[\textsuperscript{11}C]-WAY100635 PET demonstrates marked 5-HT\textsubscript{1A} receptor changes in sporadic ALS

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Summary

The pathogenesis of amyotrophic lateral sclerosis (ALS) remains obscure, but it is now clear that neuronal loss is not confined to the motor cortex, even in cases without dementia. A reliable method of assessing cortical involvement in vivo remains elusive. WAY100635 binds selectively to the 5-hydroxytryptamine (5-HT\textsubscript{1A}) receptor, which is expressed on pyramidal neurones present throughout the cortex. [\textsuperscript{11}C]-WAY100635 PET is, therefore, a potential marker of cerebral neuronal loss or dysfunction in ALS. Twenty-one ALS subjects and 19 healthy volunteers underwent [\textsuperscript{11}C]-WAY100635 PET of the brain. A cortical template consisting of multiple volumes of interest (VOI) was applied to each individual’s [\textsuperscript{11}C]-WAY100635 binding potential (BP) image to determine the regional reduction in binding in ALS patients compared to controls. There was a marked reduction (21%) in both the global cortical and raphe BP of [\textsuperscript{11}C]-WAY100635 in ALS patients ($P < 0.001$), with regional variations in the VOI analysis that ranged from 16% to 29% decrease compared with the control group, and trends to greater reductions in those with bulbar involvement. To clarify the significance of the global cortical reductions, statistical parametric mapping was used as an alternative method to identify the cortical regions with the most significant decreases in [\textsuperscript{11}C]-WAY100635 binding. SPM analysis revealed the greatest differences between ALS cases and controls in frontotemporal regions, cingulate and lateral precentral gyri. The reductions in cortical [\textsuperscript{11}C]-WAY100635 binding were not related to depression, riluzole or other drug use. We postulate that the reduction of 5-HT\textsubscript{1A} binding represents loss of, or damage to, neurones bearing these receptors although we cannot exclude the possibility that these reductions reflect alterations in receptor expression or function. Further investigation into the role of the 5-HT\textsubscript{1A} receptor and the potential of [\textsuperscript{11}C]-WAY100635 PET as a marker of cortical dysfunction in ALS is warranted.

Keywords: amyotrophic lateral sclerosis; motor neurone disease; PET; WAY100635; serotonin 1A receptor

Abbreviations: 5-HT = 5-hydroxytryptamine; ALS = amyotrophic lateral sclerosis; BP = binding potential; DSM = Diagnostic and Statistical Manual of Mental Disorders; HADS = Hospital Anxiety and Depression Score; mCi = millicuries; MNI = Montreal Neurological Institute; PET = positron emission tomography; SPM = statistical parametric mapping; SRTM = simplified reference tissue compartmental model; TAC = time-activity curve; UMN = upper motor neurone; VOI = volume of interest

Introduction

Amyotrophic lateral sclerosis (ALS) is a progressive and fatal neurodegenerative disease characterized by the degeneration of corticospinal, brainstem and spinal cord motor neurones (Rowland and Shneider, 2001). The cause of ALS is not known, with the exception of rare familial forms (Cleveland and Rothstein, 2001; Shaw et al., 2001). There are no specific diagnostic tests or reliable biological markers to assess disease progression in ALS, nor are there reliable methods for assessing the extent of cerebral lesions, including extramotor involvement (Pioro et al., 1994; Abrahams et al., 1996; Lloyd et al., 2000; Ellis et al., 2001; Turner et al., 2004; Maekawa et al., 2004). Much of the current understanding of the nature of the cerebral lesion in ALS has come from neuropathological studies (Smith, 1960), which can only reveal end-stage changes. The localization of the serotonin 5-hydroxytryptamine (5-HT<sub>1A</sub>) receptor on pyramidal neurones (Azmitia et al., 1996), which are found throughout the neocortex, makes this receptor a potential marker of motor and extra-motor cerebral involvement in ALS (Azmitia, 2001).

