the correct saccade directions for the saccade trials and were not actually displayed. Fixation-only trials are not shown in this figure.

responses followed by the generation of a voluntary action toward the opposite site of the target (Guitton et al., 1985; Shaunak et al., 1995; Luna et al., 1998; Evdokimidis et al., 2002; Munoz and Everling, 2004). Functional imaging has helped identify the oculomotor network involved in the preparation and execution of antisaccades, including frontal cortex areas, such as the frontal eye fields (FEFs) and supplementary eye fields (SEFs), and the DLPFC (Luna et al., 1998; Munoz and Everling, 2004; Anderson et al., 2012; Jamadar et al., 2013).

The aim of this study was to test the hypothesis that deficits in the control of flexible behavior in ALS are related to deficient DLPFC activation, specifically during the antisaccade preparation stage. This hypothesis is supported by previous findings suggesting that the frontal lobe participates in the inhibition of unwanted automatic saccades (Guitton et al., 1985; Pierrot-Deseilligny et al., 2003). To examine this hypothesis, we measured the implementation of flexible behaviors using the antisaccade task in a group of ALS patients and age-matched controls during a rapid event-related fMRI acquisition.

Materials and Methods

All experiments were approved by the Research and Ethics Board of Queen's University and adhered to the principles of the Canadian Tri-Council Policy Statement on Ethical Conduct for Research Involving Humans, in accordance with the principles of the Declaration of Helsinki (World Medical Association, 2013). ALS patients and age-matched control subjects participated in a rapid event-related fMRI design with prosaccade and antisaccade trials interleaved with preparatory protrials and antitrials that did not include saccade executions ("catch" trials) (Fig. 1). This design allowed us to separately examine activation related to the preparation stage for an antisaccade, referred to as the task set, from activation related to executing the antisaccade response.

Participants. Twenty-one patients with definite ALS diagnoses and with no other neurological problem, including the presence of vascular lesions, agreed to participate in this study and were compensated for their time. They were recruited from the neuromuscular clinics at Saint Mary's of the Lake and Kingston General Hospitals by M.M. and were required to participate in two sessions held within 10 d apart. Nine of these patients were unable to complete the MRI studies primarily because of breathing difficulties while lying supine in the scanner. Twelve ALS patients (ages 44–76 years, 2 females, mean ± SD age 61.6 ± 9.6 years) completed all experimental procedures and were included in the final analysis (Table 1). It should be noted that one patient had an especially long disease duration (Patient 5 in Table 1). However, an extensive clinical analysis did not reveal any other neurological condition that could explain his symptoms. His initial presentation was an upper motor neuron type involving lower limbs. A control group of 12 healthy volunteers (ages 41–76 years, 3 females, mean ± SD age 61.6 ± 10.7 years) were age- and gender-matched to the ALS patients and were included in the final analysis. Controls did not possess any neurological/psychiatric disorders as assessed by the experimenter and by scores on the Mini-Mental Status Examination (Folstein et al., 1983) or the Montreal Cognitive Assessment (MoCA) (Nasreddine et al., 2005).

Clinical evaluation of ALS patients was performed during the first session. This evaluation was modeled after a rapid screening battery used in ALS patients to measure physical function and frontal lobe impairments (Flaherty-Craig et al., 2006). The measures of physical function included pulmonary function tests of forced vital capacity, the self-administered ALS Functional Rating Scale Revised version (Cedarbaum et al., 1999), manual strength tests of maximum voluntary isometric contraction of the dominant hand, and a patient history, including age and symptoms at disease onset, disease duration, and current medications being taken by the patient. Neuropsychological testing included the MoCA, the Frontal Behavioral Inventory (Kertesz et al., 1997), the Centre for Neurologic Study Lability Scale (Moore et al., 1997), a modification of the Hospital Anxiety and Depression Scale (Bjelland et al., 2002) to exclude one question which falsely exaggerated the measure of depression due to the physical disabilities experienced by ALS patients (Abrahams et al., 2000), the Neurobehavioral Cognitive Status Examination (Mueller et al., 2001) to assess verbal reasoning and judgment, and the Controlled Oral Word Association test (Benton, 1969) to assess verbal fluency, where the letters C, A, and S were used to avoid repetition from verbal fluency tasks in the MoCA. Verbal fluency represents the average time taken to think of each word (Abrahams et al., 2000) and is designed to control for individual variations in motor speed. Previously described normative data were used as a benchmark for age-matched control performance (Tombaugh et al., 1999).

fMRI experimental design. Brain imaging was acquired during the second session. A randomly interleaved, rapid event-related design was used (Cameron et al., 2012; Hakvoort Schwerdtfeger et al., 2012; Alahyane et al., 2014), allowing the presentation of different trial types within a reasonable time period. Included in the design were full prosaccade and antisaccade trials aimed at examining both the preparatory and execution components of saccades (Fig. 1, top 2 rows), preparatory-only trials (i.e., catch trials) that exclusively measured preparatory activation (Fig. 1, bottom 2 rows), and fixation-only trials (data not shown). Participants were asked to fixate on a neutral fixation stimulus (a "gold coin") that appeared for 1000 ms at the center of the screen to start each trial. The neutral fixation stimulus would change to an instructional cue indicating to the participant that a prosaccade or an antisaccade was required. The symbols used for the instructional cue were colored diagram images: a green turtle indicated that a prosaccade was required, and a red crab indicated that an antisaccade was required. Colored diagram symbols were chosen because the rapid event-related experiment was designed for use across various patient groups that included child-aged participants, and this made the task easier for children to learn.

After the 1300 ms presentation of the instructional cue, a 200 ms gap period occurred during which the participant was presented with a black screen. The gap period was introduced to enable participants to generate more "automatic" saccades and has been associated with shorter saccadic reaction times (SRTs), more antisaccade direction errors, and more express prosaccades (Munoz and Corneil, 1995; Fischer and Weber, 1997; Munoz et al., 1998). On saccade trials, a peripheral target (gold coin) was

Table 1. Clinical and neuropsychological information for ALS patients included in the imaging study[a]

		Clinical evaluation								Neuropsychological evaluation							
											Cognistat					HADS	
Patient no.	Months since diagnosis	El Escorial criteria	Onset	FVC % pred	ALS FRS-R	MVIC (kg)	ULT (/70)	LLT (/70)	Hand	MoCA (/30)	R	J	COWA (VF)	FBI	CNS-LS	D	A
1	60	def	UL	78	38	5.5	50	58	R	25	7	6	3.37	42	10	0	5
2	20	def	LL	90	41	45	70	42	L	25	8	6	4.70	2	7	1	0
3	84	def	LL	92	29		38	70	L	24	4	5	7.00	24	11	9	9
4	12	def	UL	101	41	31	64	62	L	21	4	4	12.10	12	11	3	2
5	168	def	LL	63	42	18	67	64	R	30	8	5	2.96	2	19	2	5
6	13	def	UL	100	31	6	56	63	R	23	4	5	14.80	11	7	1	5
7	13	def	UL	62	28	3	50	60	R	29	8	6	2.93	10	15	5	5
8	22	def	LL	78	36	18	70	43	R	25	8	6	4.14	13	11	3	2
9	18	def	LL	88	42	30	69	60	R	18	7	5	3.50	5	9	3	9
10	16	def	UL	74	43	3	53	68	L	29	7	6	4.88	3	7	1	1
11	10	def	LL	77	31	50	64	26	R	23	8	5	9.11	4	9	3	7
12	11	def	Bulbar	94	33	3.3	56	65	R	26	8	5	3.83	4	13	4	1
Mean ($n = 12$)	37.3			83.1	36.3	19.3	58.9	56.8		24.8	6.8	5.3	6.1	11.0	10.8	2.9	4.3
SEM	47.1			13.1	5.6	17.4	10.1	13.0		3.5	1.7	0.7	3.9	11.7	3.6	2.4	3.1

