Executive deficits, not processing speed relates to abnormalities in distinct prefrontal tracts in amyotrophic lateral sclerosis

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Cognitive impairment in amyotrophic lateral sclerosis is characterized by deficits on tests of executive function; however, the contribution of abnormal processing speed is unknown. Methods are confounded by tasks that depend on motor speed in patients with physical disability. Structural and functional magnetic resonance imaging studies have revealed multi-system cerebral involvement, with evidence of reduced white matter volume and integrity in predominant frontotemporal regions. The current study has two aims. First, to investigate whether cognitive impairments in amyotrophic lateral sclerosis are due to executive dysfunction or slowed processing speed using methodology that accommodates motor disability. This is achieved using a dual-task paradigm and tasks that manipulate stimulus presentation times and do not rely on response motor speed. Second, to identify relationships between specific cognitive impairments and the integrity of distinct white matter tracts. Thirty patients with amyotrophic lateral sclerosis and 30 age- and education-matched control subjects were administered an experimental dual-task procedure that combined a visual inspection time task and digit recall. In addition, measures of executive function (including letter fluency) and processing speed (visual inspection time and rapid serial letter identification) were administered. Integrity of white matter tracts was determined using region of interest analyses of diffusion tensor magnetic resonance imaging data. Patients with amyotrophic lateral sclerosis did not show impairments on tests of processing speed, but executive deficits were revealed once visual inspection time was combined with digit recall (dual-task) and in letter fluency. In addition to the corticospinal tracts, significant differences in fractional anisotropy and mean diffusivity were found between groups in a number of prefrontal and temporal white matter tracts including the anterior cingulate, anterior thalamic radiation, uncinate fasciculus and hippocampal portion of the cingulum bundles. Significant differences also emerged in the anterior corona radiata as well as in white matter underlying the superior, medial and inferior frontal gyri and the temporal gyri. Dual-task performance significantly correlated with fractional anisotropy measures in the middle frontal gyrus white matter.
and anterior corona radiata. Letter fluency indices significantly correlated with fractional anisotropy measures of the inferior frontal gyrus white matter and corpus callosum in addition to the corticospinal tracts and mean diffusivity measures in the white matter of the superior frontal gyrus. The current study demonstrates that cognitive impairment in amyotrophic lateral sclerosis is not due to generic slowing of processing speed. Moreover, different executive deficits are related to distinct prefrontal tract involvement in amyotrophic lateral sclerosis with dual-task impairment associating with dorsolateral prefrontal dysfunction and letter fluency showing greater dependence on inferolateral prefrontal dysfunction.

Keywords: amyotrophic lateral sclerosis; diffusion tensor imaging, dual-task, cognition
Abbreviations: ALS = amyotrophic lateral sclerosis; FTD = frontotemporal dementia

Introduction

Cognitive profile of amyotrophic lateral sclerosis

Amyotrophic lateral sclerosis (ALS) is characterized by progressive degeneration of the upper motor neurons of the corticospinal tract and the lower motor neurons of the brain stem and spinal cord. However, it is now well recognized that ALS is a multi-system disorder, as changes in extra-motor areas of the cortex have consistently been observed (Kew et al., 1993; Abrahams et al., 1996; Geser et al., 2008). A relationship exists between ALS and frontotemporal dementia (FTD) with 5–15% of patients with ALS developing an FTD syndrome, predominantly a behavioural variant associated with transactive response DNA binding protein (TDP)-43 pathology (Neary et al., 2000; Geser et al., 2009). Moreover, a larger proportion (~35%) of non-demented patients with ALS present with specific cognitive impairment (Ringholz et al., 2005; Phukan et al. 2012) implicating a spectrum of cognitive change between FTD and ALS, and with subclinical FTD symptoms in a significant number of cases (Girardi et al., 2011; Abrahams, 2013). Recent genetic studies have revealed a hexanucleotide repeat expansion in C9orf72 that accounts for 41% of familial and 5% of sporadic cases (Byrne et al., 2012). Patients with the mutation have a distinct phenotype and an increase in FTD. Non-demented patients with ALS who have the expansion display similar levels of executive dysfunction, but less non-executive cognitive dysfunction than those without the expansion (Byrne et al., 2012).

In non-demented patients with ALS, the most commonly reported deficits are in tests of executive function (Kew et al., 1997; Abrahams et al., 2000; Lomen-Hoerth et al., 2003); the most striking and consistent impairments are on tests of letter (phonemic) fluency (Raaphorst et al., 2010), which remain even when individual variations in motor dysfunction and speed are taken into account (Abrahams et al., 2000). The tasks require intrinsic generation of responses in the absence of environmental cues (Frith et al., 1991) and are thought to include executive processes of initiation, strategy formation, sustained attention, set-shifting, inhibition and working memory (Troyer et al., 1997; Abrahams et al., 2000; Rende et al., 2002; Azuma, 2004). Patients with ALS have also been shown to have deficits on tasks in which information is manipulated rather than just held within working memory, such as the reverse digit span test (Rakowicz et al., 1998; Hanagasi et al., 2002) and the paced auditory serial addition test (Moretti et al., 2002). In terms of Baddeley’s (2000) model of working memory, it is postulated that the ‘central executive’ is impaired in ALS (Abrahams et al., 2000). This is an attention control system that coordinates independent short-term memory slave systems (the phonological loop for auditory information and the visuo-spatial sketch pad for visual information) by allocating resources and directing attention in order to maintain and manipulate information. Dual-task paradigms in which a participant performs visual and auditory tasks concurrently are commonly used to investigate this particular function (Cocchini et al., 2002). Dual-tasking has been shown to be impaired in patients with FTD (Perry and Hodges, 2000; Sebastián Gascón and Hernández-Gil, 2010) and a deficit was reported in one study of ALS (Schreiber et al., 2005). However, the performance measurement in this study was reaction time (motor speed), which may falsely exaggerate impairment in ALS due to physical disability.