Positron emission tomography permits the imaging of brain neurochemistry in vivo and provides new approaches for investigating pathological processes early in ALS disease progression (Turner and Leigh, 2000). WAY100635, (N-[2-[4-(2-methoxyphenyl)-1-piperazinyl]ethyl]-N-(2-pyridinyl)cyclohexane carboxamide), is a selective ligand for the serotonin 5-HT<sub>1A</sub> receptor (Forster et al., 1995), and [<sup>11</sup>C]-WAY100635 PET is a sensitive marker of in vivo 5-HT<sub>1A</sub> receptor binding (Rabiner et al., 2002). We therefore tested the hypothesis that [<sup>11</sup>C]-WAY100635 PET might reveal novel insights in vivo into the nature and extent of cortical pathology in ALS.

Materials and methods

Participants

Patients diagnosed as fulfilling the criteria of possible, probable or definite ALS according to revised El Escorial criteria (Brooks et al., 2000) were invited to undergo [<sup>11</sup>C]-WAY100635 PET. Results were compared with a group of healthy volunteers. The prescription of any medication known to bind to serotonin receptors was a strict exclusion factor for all subjects. The presence of a major affective disorder (according to the Diagnostic and Statistical Manual of Mental Disorders, DSMIV, criteria) was excluded in all subjects. As an additional safeguard, the ALS patients were also scored for anxiety/depressive symptoms using a modified Hospital Anxiety and Depression Score (HADS), with the aim of excluding any with high scores (>8) in either category. None of the subjects were demented (according to the Diagnostic and Statistical Manual of Mental Disorders, DSMIV criteria) and none required assisted ventilation at the time of the scan. Patients were requested to discontinue all other medication (includingriluzole where prescribed) for at least 48 hours prior to scanning.

Clinical characteristics used in correlational analyses included the site of symptom onset (limb or bulbar), the presence or absence of bulbar signs at the time of the scan, the revised ALS functional rating scale (ALSFRS-R) or a lower score indicates greater disability (Cedarbaum et al., 1999) at the time of the scan and the disease duration in months from onset of symptoms to the time of the scan. Informed written consent was obtained from all subjects and the study was approved by the Administration of Radioactive Substances Advisory Committee (ARSAC) and the Research Ethics Committees of the Hammersmith Hospitals Trust, the Institute of Psychiatry and the Kings College Hospital, London, UK.

Data acquisition

PET was performed with an ECAT966 HR ++ camera (Spinks et al., 2000), collecting 23 time frames over 95 min in list-mode. Scanning started 30 s before a bolus injection of approximately 8 mCi [<sup>11</sup>C]-WAY100635 intravenously over 30 s into an antecubital fossa vein.

Image analysis

Regional cerebral uptake of [<sup>11</sup>C]-WAY100635 was modelled kinetically using a simplified reference tissue compartmental model (SRTM) (Lammertsma and Hume, 1996; Gunn et al., 1998a). The cerebellum was chosen as a reference tissue, as it has an extremely low number of 5-HT<sub>1A</sub> receptors and so the tracer signal there represents non-specific binding (Hall et al., 1997). This type of modelling is particularly suitable for a disease such as ALS, where the cerebellum is not involved pathologically. The parameter of interest estimated for each brain region by the SRTM is the binding potential (BP = f<sub>D</sub> B<sub>AVAIL</sub>/K<sub>D</sub>, where f<sub>D</sub> is the ‘free fraction’ of the radiotracer in the non-displaceable compartment, B<sub>AVAIL</sub> is the concentration of available binding sites and K<sub>D</sub> is the equilibrium dissociation rate constant of the radioligand (Cunningham and Lammertms, 1994). The reference tissue model was applied at a voxel level using a basis function implementation (Gunn et al., 1997, 1998b), and parametric maps of BP were generated (BP images).

In order to control for artefactual changes in BP due to reference region variation, decay-corrected time-activity curves (TACs) from the cerebellar reference regions for all subjects scanned were plotted after normalization to their maxima. To avoid a potential type II error (due to decreases in BP that are due to changes in the reference region rather than the region of interest), any ALS patient lying clearly outside the control population’s TACs was excluded from the subsequent analysis.