[a]ALSFRS-R, ALS Functional Rating Scale Revised version; CNS-LS, Centre for Neurologic Studies Lability Scale; Cognistat R/J, Verbal Reasoning and Judgment questions from Neurobehavioral Cognitive Status Examination; COWA, Controlled Oral Word Association test; def, definite diagnosis; FBI, Frontal Behavioral Inventory; FVC % pred, forced vital capacity percent predicted when sitting; HADS D/A, Depression and Anxiety measures of Hospital Anxiety and Depression Scale; Hand, dominant hand as identified by Modified Edinburgh Handedness Inventory; LLT, lower limb total of Manual Muscle test; MoCA, Montreal Cognitive Assessment; MVIC, maximum voluntary isometric contraction; ULT, upper limb total of Manual Muscle test.

flashed for 100 ms to the left or right of the neutral fixation, at eccentricities of either 6° or 7° in separate trials, to signal a saccade. Participants had 1400 ms to execute the appropriate prosaccade (look toward the target location) or antisaccade (look away from the target in the opposite direction) based on the instructional cue presented in that trial. The neutral fixation stimulus (gold coin) then reappeared at the center of the screen for 500 ms, and participants were required to reestablish central fixation to initiate the next trial. Before commencing the task, participants were instructed to make a correction saccade if they generated direction errors. On catch trials, the instructional cue was presented and disappeared to initiate the gap period, but the peripheral target did not appear to signal a saccade; subjects were instead required to maintain central fixation for the remainder of the trial (1700 ms) without generating a saccade. Participants did not know whether or not the peripheral target would appear on any given trial; thus, the instruction cue would always elicit preparation for a prosaccade or antisaccade. Full saccade, catch, and fixation only trials were 4500 ms in duration. The duration of the intertrial interval was jittered, using fixation periods that spanned 1 repetition time (TR) (1.5 s; 8 times), 2 TR (3.0 s; 4 times), and 3 TR (4.5 s; 4 times) to increase the statistical efficiency and power in the rapid event-related design (Dale, 1999).

Runs consisted of 64 trials that included 8 procatch trials, 8 anticatch trials, 16 prosaccade trials, 16 antisaccade trials, and 16 fixation trials (Fig. 1; fixation trials not shown). Trial types were pseudo-randomly interleaved, and right and left prosaccade and antisaccade trials were presented in equal quantities within each run. Each participant performed 5–9 runs (depending on eye tracking success), with each run lasting 277.5 s. Each run started with an additional fixation period of 3 s, whereas fMR images were acquired, to allow the MR signal to reach a steady state. Each run ended with a 16.5 s fixation period to allow the hemodynamic response to return to baseline before commencing the next run. Each subject was given a practice run before entering the magnet.

Visual display and eye tracking. Visual stimuli were generated and controlled using E-PRIME software (Psychology Software Tools) on a personal computer. Images were back-projected onto a high-contrast rear projection screen (DA-LITE), positioned at the head end of the magnet bore, using a NEC LT265 DLP video projector with a refresh rate of 60 Hz and a resolution of 1024 × 768. Participants viewed the screen via a mirror attached to the head coil (described below). Eye position data were recorded using an ISCAN ETL-400 camera that sampled the eye position at a frequency of 120 Hz. To ensure synchronization, the MRI sequences directly triggered the E-PRIME software using a trigger signal from the scanner. An infrared fiber-optic illuminator, which was fixed to the head coil, was used to illuminate the right eye for tracking. After the anatomical MRI scan was acquired, the eye tracker was calibrated using a nine-point array that covered most of the visual field. Analysis of the eye movement data was performed off-line using custom-made MATLAB programs (MathWorks).

Imaging protocol. All imaging data were acquired at the Queen's University MRI Facility using a Siemens 3 Tesla Magnetom Trio system fitted with a 12-channel receive-only head coil. High-resolution T1-weighted whole-brain structural scans were performed on each participant using an MPRAGE sequence (TR = 1760 ms, TE = 2.2 ms, flip angle = 9°, 256 × 256 mm field of view, and 256 × 256 matrix size providing 1 mm isotropic voxels, 176 slices). Functional data were collected using a T2*-weighted EPI acquisition (TR = 1500 ms, TE = 30 ms, flip angle = 72°, 211 × 211 mm field-of-view, 64 × 64 matrix size, 3.3 mm isotropic voxel resolution, 185 volumes) for BOLD-based imaging (Ogawa et al., 1990). Twenty-four slices were acquired and positioned to include all regions of interest extending from the top of the brain to the ventral striatum (STR).

MRI preprocessing. All functional imaging runs were preprocessed using Brain Voyager 1.9. The first two volumes of each functional run were discarded before any preprocessing, to allow for steady-state magnetization. To correct for between-scan movements, all volumes within a run were realigned to the first volume of that functional run. Slice scan time correction was conducted to adjust for time differences due to multislice imaging acquisition using a cubic spline interpolation, which was based on the TR duration and order of slice scanning (ascending interleaved). 3D spatial smoothing was then performed using a 4 mm full-width at half-maximum Gaussian filter on all volumes, and each run was filtered to remove linear drift using a high-pass filter with the upper cutoff frequency corresponding to 3 cycles over the length of the run. Finally, all functional data were superimposed onto 3D anatomical images, resampled into 3 mm cubic voxels, aligned to the anterior commissure–posterior commissure axis, and transformed into Talairach space (Talairach and Tournoux, 1988).

Behavioral analyses. Behavioral data were analyzed using custom-written scripts in MATLAB 7.4 (MathWorks). SRT was defined as the time to make the first saccade away from fixation after peripheral stimulus onset. Saccades with a SRT <90 ms were considered anticipatory (Munoz et al., 1998) and thus were excluded from analysis. This value was selected because it was the point at which errors in prosaccade trials were no longer executed at chance (1:1 ratio correct: incorrect). Therefore, 90 ms was decided as the earliest time at which detection of the visual target could influence behavior. Express saccades, which are the shortest visually triggered saccades, have typically been calculated as saccades with SRTs between 90 and 135 ms (Fischer et al., 1993; Munoz et

al., 1998); however, the boundaries of this epoch change according to the participant age and stimulus conditions (Bell et al., 2006; Peltsch et al., 2011; Marino et al., 2012). In the current study, the express saccade epoch was measured between 90 and 160 ms, where 160 ms was the latency at which both groups made more correct responses than errors during antisaccade trials (data not shown). Prosaccade direction errors were defined as saccades executed away from the target during prosaccade trials; antisaccade direction errors were defined as saccades executed toward the target during antisaccade trials. Direction error rate was calculated by dividing the total number of errors by the total number of valid trials. Intrasubject variability for SRT was calculated using the coefficient of variation for correct trials (SD/mean × 100).

Valid trials consisted of all trials except for those that included the following: (1) failure to fixate during fixation trials; (2) failure to fixate during the instruction period of a full prosaccade or antisaccade trial; (3) failure to execute a saccade during the response period; (4) execution of multiple saccades during the response period; (5) saccades executed during catch trials; (6) antisaccades executed during prosaccade trials; (7) failure to correct an antisaccade direction error; and (8) trials in which eye-tracking was unsuccessful. These aforementioned excluded trials were modeled separately as "invalid trials" in the fMRI analysis described below.

Mixed-design ANOVAs were conducted to examine differences in behavior between the control and ALS groups in terms of SRT, CVSRT, percentage of express saccades, and percentage of direction errors during antsaccade- and prosaccade trials. Nonparametric two-sample Kolmogorov–Smirnov tests were conducted to compare the SRT cumulative distributions between the two groups. Group differences on saccade measures were not observed between leftward versus rightward saccades or between 6° versus 7° eccentricities ($p > 0.05$); therefore, these responses were pooled. Furthermore, 2 × 2 repeated-measures ANOVAs were used to measure between-group differences of saccade metrics, including prosaccade and antisaccade duration, amplitude, and velocity. The variables were group with two levels (ALS, controls) and task with two levels (pro, anti).

fMRI main contrast analyses. The BOLD time series for each voxel was deconvolved with the canonical hemodynamic response function to estimate the underlying time course of neural activity. The hemodynamic response function was modeled as a 13-point time series with a temporal resolution of 1.54 s. Events were modeled separately in the design matrix according to trial type, including the following: (1) anticatch trials, (2) procatch trials, (3) correct antisaccade trials, (4) correct prosaccade trials, (5) corrected antisaccade direction errors, and (6) invalid trials. Fixation trials were used as an implicit baseline.