Processing speed also plays an important role in fluency tasks. Performance in measures such as the digit symbol substitution test and letter comparison task have been found to be strong predictors of fluency performance in populations with traumatic brain injury (Bittner and Crowe, 2007), Parkinson’s disease (McDowd et al., 2011) and normal ageing (Bryan et al., 1997). Moreover, processing speed is thought to be a major deficit in other disorders characterized by white matter abnormalities, such as multiple sclerosis (Hujibregts et al., 2006; Dineen et al., 2009). Indeed, patients with ALS have been shown to be impaired on the symbol digit modalities test (Mezzapesa et al., 2007). However, processing speed is inherently difficult to measure in populations with motor dysfunction due to the reliance of most tasks on timed motor responses, or reaction times (e.g. the digit symbol substitution test, Wechsler, 1981; Digit-digit task, Salthouse, 1994) and measurement using standardized psychomotor tests will once again be confounded by motor disability.

Neural correlates of cognitive impairment in amyotrophic lateral sclerosis

Evidence for a frontotemporal syndrome in ALS comes from brain imaging data (Tsermentseli et al., 2012). Analyses of structural MRI scans of non-demented patients with ALS using manual
and automated volumetric techniques have reported reduced grey matter volume in the superior, medial and mid frontal gyri and anterior cingulate (Ellis et al., 2001; Chang et al., 2005; Kassubek et al., 2005; Grosskreutz et al., 2006; Lillo et al., 2012), as well as the inferior frontal gyri, superior temporal gyri and temporal poles (Chang et al., 2005), although not consistently (Kiernan and Hudson, 1994; Abrahams et al., 2005). Abnormalities have also been exhibited in extra-motor white matter underlying anterior frontal cortex (Kiernan and Hudson, 1994; Kassubek et al., 2005), frontotemporal association fibres implicating the superior longitudinal fasciculus, cingulum and fronto-occipital fasciculus (Abrahams et al., 2005), and the corpus callosum (Abrahams et al., 2005; Kassubek et al., 2005). The recent application of diffusion tensor imaging has proved useful in monitoring white matter integrity and disease progression in the corticospinal tract (Agosta et al., 2010; Bastin et al., 2013), and detected abnormalities in the corpus callosum (Filippini et al., 2010; Lillo et al., 2012) and uncinate fasciculus (Sato et al., 2010).

Despite these advances in brain imaging, relatively few studies have directly investigated the relationship between localized cerebral changes and patients’ cognitive profiles. Functional imaging studies provide evidence to suggest that the neural correlates of impaired fluency performance in ALS are predominantly prefrontal (Tsermentesi et al., 2012). Patients with ALS who have impaired verbal fluency scores have shown reduced cerebral blood flow in prefrontal regions including dorsolateral prefrontal cortex and anterior cingulate in letter fluency activation paradigms in PET (Abrahams et al., 1996) and functional MRI (Abrahams et al., 2004). Cerebral blood flow reductions were shown in comparison to matched control subjects, and to patients with intact fluency performance, illustrating the degree of heterogeneity within cohorts of patients with ALS. Moreover, a recent functional MRI study examined response inhibition in ALS and found evidence of altered activation patterns relative to controls in prefrontal regions including left superior gyrus, left anterior cingulum and left medial frontal gyrus (Goldstein et al., 2011). Associations between cognitive and behavioural changes and structural MRI have been investigated in a recent study in which patients with evidence of cognitive impairment (deficits across two tests of executive functions) and/or behaviour change (two non-overlapping behavioural features) showed greater grey matter atrophy than patients with ALS without such symptoms using automated voxel based analyses (Mioshi et al., 2013). Atrophy was evident across motor and somatosensory cortical regions, which extended into adjacent areas including the superior frontal and parietal gyri. Association with white matter changes has been shown in one study in which patients with impaired verbal fluency had extensive volumetric reductions in frontotemporal white matter regions, whereas those patients with ALS who were unimpaired in verbal fluency showed fewer changes, suggesting that these white matter structures may underpin poor task performance (Abrahams et al., 2005). To our knowledge, only one study has investigated the relationship between diffusion tensor imaging metrics and cognitive functioning in ALS (Sarro et al., 2011). In that study, performance on tests of executive functioning (including verbal fluency) were correlated with reduced white matter integrity in the corpus callosum, cingulum, inferior longitudinal fasciculus and uncinate fasciculus, although fluency measures were not adapted to control for motor dysfunction.