Anatomical region definition—VOI method

A normal [<sup>11</sup>C]-WAY100635 template was created from integrated PET images (0–90 min) of 19 normal subjects transformed into standard stereotaxic space (Montreal Neurological Institute space, MNI), as described previously (Rabiner et al., 2002). Anatomical regions, including the cerebellar region, were defined on a single subject MRI in MNI space, to create a generic volume of interest (VOI) map (see Hammers et al., 2002, 2003). Only the top 33% of values within the template were used to restrict the regions of interest to grey matter. The [<sup>11</sup>C]-WAY100635 template was warped onto each individual [<sup>11</sup>C]-WAY100635 integral image (0–90 min), using SPM99 (Wellcome Department of Cognitive Neurology). The transformation parameters obtained were subsequently applied to the generic VOI map, producing an individualized VOI map for each subject. The cerebellar VOI was applied to the dynamic PET image to create an input function for the calculation of parametric images. The cortical VOI map was applied to the BP images to obtain regional BP values. This technique allowed the examination of...
40 distinct cortical brain VOIs (20 in each hemisphere), which contain post-synaptic 5-HT\textsubscript{1A} receptors. A global mean BP value was also calculated by averaging all cortical regions in the analysis.

We also calculated the [\textsuperscript{11C}]WAY100635 BP for the midbrain raphe nuclei (hereafter termed raphe), a small nucleus consisting of serotonergic cell bodies with a high concentration of pre-synaptic 5-HT\textsubscript{1A} autoreceptors. The raphe is not detectable with conventional MRI but is well defined as a median midbrain ‘hotspot’ on a [\textsuperscript{11C}]WAY100635 PET image. Therefore, a VOI for the raphe was defined manually on an integral PET emission dynamic image (summed from 20 to 90 min post-injection of the radiotracer) for each individual PET scan, and then applied onto the BP images to generate the raphe BP values.

Statistical analysis in the VOI method
Statistical analyses were performed using SPSS v.11 (SPSS Inc., Chicago, IL, USA). An independent t-test was carried out between control and patient groups for the global cortical BP values, and separately for the raphe. A similar analysis was then performed using only those four ALS patients never exposed to riluzole at any time, in order to assess any potential drug effect.

To interrogate all regions simultaneously and test for potentially confounding clinical factors, such as age or gender, a repeated measures ANOVA was performed using the general linear model option in SPSS. In this analysis, all brain regions (BP values from 40 cortical regions and the raphe) were designated the ‘within-subject’ factor, and subject group (control or patient) as the ‘between-subject’ factor, in order to examine the overall variation of regional binding, and for a significant group effect. Clinical covariates of subject age, gender and injected dose were then included in the repeated measures analysis to exclude a confounding effect on the overall group differences. For the purposes of tabulation, the difference in the patient and control mean BP for each cortical region was expressed as a percentage of the mean BP value for the controls.

Single-tailed (because of the assumption that we are studying decreases in BP only) ANOVA was used to compare mean BP for each VOI plus the global binding between, initially, patients with limb and bulbar-onset disease and, subsequently, those with and without bulbar involvement at the time of the scan. A one-tailed bivariate Spearman (non-parametric) correlation coefficient was also calculated for each VOI and the global BP against ALSFRS-R (positive correlation, that is, lower BP with lower ALSFRS-R score) and separately against disease duration (negative correlation, that is, lower BP with increased disease duration). Those regions where \( P < 0.05 \) for the reduction in BP were identified. It was then necessary to apply a Bonferroni correction multiplication factor of 41 to the \( P \) values for cortical VOIs and the raphe in view of the multiple comparisons being undertaken.

Statistical parametric mapping
Statistical Parametric Mapping (SPM99, Wellcome Department of Cognitive Neurology) (Friston et al., 1991) was employed as a second and distinct method of regional analysis in order to try and localize regions of particularly decreased [\textsuperscript{11C}]WAY100635 BP in the ALS group.

Individual BP images were normalized into standard MNI space using the normal [\textsuperscript{11C}]WAY100635 template and a t-test applied at a voxel level to mean BP images from ALS patients and controls, with proportional scaling of the global BP (calculated using the default setting, including a relative threshold of 0.8). A threshold of \( P = 0.05 \) was applied to SPMs and only those clusters identified with a corrected \( P < 0.05 \) were displayed within the standard ‘glass brain’ format, rendered also to a generic T1 MRI to aid localization.

Results
Quality control
Twenty-four ALS patients underwent [\textsuperscript{11C}]WAY100635 PET. Three ALS patients’ cerebellar reference region TACs lay significantly outside the range of the control population. These subjects had BP values notably lower than the rest of the ALS group and, therefore, were excluded from the subsequent analysis to avoid a potential type II error. There were no obvious differences phenotypically between these three ALS patients and the remaining group however.