Several statistical parametric maps were computed for each group, reflecting the statistical significance of the response consistency for each voxel within each trial type, as defined above. To identify the saccade-related neural network, we looked at correct full antisaccade trials and full prosaccade trials over BOLD time points 5–7 (7.7, 9.3, and 10.8 s from trial onset), which corresponded to the time intervals of the peak of the BOLD responses from the instructional cue presentation to the execution of the saccade. These analyses resulted in group-level statistical maps that were generated at a false discovery rate corrected threshold of $p < 0.01$ (T value = 5.0). To identify the most reliable responses, using the cluster threshold estimator plugin for BrainVoyager QX, we also calculated the minimum cluster size necessary to achieve a false activation probability $\alpha = 0.05$ (Forman et al., 1995). This procedure excluded clusters <49 contiguous voxels. These statistical maps constitute the main contrast, and were used for subsequent ROI second-level analyses pertaining to task set establishment and response execution.

fMRI ROI analyses. ROIs were chosen based on previous functional imaging studies that showed consistent activation in these areas during prosaccade and antisaccade execution and preparation (Luna et al., 1998; DeSouza et al., 2003; Connolly et al., 2005; Ford et al., 2005; Brown et al., 2006, 2007; Raemaekers et al., 2007; Cameron et al., 2012; Hakvoort Schwerdtfeger et al., 2012; Jamadar et al., 2013). The following ROIs were selected from the main contrast to perform second level analyses and are known to participate in the saccade network: the frontal pole (FP), DLPFC, the insula, the anterior cingulate cortex (ACC), the STR, the SEFs, the FEF, the precuneus (PCu), and the parietal eye fields (PEFs). ROI analyses were conducted using random-effects Gaussian linear models to extract β-weight parameter estimates of BOLD signal change during saccades from each ROI. ROIs were identified using anatomical landmarks and known locations in Talairach space. Each ROI was defined as the 125 contiguous voxels (5 × 5 × 5) within a cubic cluster centered on the point of peak activation within the selected region. Peak preparatory activation was measured as the mean β-weight values from the fifth and sixth time points following catch trial onset. For analysis of the saccade execution processes, the time points were shifted by 1.5 s to include the sixth and seventh time points following saccade trial onset, as the presentation of the peripheral target occurs 1.5 s (one time point) after the appearance of the instruction (Brown et al., 2007; Alahyane et al., 2014). Mixed-design Split-Plot ANOVAs with one within-subjects factor (with two levels: pro and anti) and one between-subjects factor (with two levels: control group and ALS group) were then conducted to examine differences in mean β-weight values for all ROIs. Paired Student's t tests were conducted to analyze the preparatory differences between correct and error trials within the ALS group using the β-weight averages of the fifth and sixth time points. Finally, to evaluate the relationship between BOLD signal change and task performance, Pearson's correlations were performed between β-weight values and behavioral measurements, including SRT, CV, and proportion of direction errors.

Results

Clinical and neuropsychological evaluation

Scores from the clinical and neuropsychological evaluations of ALS patients are summarized in Table 1. All patients included in the study met the El Escorial criteria for "definite" ALS diagnosis. Five patients had upper limb onset, six had lower limb onset, and only one had bulbar onset. The mean disease duration for all patients at the time of the clinical evaluation was 37.3 months (range, 10–168 months), and the mean vital capacity was 83.1% predicted (forced vital capacity range, 62–101). The mean ± SD ALS Functional Rating Scale Revised version evaluation of physical disability was 36.3 ± 5.6.

The neuropsychological tests revealed considerable deficits in the ALS patients. The mean MoCA score was 24.8 ± 3.5 of 30 points, which is above the average mild cognitive impairment average of 22 points (Nasreddine et al., 2005). The ALS patients performance on each MoCA domain was as follows: visuospatial/executive 4.33 ± 0.77 (of 5), naming 2.91 ± 0.28 (of 3), attention (sum) 5.16 ± 1 (of 6), language (sum) 2.41 ± 0.9 (of 3), abstraction 1.75 ± 0.45 (of 2), delayed recall 3 ± 1.47 (of 5), and orientation 5.91 ± 0.28 (of 6).The Neurobehavioral Cognitive Status Examination reasoning and judgment scores (6.8 ± 1.7 and 5.3 ± 0.7, respectively) fell within the average ranges reported for healthy adults (Kiernan et al., 2011). The mean verbal fluency score was 6.1 ± 3.9 (range 2.9–14.8), which is within the lower range of previous reports (Massman et al., 1996; Abrahams et al., 2000; Ahn et al., 2011).

The ALS patients Frontal Behavioral Inventory score ranged from 2 to 42, with an average of 11.0 ± 11.7. One patient had a score of ≥27, which is required for a diagnosis of frontal lobe dementia (Kertesz et al., 1997). Mean Centre for Neurologic Study Lability Scale emotional lability score was 10.8 ± 3.6. A score of ≥13 suggests emotional lability (Moore et al., 1997). ALS participants did not display signs of depression or anxiety in the Hospital Anxiety and Depression Scale test. All but one patient fell within the normal range for anxiety. The average depression score of 2.9 ± 2.4 and anxiety score of 4.3 ± 3.1 fell within the normal range of 0–7 points (Zigmond and Snaith, 1983).