The current study therefore aimed to: (i) dissociate impairments in executive functions from slowed processing speed in ALS using tasks which account for motor dysfunction; and (ii) gain an understanding of the changes in white matter integrity in distinct pathways that may be associated with any cognitive abnormality. The current investigation employed tasks that do not use reaction time as their output measure but rather manipulate stimuli presentation times in order to estimate processing speed. Two paradigms were devised; one for meaningful and one for abstract visual information. Similar paradigms of abstract visual information have been used to investigate processing speed in cognitive ageing and Parkinson’s disease (Johnson et al., 2004; Edmonds et al., 2008). Moreover, in line with the recent emphasis of linguistic impairment in ALS (Abrahams, 2013; Taylor et al., 2013), the processing speed of linguistic material was also investigated using rapid serial visual letter identification. This methodology is analogous to inspection time paradigms that have been used consistently to provide measures of perceptual speed (Schneider and Shiffrin, 1977; Broadbent and Broadbent, 1987). Executive function was investigated by way of a dual-task paradigm that employed processing speed and working memory tasks and used a preload methodology to minimize demands on the motor system. A participant’s performance level on each sub-task was titrated to ensure that each task was of equal difficulty before undertaking them together. Similar paradigms have been used to investigate dual-task abilities over the lifespan (Cocchini et al., 2002; Anderson et al., 2011) and allow processing speed and working memory to be determined both independently and under dual-task conditions.

Materials and methods

Participants

The patient group consisted of 30 people with ALS recruited from regional ALS services at the following National Health Service sites: Western General Hospital, Edinburgh; Southern General Hospital, Glasgow; and Ninewells Hospital, Dundee, Scotland and from the Motor Neurone Disease register for Scotland, University of Edinburgh. All had clinical and electrophysiological evidence of combined upper and lower motor neurone involvement and fulfilled the revised El Escorial criteria for clinically definite or probable ALS (Brooks et al., 2000). Twenty-six patients had sporadic ALS and four had a history of suspected ALS in a first degree relative. Genetic screening was available in 11 patients, of whom two were found to be positive for C9orf72 with neither having a family history of the disease. Ten patients had bulbar onset, 11 had upper limb onset and nine had lower limb onset. Exclusion criteria for patients included the presence of another neurological disorder, history of psychiatric disorder or high cardiovascular risk factors. No patient had evidence of dementia in clinical notes or on initial discussion, although one patient was subsequently found to fulfil the criteria for possible behavioural variant FTD (Rascovsky et al., 2011) after a detailed interview with a caregiver. Disease progression and severity was assessed in patients by administering the ALS functional rating scale-revised (Cedarbaum et al., 1999). Respiratory functioning was assessed with forced vital capacity...
predicted/expected ratio and the Epworth sleepiness scale (Johns, 1991). Thirty age- and education-matched healthy controls were recruited from the volunteer participant panel of the University of Edinburgh Psychology Department or from spouses of patients. All participants had normal hearing and corrected vision and did not have significant neurological or psychiatric comorbidity. This study was approved by the National Health Service Scotland Research Ethics Committee and the Department of Psychology, University of Edinburgh and undertaken in accord with the Declaration of Helsinki.

Background assessment

The Wechsler Test of Adult Reading (Wechsler, 2001) was used to estimate premorbid intelligence. The Hospital Anxiety and Depression Scale (Zigmond and Snaith, 1983) was used to exclude the possibility that an abnormal cognitive profile might be related to emotional state. The question ‘I feel as if I am slowed down’ has exaggerated salience to patients who experience motor dysfunction and was therefore not included in the score (Abrahams et al., 2005). The Graded Naming Test (McKenna and Warrington, 1983) was used to provide a measure of confrontation naming. The digit span, spatial span and logical memory subtests of the Wechsler Memory Scale-III (Wechsler, 1999) were used to assess verbal working memory, spatial working memory and immediate and delayed recall of verbal information respectively. Executive functioning was assessed by two tests: (i) the Brixton Spatial Anticipation test (Burgess and Shallice, 1997) is a commonly used measure of executive functioning that requires minimal motor output and assesses rule detection and set shifting; and ii) the written and spoken letter fluency tests were employed to assess initiation, strategy formation, set-shifting and monitoring; both fluency tests accommodate for physical disability by producing a fluency index (Abrahams et al., 2000), which controls for speaking and writing time, and produces a measure of thinking time.

Experimental cognitive testing

Processing speed

Using the visual inspection time test (adapted from Edmonds et al. (2008)), participants were required to make a forced choice decision regarding an abstract visual stimulus presented briefly on a computer screen. The target stimulus was a simple geometric figure with a clearly elongated ‘tail’ on either the left or right side. Following brief presentation, participants were required to indicate whether they saw a longer tail on the left or right side. During the test participants did not receive feedback and the duration time of the stimulus was manipulated in a fixed pseudorandom order at durations of 150, 125, 102, 85, 68, 51, 34 and 17 ms. A total of 80 trials were completed, 10 at each stimulus duration.

In the rapid serial letter identification task, adapted from Hoffman (1978), participants were presented with a sequence of six consecutive letters. Five of the letters within the stream were black, and one, the target, was red, which was presented in random positions. Participants were required to identify the target letter verbally. Processing speed was investigated by manipulating the duration of the presentation of the letter stimuli in any one sequence in a fixed pseudorandom order (150, 125, 102, 85, 68, 51, 34 or 17 ms). There were 40 trials in total, five at each duration, and the response accuracy was recorded.

Executive functioning: Dual Task Paradigm or Dual Task Procedure

The dual-task paradigm consisted of combining the visual inspection time test (described above) with the delayed digit recall test, both of which were titrated to an individual’s abilities. A similar paradigm has been used to investigate dual-task ability, without the confounds of reaction time-based tasks, in children and older adults (Anderson et al., 2011). The dual-task employed a preload paradigm (Cocchini et al., 2002) as typical concurrent dual-task paradigms are likely to put high demands on response selection and initiation and thus result in interference, especially in populations with motor difficulties. All participants completed the dual-task procedure in the same order as follows.