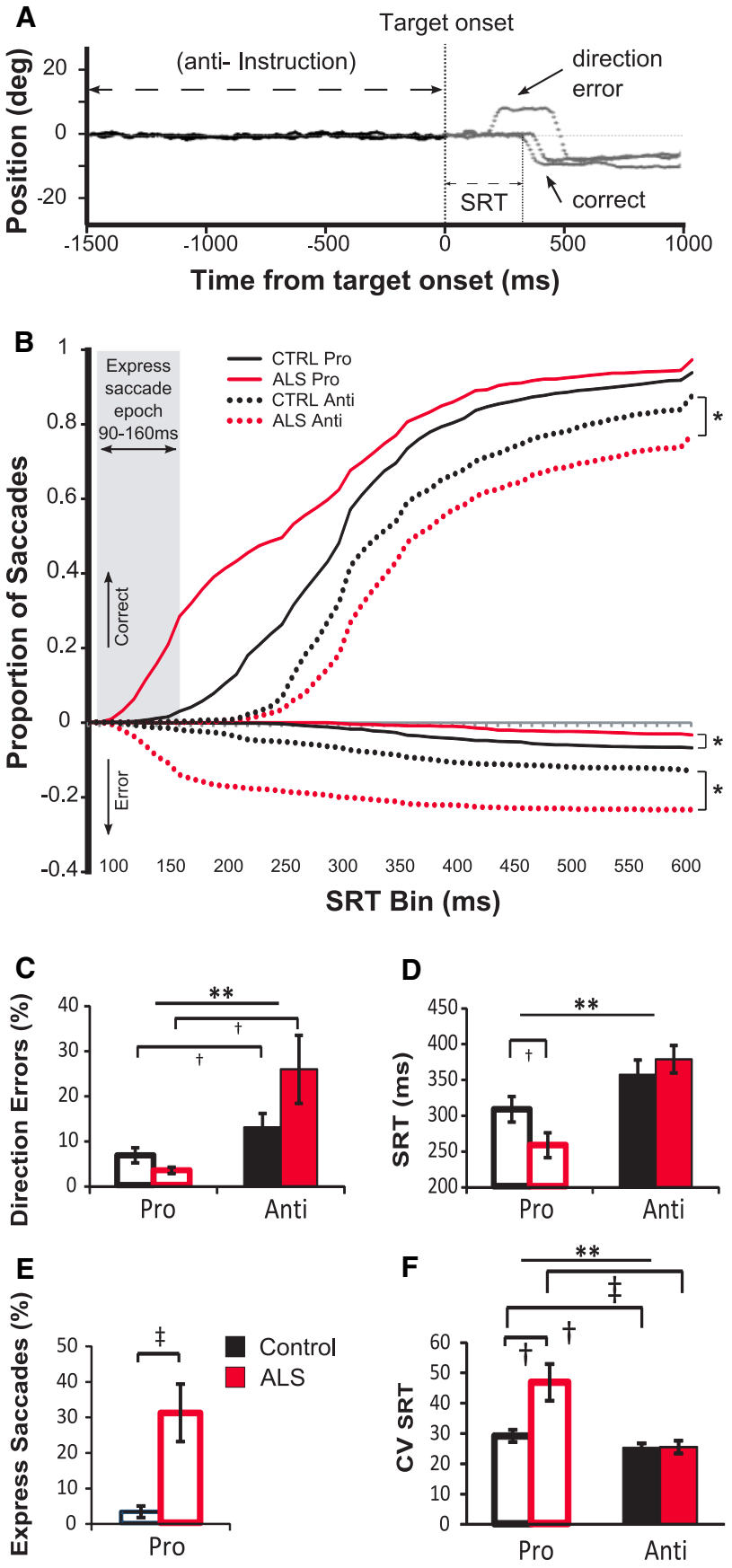

Figure 2. Eye movement behavioral data. ***A***, Sample eye traces depicting correct antisaccade trials and an erroneous antisaccade trial (direction error) followed by a correction. ***B***, Cumulative probabilities of saccade distributions for the two groups (pooled SRT across subjects). Positive *Y* values indicate correct saccades, whereas negative *Y* values indicate direction errors; dashed lines indicate antitrials; solid lines indicate protrials; black lines indicate control; red lines indicate ALS patients. Gray shaded region represents the region categorized as "express saccades" ($90 \leq SRT \leq 160$ ms). Asterisks indicate significant shifts in error rates between the control group and the ALS group. ***C***, Mean percentage direction errors (initial saccade away from target on prosaccade trial, toward target on antisaccade trial). ***D***, Mean SRTs on correct trials. ***E***, Mean percentage of express saccades (90–160 ms). ***F***, Mean intrasubject CVSRT. Error bars indicate SEM. †$p < 0.05$, significance for group × task interactions only. ‡$p < 0.01$, significance for group × task interactions only. *$p < 0.05$. **$p < 0.01$, significance for main effects of task.

Eye movement behaviors

Eye movement behavioral data are shown in Figure 2. Sample control eye traces depicting correct antisaccade trials and an erroneous antisaccade trial (direction error) followed by a correction are shown in Figure 2*A*.

SRT cumulative distribution

The cumulative distribution of SRTs for the prosaccade and antisaccade tasks are displayed as a proportion of the total number of trials, where the latencies of correct and incorrect saccades were categorized into SRT bins of 10 ms increments (Fig. 2*B*). In the prosaccade task, ALS subjects were much faster than controls and many responses fell within the express saccade epoch (Fig. 2*B*, gray bar). In contrast, on the antisaccade task, control subjects were faster at responding than ALS patients. Cumulative SRT distributions using the nonparametric two-sample Kolmogorov–Smirnov test were significantly different across ALS and control groups for correct antisaccades ($K = 1.435$, $p < 0.033$), incorrect antisaccade trials ($K = 4.489$, $p < 0.001$), and incorrect prosaccade trials ($K = 2.490$, $p < 0.001$). Distributions of correct prosaccade SRTs between ALS and controls were not significantly different when all SRT bins were included ($K = 1.272$, $p < 0.079$); however, SRT distributions in the express saccade epoch only (90–160 ms) were significantly different between groups ($K = 1.500$, $p < 0.022$) (Fig. 2*B*).

Saccade direction errors

The ALS group made significantly more direction errors on the antisaccade task than in the prosaccade task ($t_{(11)} = -2.61$, $p = 0.02$) (Fig. 2*C*), whereas the control group direction errors on the antisaccade task showed a large trend toward significance ($t_{(11)} = -2.12$, $p = 0.057$) compared with the prosaccade task. The analysis of group by condition interaction

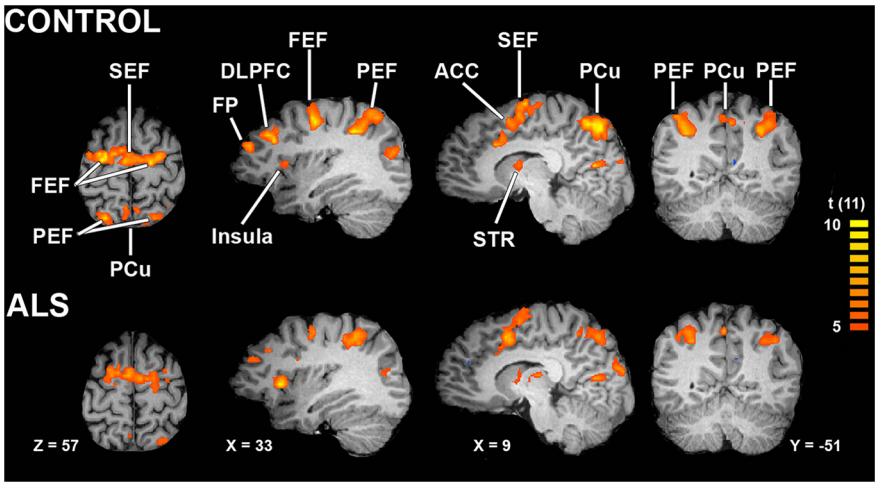

Figure 3. Saccade network. Contrast map of combined correct prosaccade trials and antisaccade trials corrected using false discovery rate at $p < 0.01$ (t value = 5.0, df = 11). The identified ROIs were cluster-corrected across the population of voxels with $p < 0.05$ (49 contiguous voxels, as estimated by Brain Voyager's Cluster-level Statistical Threshold Estimator with 1000 iterations).

Table 2. Regions of interest[a]

		Control				ALS			
ROI	CH	*X*	*Y*	*Z*	T value	*X*	*Y*	*Z*	T value
FP	L	−35	43	19	4.18	−35	41	19	5.55
	R	28	44	22	7.93	35	41	26	7.78
DLPFC	L	−40	26	32	4.14	−39	31	33	5.99
	R	36	27	31	9.54	36	31	33	7.28
Insula	L	−29	26	7	8.76	−28	20	6	10
	R	30	21	9	9.56	31	18	7	10.11
ACC	L	—	—	—	—	—	—	—	—
	R	8	3	45	7.59	9	8	44	9.61
STR	L	−21	−3	7	8.44	−27	−1	8	9.48
	R	21	−7	10	9.47	24	1	7	7.5
SEF	L	−6	−11	56	8.28	−6	−3	54	9.65
	R	3	−11	57	10.03	3	−1	57	8.82
FEF	L	−20	−13	57	10.79	−19	−7	62	12.81
	R	23	−5	54	16.18	24	−6	52	8.56
PCu	L	−24	−66	48	10.59	−26	−57	46	6.81
	R	27	−62	42	17.03	27	−58	45	6.78
PEF	L	−24	−66	48	13.42	−26	−57	46	6.81
	R	27	−62	42	15.78	27	−58	45	7.28

[a]Talairach coordinates (*X*,*Y*,*Z*) of cubic clusters containing the 125 most significant voxels centered around peak activation in GLM contrast maps for antisaccade + prosaccade contrast (Fig. 3). All ROIs were 125 voxels taken at local maxima. CH, Cerebral hemisphere.

also resulted in a trend between group and task ($F_{(1,22)} = 3.21$, $p = 0.08$, $\eta_p^2 = 0.127$). Analysis of the direction errors revealed no main effect of group ($F_{(1,22)} = 0.842$, $p = 0.37$, $\eta_p^2 = 0.037$). However, there was a significant main effect of task ($F_{(1,22)} = 10.01$, $p < 0.01$, $\eta_p^2 = 0.313$).

Saccadic reaction times

Analysis of SRTs (Fig. 2*D*) showed a significant main effect of task ($F_{(1,22)} = 27.76$, $p < 0.01$, $\eta_p^2 = 0.558$), with significantly prolonged antisaccade latencies as previously reported (Shaunak et al., 1995; Donaghy et al., 2010). However, there was no main effect of group ($F_{(1,22)} = 0.617$, $p = 0.44$, $\eta_p^2 = 0.027$), or group × task interactions ($F_{(1,22)} = 3.94$, $p = 0.06$, $\eta_p^2 = 0.152$).