Individual ability levels

Delayed digit recall test

The participant’s individual delayed digit recall level was determined by presenting them with increasing sequences of numbers to recall from two items with three trials at each length. Participants were presented with digits aurally at a rate of one per second, and asked to recall them after a 15 s delay. If two out of three trials were completed accurately, the number of items per sequence was increased. The delayed digit recall level was taken as the maximum number of items that were recalled accurately in at least two out of three trials.

Visual inspection time

Visual inspection time level was determined by presenting participants with stimuli at increasingly shorter durations (150, 125, 102, 85, 68, 51, 34 and 17 ms). Six visual inspection time trials were presented at each stimulus duration—if participants accurately responded to five, they moved on to the next shortest duration. The stimulus duration at which participants failed to reach the accuracy criterion (five of six trials correct) was taken as their visual inspection time level.

Single tasks

Delayed digit recall

The delayed digit recall single task consisted of eight trials made from digit sequences of one less than their delayed digit recall level—this procedure was adopted following a pilot dual-task study which revealed that performing the dual-task at maximum ability was overly difficult. The total number of digits correctly recalled was converted to a percentage of the total number of digits presented to give the delayed digit recall single task performance (% correct = digits recalled/total digits).

Figure 1  Schematic of visual inspection time task (adapted from Edmonds et al., 2008). ISI = interstimulus interval.
for the single-shot spin-echo echo-planar imaging sequence were locations giving 2 mm isotropic voxels. The repetition and echo times/C2 matrix 128/C2 weighted (b = 1000 s/mm2) whole brain single-shot spin-echo echo-planar imaging volumes acquired with diffusion encoding gradients multiple small square regions of interest to be placed on the T2-weighted (b = 1000 s/mm2) and sets of diffusion-weighted (b = 1000 s/mm2) whole brain single-shot spin-echo echo-planar imaging volumes acquired with diffusion encoding gradients applied in 64 non-collinear directions (Jones et al., 2002). The acquisition parameters were: (i) field of view 256 × 256 mm; (ii) imaging matrix 128 × 128; and (iii) 72 × 2 mm thick contiguous axial slice locations giving 2 mm isotropic voxels. The repetition and echo times for the single-shot spin-echo echo-planar imaging sequence were 16.5 s and 98.3 ms, respectively. The examination took ~20 min.

**Visual inspection time**

The visual inspection time single task consisted of eight 15 s blocks of visual inspection time trials with durations one step below their visual inspection time level. The total number of correct responses was converted to a percentage of total number of trials to give visual inspection time single task performance (% correct = correct responses/total trials).

**Dual-task: delayed digit recall and visual inspection time**

Participants completed eight trials which consisted of first presentation of the digit sequence followed immediately by the visual inspection time trials (15 s) and subsequent recall of the digits. Percentage correct (as described above) was calculated for the delayed digit recall and visual inspection time dual-task respectively. Finally, a measure of dual-task cost for each task was calculated by comparing performance in the single to dual-task conditions:

\[
\text{Dual-task cost} = \left[ \frac{\text{[Dual-task]} \% \text{ correct} - \text{Single task} \% \text{ correct}}{\text{Single task} \% \text{ correct}} \right] \times 100
\]

The average of the two component tasks is a better representation of performance as it controls for strategic prioritizing of one task over the other, thus finally combined average accuracy scores were computed for single task performance, dual-task performance, and dual-task cost.

**Magnetic resonance imaging**

All MRI data were acquired using a GE Signa Horizon HDxt 1.5 T clinical scanner (General Electric) equipped with a self-shielding gradient set (33 mT/m maximum gradient strength) and manufacturer-supplied 8-channel phased-array head coil. The diffusion MRI protocol consisted of seven T2-weighted (b = 0 s/mm²) and seven sets of diffusion-weighted (b = 1000 s/mm²) whole brain single-shot spin-echo echo-planar imaging volumes acquired with diffusion encoding gradients applied in 64 non-collinear directions (Jones et al., 2002). The acquisition parameters were: (i) field of view 256 × 256 mm; (ii) imaging matrix 128 × 128; and (iii) 72 × 2 mm thick contiguous axial slice locations giving 2 mm isotropic voxels. The repetition and echo times for the single-shot spin-echo echo-planar imaging sequence were 16.5 s and 98.3 ms, respectively. The examination took ~20 min.

**Regions of interest**

Semi-automated region of interest analysis was performed using in-house software written in MATLAB (MathWorks), which allowed multiple small square regions of interest to be placed on the T2-weighted echo-planar imaging volumes and then overlaid on the co-registered mean diffusivity and fractional anisotropy maps either by hand or automatically using locations defined in Montreal Neurological Institute (MNI; http://www.bic.mni.mcgill.ca) standard space. In the latter, the software allows the user to interactively move regions of interest if standard to native space registration errors cause white matter regions of interest to be placed on CSF or grey matter structures.