Express saccades

The express saccade epoch was defined within the 90–160 ms interval. The ALS group made a significantly greater proportion of express saccades on prosaccade trials compared with controls ($t_{(22)} = -2.37$, $p = 0.027$, d = −1.3) (Fig. 2*E*).

SRT variability

SRT intrasubject variability was expressed as a CV (Fig. 2*F*). A main effect of task was found for CVSRT ($F_{(1,22)} = 17.57$, $p < 0.01$, $\eta_p^2 = 0.444$) such that prosaccade SRTs were significantly more variable within subjects than antisaccades. A significant group effect was also observed ($F_{(1,22)} = 6.85$, $p = 0.016$, $\eta_p^2 = 0.237$), where ALS patients had more variability for CVSRT than controls. The interaction between group and task also reached significance ($F_{(1,22)} = 9.7$, $p < 0.01$, $\eta_p^2 = 0.306$), likely as a result of the increased prosaccade variability in the ALS group.

Saccade metrics

An ANOVA revealed no significant main effect of group on saccade amplitude ($F_{(1,22)} = 0.66$, $p = 0.42$, $\eta_p^2 = 0.029$) or saccade velocity ($F_{(1,22)} = 1.24$, $p = 0.27$, $\eta_p^2 = 0.053$), suggesting that ALS patients did not have significantly altered saccade metrics. A main effect of task was observed for saccade amplitude ($F_{(1,22)} = 7.96$, $p = 0.01$, $\eta_p^2 = 0.266$), where antisaccades had significantly greater amplitudes than prosaccades. A significant interaction effect between group and task on saccade amplitude ($F_{(1,22)} = 9.37$, $p < 0.01$, $\eta_p^2 = 0.299$) was followed by pairwise comparisons, which revealed that antisaccades had significantly greater amplitudes than prosaccades only within the ALS group ($p = 0.003$). Saccade velocity showed no main effect of task ($F_{(1,22)} = 1.3$, $p = 0.265$, $\eta_p^2 = 0.056$) but displayed a significant interaction between group and task ($F_{(1,22)} = 7.92$, $p = 0.01$, $\eta_p^2 = 0.265$).

Behavioral correlations

Prosaccade and antisaccade SRTs and direction errors were correlated with neuropsychological test scores from Table 1. All correlations of antisaccade SRTs were found to be not significant (p values >0.11). All correlations of antisaccade direction errors were also found to be not significant (p values >0.062), with the exception of the MoCA score where a two-tailed Pearson's correlation revealed a significant negative correlation between the ALS group's performance on the MoCA and the percentage of antisaccade errors made ($r_{(12)} = -0.686$, $p < 0.01$), where antisaccade error rates increased as MoCA scores worsened.

fMRI results

The saccade network

An initial analysis was performed to identify regions involved in the preparation and execution of prosaccade and antisaccades. This global analysis identified an oculomotor network that is consistent with previous reports using fMRI to delineate saccade-related areas (Luna et al., 1998; Connolly et al., 2002; Ford et al., 2005; Brown et al., 2006, 2007; Anderson et al., 2012; Jamadar et al., 2013). Our results included FP, DLPFC, insula, SEF, FEF, STR, PCu, and PEF. Figure 3 depicts the most relevant slices for this network for the control and ALS groups, and Table 2 lists the Talairach locations of peak activation for all key ROIs. Both groups recruited all predefined ROIs, suggesting that the observed behavioral deficits in the ALS group likely were attributed

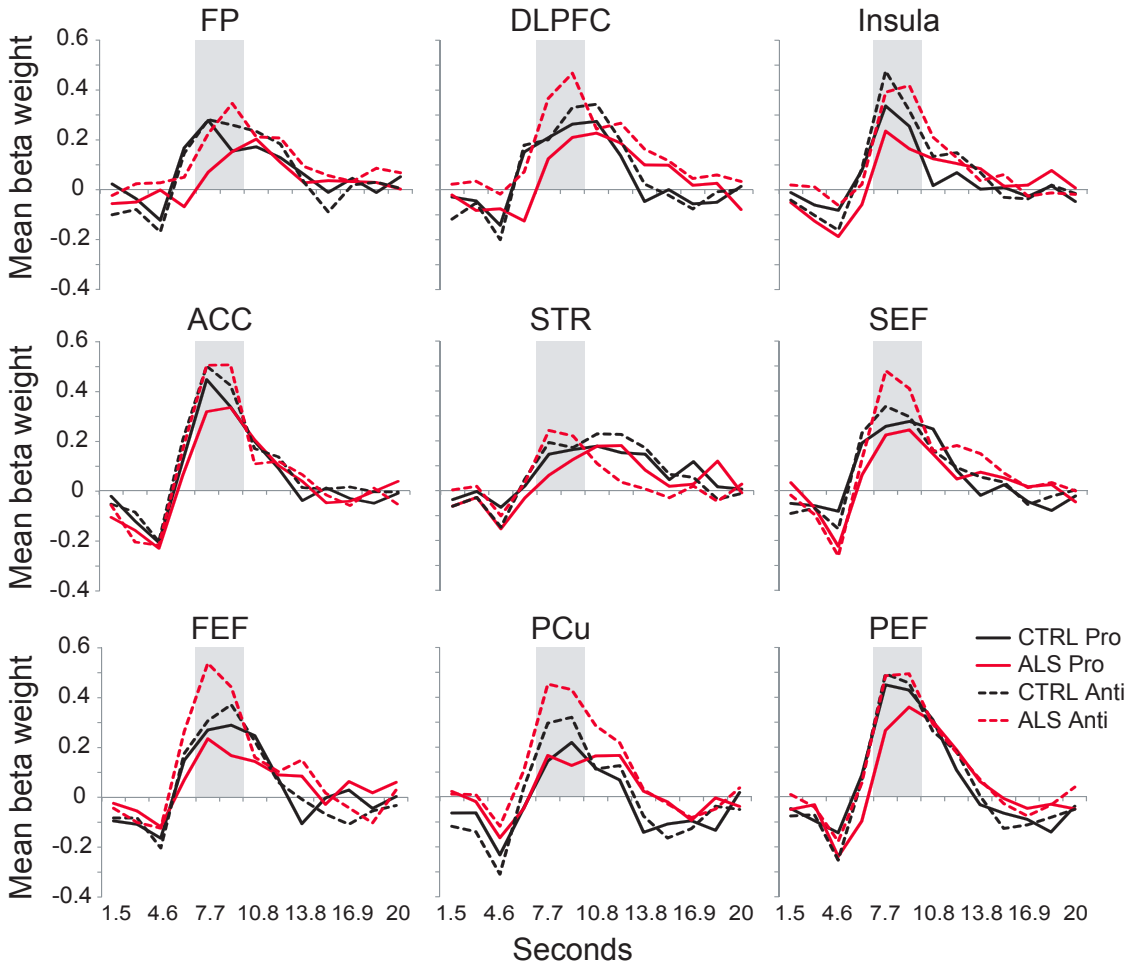

Figure 4. ROI activation time course. Average activity time courses in the preparatory network ROIs during pro (solid lines) and anti (dashed lines) for control (black) and ALS patients (red). Gray bar represents the time points used for subsequent analysis.

to critical differences in subprocesses of prosaccade and antisaccade control (i.e., saccade preparation or execution). The ROIs obtained from this analysis were then selected for second-level analyses to dissect the differential contribution of the preparatory and execution processes.

The preparatory network

Once the saccade network ROIs were defined, the BOLD signal time courses corresponding to the catch trials were obtained (Fig. 4). To dissect the effect of ALS pathology on the processes specifically involved in the preparation of the oculomotor network to an impending prosaccade or antisaccade, we analyzed the fifth and sixth time points (7.7 and 9.3 s from trial onset) of the procatch and anticatch trials (Fig. 5). Group comparisons of the average of these time points obtained from the local maxima β weight values at all ROIs are shown in Figure 5. We were especially interested in analyzing whether ALS patients responded differently from controls when they had to inhibit the automatic response in contrast to simply produce the automatic response (i.e., we were interested in the interaction between these two conditions). Prosaccade and antisaccade catch trials were evaluated to determine which oculomotor regions were recruited by the ALS group compared with controls when preparing a saccade response to the prosaccade or antisaccade visual cue. The group (control and ALS) by condition (prosaccade and antisaccade) interaction was significant for all ROIs with the exception of the ACC (Fig. 5). A significantly greater preparatory response was evoked on antisaccade catch trials by the ALS group in the following regions: FP ($F_{(1,46)} = 4.27$, $p = 0.04$, $\eta_p^2 = 0.089$), DLPFC ($F_{(1,46)} = 16.14$, $p < 0.01$, $\eta_p^2 = 0.241$), insula ($F_{(1,46)} = 11.10$, $p < 0.01$, $\eta_p^2 = 0.202$), ACC ($F_{(1,23)} = 16.14$, $p < 0.01$, $\eta_p^2 = 0.241$), STR ($F_{(1,46)} = 5.22$, $p = 0.02$, $\eta_p^2 = 0.106$), SEF ($F_{(1,46)} = 8.00$, $p < 0.01$, $\eta_p^2 = 0.154$, FEF ($F_{(1,46)} = 13.96$, $p < 0.01$, $\eta_p^2 = 0.241$), PCu ($F_{(1,46)} = 11.67$, $p < 0.01$, $\eta_p^2 = 0.210$), and PEF ($F_{(1,46)} = 4.94$, $p = 0.03$, $\eta_p^2 = 0.101$). The analysis of the condition (prosaccade vs antisaccade) yielded significant differences in all areas: FP ($F_{(1,44)} = 15.41$, $p < 0.01$, $\eta_p^2 = 0.25$), DLPFC ($F_{(1,44)} = 25.94$, $p < 0.01$, $\eta_p^2 = 0.37$), insula ($F_{(1,44)} = 8.32$, $p < 0.01$, $\eta_p^2 = 0.15$), ACC ($F_{(1,44)} = 16.06$, $p < 0.01$, $\eta_p^2 = 0.42$), STR ($F_{(1,44)} = 11.84$, $p < 0.01$, $\eta_p^2 = 0.21$), SEF ($F_{(1,44)} = 20.9$, $p < 0.01$, $\eta_p^2 = 0.32$), PCu ($F_{(1,44)} = 6.37$, $p = 0.01$, $\eta_p^2 = 0.12$), PEF ($F_{(1,44)} = 11.34$, $p < 0.01$, $\eta_p^2 = 0.20$.

To analyze the effect of the heightened antisaccade preparatory activation in the ALS group on saccade behavior, we correlated the mean β weights from anticatch trials to the antisaccade reaction times for each ALS patient (Fig. 6). Significant negative correlations were found between antisaccade reaction times and mean β weights in all preparatory oculomotor ROIs, with the exception of the ACC, PCu, and PEF, suggesting that subjects

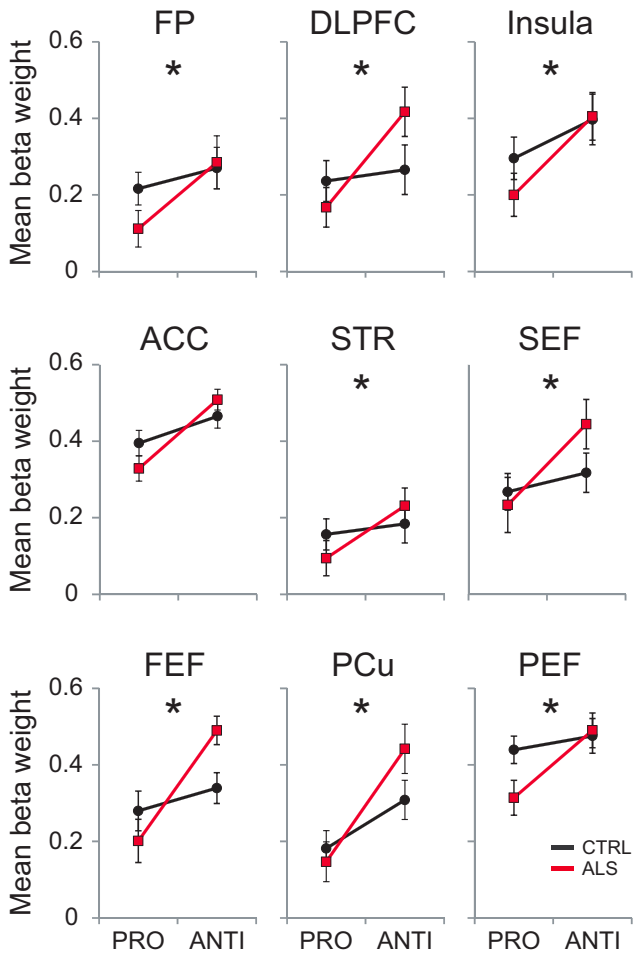

Figure 5. ROI procatch and anticatch trials analysis. Group × condition interaction analysis using the mean β weight peak activity during procatch and anticatch trials for control and ALS groups. Note the heightened activity increase in the anticatch condition in all areas in the ALS group. ACC also showed a significant activity increase in the anticatch condition; however, the group × condition interaction did not reach significant levels. Error bars indicate SE. *$p < 0.05$.

who showed heightened activation when preparing to make an antisaccade were able to execute faster antisaccade reaction times (Fig. 6).

The saccade execution network

To isolate ROI activation involved in the execution of a prosaccade or antisaccade, we analyzed the mean peak β weights of each region using the sixth and seventh time points (9.3 and 10.8 s from trial onset) of prosaccade and antisaccade trials minus the preparatory activation from catch trials reported above. The group × condition interaction analysis was significant in the insula ($F_{(1,44)} = 4.84$, $p = 0.03$, $\eta_p^2 = 0.09$), and showed a trend in PEF ($F_{(1,44)} = 3.09$, $p = 0.08$, $\eta_p^2 = 0.06$). The analysis of the condition (prosaccade vs antisaccade) yielded significant differences in the ACC ($F_{(1,44)} = 5.65$, $p = 0.027$, $\eta_p^2 = 0.20$), STR ($F_{(1,44)} = 10.15$, $p < 0.01$, $\eta_p^2 = 0.18$), SEF ($F_{(1,44)} = 10.92$, $p < 0.01$, $\eta_p^2 = 0.19$), PCu ($F_{(1,44)} = 21.58$, $p < 0.01$, $\eta_p^2 = 0.32$), PEF ($F_{(1,44)} = 20.39$, $p < 0.01$, $\eta_p^2 = 0.31$), but not in FP, DLPFC, insula, or FEF. Finally, the analysis of the group effect resulted in a significant decrease only in PEF ($F_{(1,44)} = 5.09$, $p = 0.02$, $\eta_p^2 = 0.10$). These results show little disruption to the saccade execution network, suggesting that the main detrimental ALS effect is associated with saccade preparation.

Direction errors

To analyze the underlying deficits in saccade preparation that correlated with antisaccade errors in the ALS group, we analyzed the fifth and sixth time points of the antisaccade error trials and compared them with their respective time points obtained from correct antisaccade trials for each ROI. ALS patients showed a significant decrease in DLPFC activation ($t_{(23)} = 2.45$, $p = 0.02$), an increase in insula activation ($t_{(23)} = -2.16$, $p = 0.041$), and an increase trend in SEF ($t_{(23)} = -1.92$, $p = 0.06$) (Fig. 7, left column) on antisaccade error trials compared with correct trials. There were no significant differences between correct and error antisaccade trials in the other areas: FP ($t_{(23)} = 1.53$, $p =$ not significant), ACC ($t_{(23)} = -.03$, $p =$ not significant), STR ($t_{(23)} = 1.73$, $p =$ not significant), FEF ($t_{(23)} = -1.45$, $p =$ not significant), PCu ($t_{(23)} = .09$, $p =$ not significant), and PEF ($t_{(23)} = -1.24$, $p =$ not significant). A similar analysis in the control subjects showed significant decreases in activation on error trials only in FEF ($t_{(23)} = 2.3$, $p = 0.03$), and PEF ($t_{(23)} = 2.8$, $p = 0.01$).