The procedure for obtaining mean diffusivity and fractional anisotropy values for each region of interest was as follows. First, MNI coordinates were defined in standard space for each region of interest using the International Consortium for Brain Mapping DTI-81 white matter atlas (Oishi et al., 2011). Typically, between 6 and 12 square regions of interest were defined for each white matter structure in axial view, sizes of which were 3 × 3 × 1 or 2 × 2 × 1 voxels depending on the white matter region. Several regions of interest were used for each white matter region in order to reduce the effects of differences in individual region of interest placement. Next, the coordinates were mapped from standard to native space using the affine transformation matrices derived by registering each subject’s T2-weighted echo-planar imaging volume to MNI standard space. The placement of the regions of interest in individual images was then checked manually to ensure minimal contribution of grey matter and CSF signal to the mean diffusivity and fractional anisotropy measurements. To ensure unbiased measurements of mean diffusivity and fractional anisotropy, all regions of interest were defined on the T2-weighted echo planar imaging volumes (Bozzali and Cherubini, 2007) by an investigator blind to each subject’s clinical status. Values for mean diffusivity and fractional anisotropy were then obtained for each square region of interest and averaged to give mean values for each white matter structure of interest. Finally, blinded to the original region of interest selection, the investigator also performed an assessment of intra-rater reliability of region of interest placement by repeating the above analysis in 10 subjects (five patients and five control subjects) chosen at random from the study cohort.

Based on previous studies, a set of white matter structures that are potentially affected in ALS were chosen for investigation (Abrahams et al., 2005; Sarro et al., 2011). These included major white matter fibre bundles of the anterior thalamic radiation, uncinate fasciculus, superior longitudinal fasciculus, inferior longitudinal fasciculus and corticospinal tract as well as the genu and splenium of corpus callosum. Also measured were the anterior cingulum and the cingulum and...
ventral cingulum bundle connected to the hippocampus. White matter underlying subregions of prefrontal cortex were investigated: (i) superior frontal gyrus white matter (near cortical area BA10), (ii) middle frontal gyrus white matter (near cortical area BA9/46) and (iii) inferior frontal gyrus white matter (near cortical area BA44/45). In addition, white matter within the corona radiata (superior and posterior regions), anterior corona radiata, superior parietal lobule and temporal gyri (superior, middle and inferior) were also measured. Finally, areas that were not expected to be implicated in ALS were included, namely the optic radiation and white matter within occipital gyri.

Corpus callosum segmentation
As abnormalities in corpus callosum structural integrity have been shown to be a consistent feature of cerebral pathology in ALS (Yamauchi et al., 1995; Filippini et al., 2010), the corpus callosum was extracted at midline using an automatic segmentation tool (Fig. 5). Briefly, the method takes the fractional anisotropy and principal eigenvector volumes and registers them to the John Hopkins University International Consortium for Brain Mapping 2 mm fractional anisotropy template (http://cmrm.med.jhmi.edu) using affine registration to ensure the midline is in the centre of the x-axis. The midline corpus callosum is then identified by applying a threshold between 0.2 and 0.4 to the fractional anisotropy volume, and identifying those voxels whose principal eigenvectors have a predominantly left–right orientation (red) from the red/green/blue principal eigenvector colour map. The resulting segmentation mask is then applied to the mean diffusivity and fractional anisotropy volumes to provide average values for these water diffusion biomarkers in this structure of interest.

Statistical analyses
The cognitive data were explored for normal distribution using histograms, boxplots and the Shapiro-Wilks test of normality. In addition, all variables were checked for homogeneity of variance. Comparative group analyses of demographic and cognitive data were performed using t-tests in normally distributed data, Mann-Whitney U-tests in populations that were not normally distributed, and a chi-square test for categorical variables. The one-tailed probability level was adopted for tests with a predicted directional result. White matter region of interest data was investigated in the patient group only using Pearson’s r correlations.

Results
Participants
Demographic data are detailed in Table 1. There were no significant differences between groups in age, gender or premorbid IQ as assessed by the Wechsler Test of Adult Reading. In addition, there was no difference between patients and healthy controls in their self-reported levels of anxiety and depression. All patients had a forced vital capacity of >70% of their predicted value.

Background assessment
Patient and control data for performance in the background neuropsychological tests are detailed in Table 2. The ALS patient group was significantly impaired relative to controls in logical memory (immediate, delayed, percentage retained and recognition), forward and reverse digit span, spoken letter fluency test, with a tendency towards significantly increased fluency indices on the written letter fluency test. Case by case analysis revealed that four patients met the Strong et al. (2009) criteria for ALS with cognitive impairment (fifth percentile or worse on at least two separate tests of executive function) based on their performance in the fluency tasks, Brixton spatial anticipation test and reverse digit span. Of the four patients who met the criteria for ALS with cognitive impairment, two also displayed memory impairment. A further three patients with ALS who did not fulfil criteria for ALS with cognitive impairment displayed poor memory performance. Forced vital capacity predicted percentage values were not associated with scores in any of the background tests.
Experimental tests

Details of participant’s performance in the processing speed tasks are shown in Table 3. Patients and controls performed comparably in the visual inspection time task and the Rapid Serial Letter Identification (RSLI) task, with no significant difference observed between the groups in terms of the amount of errors made on either task.

Details of participant performance in the dual-task paradigm are displayed in Table 4 and Fig. 6. Patients and controls performed comparably in the initial measurement of individual level in both the delayed digit recall and visual inspection time tasks. In the combined single task scores there was no significant difference between patients and controls; \( t(58) = -0.615, P = 0.541 \), however, in the combined dual-task score and more crucially in the combined dual-task cost there were significant differences between patients and controls; \[ \text{dual-task score } t(58) = -2.289, P = 0.026; \text{dual-task cost } t(58) = 2.387, P = 0.020. \] Figure 6 demonstrates that patients exhibited a drop in performance from single to dual-task that was more than twice that of controls in both subtasks. Forced vital capacity predicted percentage values were not associated with scores in any of the experimental tests.

Magnetic resonance imaging regions of interest analyses

The intra-rater reliability analysis indicated excellent reproducibility of region of interest measurements with the standard deviation of the difference between repeated measures of mean diffusivity and fractional anisotropy being \( 21 \times 10^{-6} \text{ mm}^2/\text{s} \) (mean of measurements \( 698 \times 10^{-6} \text{ mm}^2/\text{s} \)) and 0.012 (mean 0.411), respectively. This yielded coefficients of variation of 2.9% for both mean diffusivity (range 0.6 for cingulum to 6.1% for genu) and fractional anisotropy (range 0.5 for superior longitudinal fasciculus to 4.4% for frontal white matter), which compares well with values for other studies using region of interest analysis (Shenkin et al. 2005).

Region of interest analyses are displayed in Table 5. Significant group differences were found in the following frontal tracts: the...
anterior cingulate (fractional anisotropy and mean diffusivity); anterior thalamic radiation (fractional anisotropy and mean diffusivity); and uncinate fasciculus (mean diffusivity). In addition, group differences in white matter integrity were observed in the following prefrontal regions: superior frontal gyrus white matter (fractional anisotropy and mean diffusivity); inferior frontal gyrus white matter (fractional anisotropy and mean diffusivity); middle frontal gyrus white matter (fractional anisotropy and mean diffusivity); and anterior corona radiata (mean diffusivity). Large differences were observed in the corticospinal tract (fractional anisotropy and mean diffusivity). Further significant differences were also found in temporal areas; the hippocampal portion of the cingulum (mean diffusivity), temporal gyri white matter (fractional anisotropy), and in the inferior longitudinal fasciculus (fractional anisotropy) and radiata (fractional anisotropy). Investigation of white matter integrity in posterior brain regions (occipital gyri white matter and optic radiation) revealed no group differences. Forced vital capacity predicted percentage values were not associated with fractional anisotropy or mean diffusivity in any white matter regions.

Corpus callosum segmentation

Analysis of covariances revealed a significant difference in fractional anisotropy values between patients [mean = 0.56, standard deviation (SD) = 0.046] and controls [mean = 0.59, SD = 0.043; F(1, 47) = 6.12, P = 0.012], as well as mean diffusivity values between patients (mean = 780, SD = 86 x 10^{-6} mm^2/s) and controls (mean = 740, SD = 61 x 10^{-6} mm^2/s; F(1, 47) = 4.045, P = 0.050).

Onset site

Comparative analyses were employed to investigate the effect of onset site (bulbar versus limb) in all cognitive tests and all white matter regions/tracts; there were so significant group differences in any of the measures.

Magnetic resonance imaging correlates with neuropsychological performance

Correlations were investigated in the patient group only, and in regions/tracts in which there were significant group differences. As such the reported P-values are one-tailed.

Experimental measures

Combined dual-task cost significantly correlated with white matter integrity in middle frontal gyrus fractional anisotropy; r = −0.48, P = 0.004 (Fig. 7), and anterior corona radiata fractional anisotropy; r = −0.32, P = 0.040 (Fig. 8)
Background measures

Spoken letter fluency test significantly correlated with white matter in the superior frontal gyrus mean diffusivity; \( r = 0.42, P = 0.012 \), inferior frontal gyrus fractional anisotropy; \( r = -0.40, P = 0.017 \), corticospinal tract fractional anisotropy; \( r = -0.43, P = 0.010 \), and corpus callosum fractional anisotropy; \( r = -0.35, P = 0.049 \) (Fig. 8). In addition, there was an association between reverse digit span and white matter integrity in the hippocampal portion of the cingulum mean diffusivity; \( r = -0.41, P = 0.018 \). Relationships between the cognitive tasks were also investigated to deduce whether there was any overlap between performance on different cognitive measures. There was no correlation between the combined dual-task cost and spoken letter fluency test; \( r = 0.06, P = 0.745 \), or any of the significant cognitive measures.

Discussion

These data show that patients with ALS exhibited impairments in dual-task performance while processing speed remained preserved. This selective deficit indicates the presence of executive dysfunction in ALS in the absence of generic cognitive slowing.
The tests employed assessed processing speed for abstract visual and verbal information by manipulating stimulus duration, and as such account for motor disability with untimed responses. Inconsistencies between these and previous studies may be a reflection of the type of tasks employed; the Trail Making Test part A (Hanagasi et al., 2002) and the Digit Symbol Test (Mezzapesa et al., 2007) place considerable demand on the motor (hand) functions. The current findings suggest that studies that report deficits in psychomotor speed (Raaphorst et al., 2010) are most likely a reflection of motor impairment rather than cognitive slowing, and that patients with ALS are able to process simple visual information at a normal rate. Processing speed, when isolated from motor functioning and other high-order processes, is not impaired in patients with ALS and does not contribute to other observed cognitive impairments.

In contrast, ALS patient performance in the dual-task was significantly impaired compared with controls; the accuracy cost incurred by performing two tasks concurrently was more than twice as high in patients compared with controls. The individual tasks were matched to each participant’s individual ability level so

Figure 7 Relationship between fractional anisotropy in white matter of the middle frontal gyrus and combined dual-task cost in patients with ALS.