To investigate the effect of antisaccade preparation on saccade behavior, we correlated the preparatory BOLD signal of each subject with the individual direction error performance during the antisaccade task in all ROIs of the saccade network. The analyses resulted in two significant negative correlations: one in SEF ($r = -0.66$, $p = 0.03$) and the other in FEF ($r = -0.77$, $p < 0.01$) ($r = -0.66$, $p =$ not significant) (Fig. 7, right column). The correlation analyses in all the other regions were not significant: FP ($r = 0.28$, $p =$ not significant), DLPFC ($r = -0.13$, $p =$ not significant), insula ($r = -0.56$, $p =$ not significant), ACC ($r = -0.48$, $p =$ not significant), STR ($r = 0.48$, $p =$ not significant), PCu ($r = -0.35$, $p =$ not significant), and PEF ($r = -0.40$, $p =$ not significant).

Discussion

We tested whether deterioration in the implementation of flexible behaviors in ALS is directly associated with DLPFC dysfunction. To this end, we used a prosaccade and antisaccade task coupled with fMRI and eye tracking. We found that ALS patients were significantly impaired when implementing flexible behavior, as demonstrated by a greater proportion of antisaccade direction errors (Fig. 2*B*,*C*). This error-related impairment was accompanied by a significant reduction in DLPFC activity (Fig. 7). A bias toward automatic responses in ALS was also demonstrated by a greater proportion of express saccades and more variable prosaccade SRTs (Fig. 2*E*,*F*). The results also showed functional changes that included significant increased activation in critical areas of the antisaccade network, such as FEF, SEF, and DLPFC, during saccade preparation. ALS patients showing these heightened responses during this task set establishment period also showed better performance during the antisaccade task (Fig. 6).

Behavioral deficits in ALS

Our results confirmed that ALS patients had antisaccade impairments (Shaunak et al., 1995; Evdokimidis et al., 2002; Donaghy et al., 2010) joining a body of research that has confirmed that ALS patients show oculomotor deficits in addition to the evident motor deterioration related to the motor neuron degeneration (Jacobs et al., 1981; Leveille et al., 1982; Ohki et al., 1994; Shaunak et al., 1995; Donaghy et al., 2010; Sharma et al., 2011; Burrell et al., 2013). The oculomotor deficits documented in ALS include ophthalmoplegia (Harvey et al., 1979), defective pursuit (Jacobs et al.,

1981), saccadic impairments (Shaunak et al., 1995; Donaghy et al., 2010), nystagmus (Kushner et al., 1984), and abnormal Bell phenomenon (Esteban et al., 1978). Defects in pursuit have been attributed to nonnuclear involvement of extrapyramidal or corticobulbar components of the oculomotor system. Saccadic impairments reported include slowing of vertical saccades, increased incidence of errors, and increased latency on the antisaccade task. Surprisingly, reflexive saccades appear to remain relatively intact, with bulbar onset demonstrating somewhat slower reflexive saccades than limb onset patients (Donaghy et al., 2010).

The significant increase of express saccades in ALS patients is a novel finding. Express saccades are elicited when high levels of pretarget activity combine with visual responses in saccade-related neurons of the superior colliculus (Dorris et al., 1997; Dorris and Munoz, 1998; Everling et al., 1998, 1999). During the gap period, pretarget preparatory activity is elevated and conditions are optimal for express saccade generation, making suppression of an unwanted saccade on an antisaccade trial very difficult, unless sufficient inhibition from the frontal lobes is exerted on saccade neurons in superior colliculus (Everling et al., 1998, 1999; Munoz and Everling, 2004). ALS patients show defective intracortical inhibition, leading to deficits in inhibitory interneuronal circuits that result in hyperexcitable cortical neurons (Ziemann et al., 1997). Therefore, the ALS patients' bias toward automatic express saccades could be the result of high levels of motor preparation activity because of defective intracortical inhibition, combined with poor executive control resulting from DLPFC impairment.

We also investigated whether there was any correlation between the eye movement behaviors and the neuropsychological tests. The only significant finding was that the ALS antisaccade error rate was inversely correlated with the MoCA score, which places more emphasis on frontal executive and attentional process than the more traditional Mini-Mental Status Examination (Smith et al., 2007). However, we did not find correlations with other general measurements of frontal lobe function, such as Frontal Behavioral Inventory or Controlled Oral Word Association. Previous findings show a large variation in this regard. Some studies have reported a lack of correlations between clinical data and oculomotor measurements (Shaunak et al., 1995), whereas others have shown significant correlations with frontal lobe dependent tasks, such as the Wisconsin Card Sorting Test (Evdokimidis et al., 2002) or the Stroop task (Donaghy et al., 2010). However, in the last study, the correlations with the Stroop measures were only found in bulbar onset patients. It also should be noted that ALS patients' cognitive performance varied considerably (Table 1), suggesting a continuum of impairments in this group of patients.

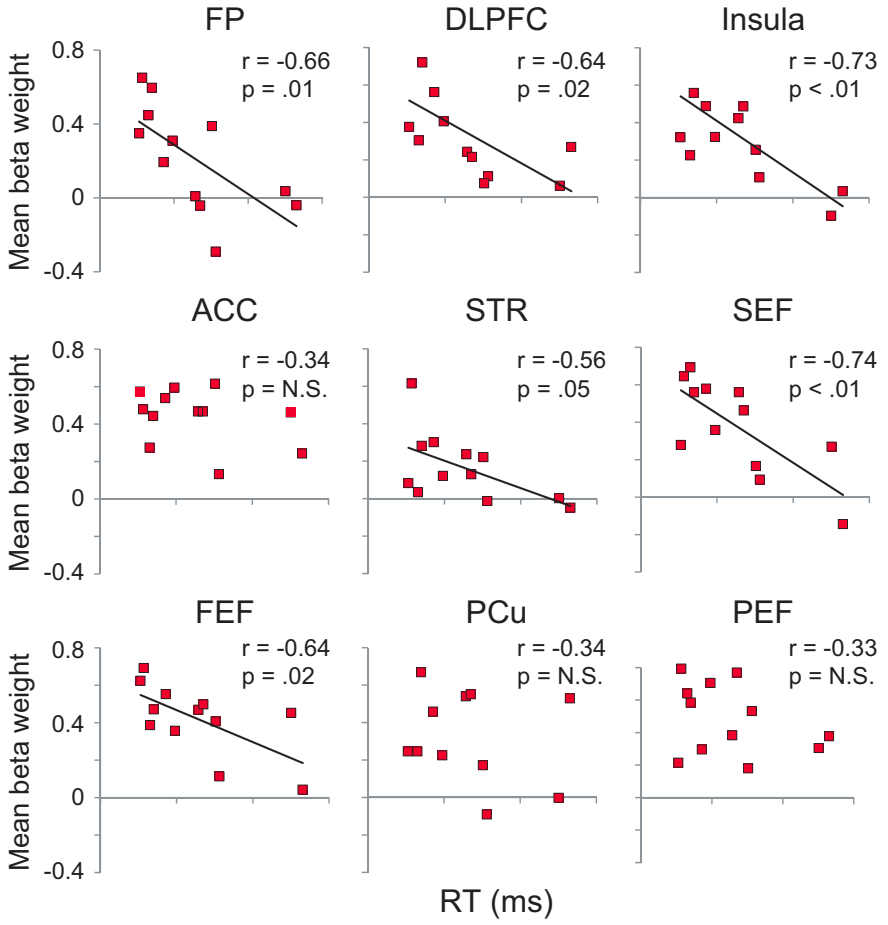

Figure 6. Correlations of activation in selected ROIs of the saccade network with SRT. Pearson's correlations between mean β weight peak activity during anticatch trials and SRT during antisaccades in all ALS patients. Note how individuals with a larger average activity during the preparatory phase in many areas, including DLPFC, show faster reaction times when executing correct antisaccades.

Saccade network activity

To perform a successful antisaccade, several cortical and subcortical brain regions must be recruited, including DLPFC (Guitton et al., 1985; Pierrot-Deseilligny et al., 2003), FEF, PEF, and SEF (Connolly et al., 2002; Curtis and D'Esposito, 2003; DeSouza et al., 2003; Ford et al., 2005; Brown et al., 2007), and basal ganglia (Cameron et al., 2009; Ford and Everling, 2009; Watanabe and Munoz, 2010, 2011, 2013). It is well documented that preparatory neural activity established before the appearance of the peripheral target presets the motor system to execute the appropriate action (Everling and Munoz, 2000; Curtis and D'Esposito, 2003; DeSouza et al., 2003). Here we showed that ALS patients recruited a similar neural network during both the preparatory and the execution stages of prosaccades and antisaccades. However, the results from our study revealed critical differences in activation not only between ALS and control group, but also within correct and incorrect antisaccade trials of ALS patients, which can help explain the observed oculomotor deficits in this group of ALS patients.

The analysis of the preparatory activity during prosaccades and antisaccades yielded significant between-group differences for the entire preparatory saccade network. These differences were driven by a significant increase of preparatory activity in response to the antisaccade cue compared with the prosaccade cue in the ALS group. Previous ALS imaging studies using block

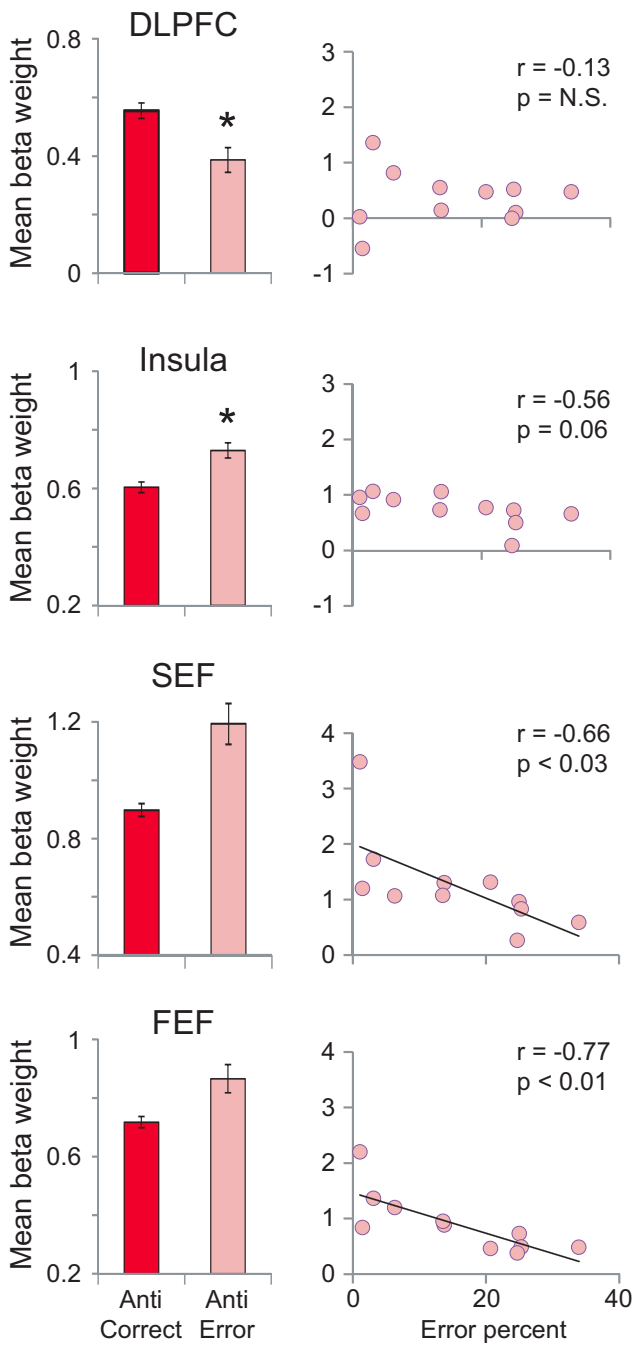

Figure 7. Direction errors analysis. Left, Mean β weight peak activity during correct and error trials in the antisaccade task for the ALS group. Note the significant reduced activity in DLPFC during error trials. Right, Correlations between mean β weight peak activities during antisaccade error trials with the percentage of errors. Note how the increased activity in the insula, SEF, and FEF correlated with fewer errors. Error bars indicate SE. *$p < 0.05$.

designs have found significant increases of activity during various tasks in patients (Mohammadi et al., 2011; Cosottini et al., 2012; Poujois et al., 2013), suggesting a possible compensatory mechanism (Konrad et al., 2002, 2006; Schoenfeld et al., 2005; Han and Ma, 2006; Lulé et al., 2007; Douaud et al., 2011). The correlation analysis between antisaccade preparatory activation and antisaccade reaction time showed significant negative correlations in the FP, DLPFC, insula, STR, SEF, and FEF, suggesting that greater preparatory activity in these ROIs corresponded to faster reaction times (Braun et al., 1992; Everling and Munoz, 2000; Connolly et al., 2005; Hakvoort Schwerdtfeger et al., 2012). Interestingly, although the insula has not traditionally been related to the saccade network, brain imaging studies have shown that it is constantly active during antisaccade tasks (Brown et al., 2006; Raemaekers et al., 2007), possibly because of its involvement with saliency processing (Menon and Uddin, 2010). In relation to the heightened activity, our results in various ROIs of the saccade network contrast with previous studies in other patient populations, such as Parkinson's disease and attention deficit hyperactivity disorder, which showed reduced preparatory activity in critical areas of the network, including SEF, FEF, PEF, STR, and DLPFC (Rieger et al., 2008; Cameron et al., 2012; Hakvoort Schwerdtfeger et al., 2012). This strongly suggests that ALS pathology has a main effect on antisaccades at the preparatory stage. Regarding the execution activity, the interaction analysis results showed that the core of the antisaccade network was similar for both groups. This is interesting because previous functional imaging reports have suggested changes in cortical activation boundaries (Kew et al., 1993). Although our experiment did not directly address this issue, the activity changes that we found point to a compensatory activation instead. However, it remains to be determined whether this activation increase is a byproduct of a decrease in interneuron inhibitory activity (Turner and Kiernan, 2012).

Direction error activity

In contrast to a block design, event-related studies allow for BOLD response analyses on a trial-by-trial basis. Exploiting this, we analyzed the BOLD signal from antisaccade trials where ALS patients made directional errors. We found a significant reduction in DLPFC preparatory activity during erroneous antisaccades, in contrast to higher than normal activity levels during correct antisaccades (discussed above), which supports our hypothesis that faulty antisaccade implementation in ALS is related to DLPFC dysfunction. Frontal lobe functional changes have been reported mainly on tasks related to language (Abrahams et al., 1996, 2004) and hand movement tasks (Kew et al., 1993; Konrad et al., 2002; Schoenfeld et al., 2005; Stanton et al., 2007; Mohammadi et al., 2011). The majority of those studies tested simple motor tasks; however, in one study ALS patients were instructed to make joystick movements in freely selected random sequences that are self-initiated responses that require some planning (Stanton et al., 2007). Although ALS patients showed increased activity centered in the primary sensorimotor cortex, they also showed reduced DLPFC activity (Stanton et al., 2007). These findings are similar to our results in that we found increased activity in a number of areas of the saccade network but reduced activity in the DLPFC when a subject made antisaccade errors. However, our experimental design allowed us to clearly distinguish the processes that were associated with these changes. Our analyses of the areas that showed activity increases during erroneous antisaccade trials showed that this heightened activity correlated with a smaller number of errors, reaching statistical significance in SEF and FEF. In contrast, the reduced DLPFC activation was specifically related to a faulty implementation of the task set.

In conclusion, our findings show that, in ALS patients, an abnormal DLPFC activation specifically during the establishment of the task set, is related to a deficit in the inhibition of automatic responses, a crucial process within the executive system. These results provide a direct link between a particular impairment of cognitive process and a functional deficit in the prefrontal cortex in ALS. Our results also show that ALS patients' heightened functional activity found in specific areas of the sac-

cade network correlate with better responses, which fits with the concept of functional compensatory plasticity subsequent to the ALS neurological impairment. Further research should explore the mechanisms resulting in this compensatory plasticity and whether it could be exploited for therapeutic purposes.

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