Figure 8 Significant correlations between cognitive tests and white matter regions of interest in patients with ALS. Montreal Neurological Institute coordinates (a) Right (x 22, y –18, z 12), Left (x –22, y –18, z 12); (b) Right (x 37, y 20, z 15), Left (x –37, y 20, z 15); (c) Right (x 12, y 48, z 30), Left (x –12, y 48, z 30); (d) Right (x 32, y 39, z 17), Left (x –31, y 39, z 17); (e) Right (x 27, y 37, z 1), Left (x –26, y 37, z 1). (D) = mean diffusivity; FA = fractional anisotrophy.
the dual-task effect could not be the result of single task difficulty (Anderson et al., 2011). This was confirmed by similar accuracy scores achieved by patients and controls in both single task conditions. Furthermore, patients and controls showed no difference in terms of the individual ability levels on each task and so were able to perform as well as controls. Thus, the dual-task decrement exhibited by patients is likely to be a reflection of a specific impairment in the ability to co-ordinate cognitive resources appropriately between the two tasks, and reflect dysfunction of the central executive. Dual-task impairments are observed in Alzheimer’s disease (Logie et al., 2004; MacPherson et al., 2007) and have also been reported to a lesser extent in FTD (Perry and Hodges, 2000; Sebastián Gascón and Hernández-Gil, 2010) and in a previous study of ALS based on reaction time measures (Schreiber et al., 2005). The current investigation employed a preload methodology to minimize motor demands (Cocchini et al., 2002), and constituent tasks for which accuracy was the outcome measure, and as such is the first demonstration of a dual-task impairment in ALS that independent of single task difficulty, processing speed and motor functioning.

Dual-task performance correlated with fractional anisotropy values in middle frontal gyrus white matter and corona radiata, whereby poor performance was associated with lower white matter integrity. The middle frontal gyrus (or dorsolateral prefrontal cortex) is thought to be highly influential in the regulation of executive processes such as strategy formation, set-shifting and working memory (Royall et al., 2002; Stuss, 2002) and was previously identified as a site of dysfunction in ALS in functional imaging (Abrahams et al., 1996, 2004). The white matter regions identified by the current study correspond well to the prefrontal areas that were suggested to reflect central executive activity in functional imaging studies of dual-tasking in healthy adults (D’Esposito et al., 1995; Wager and Smith, 2003). The neural correlates of dual-tasking are hotly debated (Erickson et al., 2005); however, functional imaging studies have demonstrated the importance of the bilateral frontal gyri (including the dorsolateral prefrontal cortex) in managing and coordinating response selection and interference (MacDonald et al., 2000; Wager and Smith, 2003), and it is possible that the white matter findings presented in this study underpin dysfunction in these processes. It has also been suggested that executive functioning is best understood in terms of interactions between networks of regions (Collette and Van der Linden, 2002), and that dual-tasks impose higher processing demands on cortical areas already involved in the component single tasks. Thus, it is also possible that the correlation with white matter underlying dorsolateral prefrontal cortex may be a reflection of the high demands on working memory that are required to maintain performance on the delayed digit recall task while completing a concurrent task.

Of particular interest is the finding that performance on the dual-task was associated with different white matter pathways than the commonly reported letter fluency deficits. The finding of letter fluency deficits is consistent with numerous others studies reporting this as a striking feature of the cognitive profile of ALS (Moretti et al., 2002; Lomen-Hoerth et al., 2003; Abrahams et al., 2004), confirming that verbal fluency impairments are one of the core deficits that characterize cognitive change in ALS. Longer fluency index times in the spoken letter fluency test were associated with reduced fractional anisotropy in white matter in the inferior frontal gyrus (adjacent to Broca’s/Brodmann area 44), superior frontal gyrus (adjacent to Brodmann area 10), corpus callosum and corticospinal tract, but not the middle frontal gyrus. These findings are generally consistent with a previous study showing that ALS patients with impaired verbal fluency had reduced white matter volumes within the superior and medial frontal lobes (Abrahams et al., 2005), but, moreover, further builds on these findings to demonstrate firstly a direct correlation and secondly changes in integrity of specific structural pathways. The white matter correlates of verbal fluency performance identified by the current study are also concordant with functional imaging studies in ALS which have shown decreased blood-oxygen response in both the anterior prefrontal cortex (Brodman area 10) and inferior frontal gyrus (Brodman area 44) in response to fluency tasks (Abrahams et al., 2004). Studies in healthy participants of fluency have also highlighted the importance of a prefrontal network including Broca’s area and left inferior frontal gyrus (Abrahams et al., 2003; Hirshorn and Thompson-Schill, 2006). Furthermore, this region has been noted as a site of pathological focus in patients with ALS-aphasia syndrome (Bak et al., 2001). The correspondence between studies suggests that disruption to these white matter pathways particularly in the inferior frontal gyrus may underpin the cognitive changes observed in patients with ALS with impaired verbal fluency.

The correlation between verbal fluency and white matter integrity in the corpus callosum also highlights this area as sensitive to cognitive change in patients with ALS. Previous studies have indicated some association between reduced corpus callosum integrity and cognitive impairment (Yamauchi et al., 1995; Abrahams et al., 2005), with the former study suggesting that pathology in the anterior portion of this structure was associated with cognitive and behavioural changes. The methods employed by the current study were unable to differentiate between discrete portions of the corpus callosum, although the trend toward low fractional anisotropy in the genu, but not splenium, may indicate the presence of an anterior gradient of corpus callosum involvement as postulated by others (Filippini et al., 2010). Indeed, corpus callosum changes have been reported in diffusion tensor imaging studies of FTD (Matsuo et al., 2008) suggesting that this structure may be influential in the cognitive changes that characterise this disorder, further highlighting the pathological link between ALS and FTD.

The observed correlation between verbal fluency performance and corticospinal tract integrity suggests that fluency performance may be sensitive to disease severity. This may be related to the propensity for patients with bulbar onset to show more upper motor neuron degeneration, as well as being more likely to exhibit cognitive impairment (Abrahams et al., 1997), although other studies have suggested that cognitive impairment occurs early in the disease and does not progress at the same rate as motor dysfunction (Schreiber et al., 2005).

Thus, performance in these two tests of executive functioning appears to be underpinned by different white matter pathways within the prefrontal cortex. Moreover, dual-task and verbal fluency performance were highly uncorrelated with each other, suggesting that impairments in executive functions are indeed
dissociable in patients with ALS. Further support for this proposal is provided by research which postulates that executive functions are dissociable at a behavioural and neuroanatomical level (Goldman-Rakic, 1995; Jurado and Roselli, 2007), and particularly by studies that suggest that verbal fluency and dual-tasking do not share the same cognitive processes, making them less likely to rely on the same neural pathways (Miyake et al., 2000).

Patients also exhibited impairment in the digit span measures, indicative of a deficit in working memory that has been reported in other studies (Rakowicz and Hodges 1998; Abrahams et al., 2000). A correlation was observed between performance on the reverse digit span task and increased mean diffusivity in the hippocampal portion of the cingulum. The hippocampal portion of the cingulum is traditionally associated with episodic and long-term memory (Kohler et al., 1998), indeed it has been shown to correlate with cognitive functioning in Alzheimer’s disease (Nakata et al., 2009). In the present study, in addition to executive dysfunction, memory impairment was detected. Memory dysfunction in ALS has been previously highlighted (Raaphorst et al., 2010), although typically related to secondary executive retrieval dysfunction. Here the patient sample showed deficits not only in measures of immediate memory, but also in retention and recognition, and as such are unlikely to be explained through an executive dysfunction route alone. This suggests a primary memory deficit in some patients with ALS. Increasingly, however, the hippocampus has been shown to have a role in working memory paradigms (Ranganath and D’Esposito, 2001), and together with the posterior cingulate, has been associated with tasks assessing multiple cognitive domains suggesting that it may be a crucial structure in complex cognitive circuitry (Kantarci et al., 2011). Moreover, neuropathological studies in patients with ALS with and without dementia have shown abnormalities in the hippocampus (Okamoto et al., 1991; Takeda et al., 2009).

The current study employed a prevalent sampling method, and as a result included a small number of patients ($n=2$) with disease duration of over 5 years, although patients with significant respiratory dysfunction were excluded. Large population studies of cognitive impairment in ALS using incidence sampling methods have shown similar rates of impairment as those from prevalent samples, but they have also revealed a more heterogeneous presentation not only of executive dysfunction but including changes in language and memory (Phukan et al., 2012; Goldstein and Abrahams, 2013). Although there was no evidence of language dysfunction (given that only one naming test was performed), memory dysfunction was present which is consistent with the findings from incident samples (Phukan et al., 2012).

The white matter region of interest analyses revealed extensive changes in structural integrity in patients with ALS relative to controls. Reduced structural integrity was found in the uncinate fasciculus, which connects the temporal lobes and amygdala to orbitofrontal and inferior regions of the prefrontal lobes, a result that is consistent with other studies in ALS (Agosta et al., 2010; Sato et al., 2010) and FTD (Matsuo et al., 2008). Further changes were shown in the patient group in white matter underlying the superior, middle and inferior frontal gyri, and in the temporal gyri. In addition, changes were evident in the anterior thalamic radiation which connects the thalamus to areas of the prefrontal cortex, and in the anterior and hippocampal portions of the cingulum, which connects subcallosal regions to the hippocampus and frontal lobes, respectively. The observed changes in prefrontal associative fibres and cingulum are largely consistent with those indicated by a previous study showing volumetric white matter reductions in patients with ALS using less refined automated volumetric estimations (Abrahams et al., 2005). In agreement with other studies (Abrahams et al., 2005; Filippini et al., 2010), reduced structural integrity was observed in the corpus callosum, as well as corticospinal tract, indicative of upper motor neuron pathology that has been consistently shown in previous diffusion tensor imaging investigations (Abe et al., 2004; Ciccarelli et al., 2006; Agosta et al., 2010). Concordant with a recent diffusion tensor imaging investigation (Sarro et al., 2011), patients with ALS showed reduced structural integrity in the inferior longitudinal fasciculus, one of the long association bundles connecting the temporal and occipital lobes. The diffusion tensor imaging data from the current study lends support to the postulated continuum between classical ALS and FTD (Lomen-Hoerth et al., 2003; Abrahams et al., 2004; Murphy et al., 2007; Sage et al., 2007; Raaphorst et al., 2010), as it highlights a preponderance for frontotemporal white matter pathology, which may play a crucial role in the multi-system involvement observed in this disorder.

In summary, the findings of the current study show a pattern of cognitive impairment in ALS with predominant executive dysfunction characterised by a deficit in the central executive with no slowing of processing speed. Moreover impairments in different types of executive functions correlated with white matter integrity in distinct prefrontal pathways, with dual-task impairment associated with dysfunction of middle frontal gyrus, whereas letter fluency deficits appear more dependent on the superior and inferior gyri and corpus callosum. Whether such deficits occur at different stages of disease and whether they may be used as tools to map out cerebral spread of the disease may be of a matter for future investigation.

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