Group characteristics
Twenty-one ALS patients were available for further analysis (4 female, 17 male; mean age 56 \pm 9 years (range 39–74). Of these, 11 were classified as definite, seven as probable and three as possible ALS according to El Escorial criteria at the time of the scan. The subsequent course of the disease in all cases has remained consistent with ALS. No ALS patients scored in the high range for either anxiety or depression scores with the HADS. Eighteen of the patients were categorized as having limb-onset disease, three as bulbar-onset. Twelve of the patients had bulbar signs at the time of the scan. The mean duration of disease was 27 \pm 23 months (range 8–89). The mean revised (ALSFRS-R) score of the ALS patients was 38 \pm 6 (range 26–46).

Data from 19 healthy volunteers were available for comparison (5 female, 14 male; mean age 47 \pm 14 years (range 18–74). The mean injected dose of [\textsuperscript{11C}]WAY100635 for the ALS patients and the controls were 8.2 \pm 1.0 mCi (range 6.7–10.1) and 7.3 \pm 1.0 mCi (range 4.0–8.0), respectively.

VOI method group comparison
The global cortical 5-HT\textsubscript{1A} BP was significantly lower in the ALS compared with the control group (mean 3.27 versus 4.15, \( P < 0.001 \)), as was the raphe BP (mean 2.90 versus 3.67, \( P < 0.001 \)). In an analysis of the four patients never exposed to riluzole versus controls, BP values were also significantly lower than controls (global cortical mean 3.38 versus 4.15, \( P < 0.04 \); raphe mean 3.02 versus 3.67, \( P < 0.05 \)).

The repeated measures ANOVA demonstrated a significant effect of brain region on [\textsuperscript{11C}]WAY100635 BP (\( F = 121, \ d.f. = 8, P < 0.001 \)), as well as an effect of group (\( F = 25, \ d.f. = 1, P < 0.001 \)). There was a significant interaction of brain region BP and subject group (\( F = 4, \ d.f. = 8, P < 0.001 \)). Covariates of subject age, gender and injected dose did not confound these results.
Both mean global cortical post-synaptic and mean raphe autoreceptor binding of $[11C]$-WAY100635 were on average 21% less in the ALS patient group compared with healthy controls. Inspection of mean group BPs for individual cortical regions revealed that all regions were lower in the ALS group compared with healthy controls (range 15–29% reduction), with the largest reductions seen in the fusiform, parahippocampal (particularly left), medial inferior temporal, precentral, intermediate frontal and orbito-frontal gyri (Table 1).

<table>
<thead>
<tr>
<th>Region</th>
<th>Controls Left mean BP</th>
<th>Controls Right mean BP</th>
<th>ALS patients Left mean BP</th>
<th>ALS patients Right mean BP</th>
<th>Left percentage reduction</th>
<th>Right percentage reduction</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fusiform</td>
<td>5.34 (1.06)</td>
<td>5.50 (1.01)</td>
<td>3.81 (0.89)</td>
<td>4.06 (0.65)</td>
<td>29</td>
<td>26</td>
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<tr>
<td>Parahippocampal*</td>
<td>5.62 (1.05)</td>
<td>5.57 (1.00)</td>
<td>4.06 (0.83)</td>
<td>4.34 (0.73)</td>
<td>28</td>
<td>22</td>
</tr>
<tr>
<td>Medial inferior temporal*</td>
<td>4.76 (0.82)</td>
<td>4.78 (0.91)</td>
<td>3.61 (0.78)</td>
<td>3.66 (0.66)</td>
<td>24</td>
<td>24</td>
</tr>
<tr>
<td>Precentral*</td>
<td>4.32 (0.53)</td>
<td>4.42 (0.64)</td>
<td>3.31 (0.63)</td>
<td>3.47 (0.58)</td>
<td>23</td>
<td>21</td>
</tr>
<tr>
<td>Intermediate frontal*</td>
<td>3.76 (0.53)</td>
<td>3.98 (0.53)</td>
<td>2.91 (0.51)</td>
<td>3.19 (0.57)</td>
<td>23</td>
<td>20</td>
</tr>
<tr>
<td>Orbito-frontal*</td>
<td>4.41 (0.90)</td>
<td>4.77 (0.75)</td>
<td>3.45 (0.68)</td>
<td>3.69 (0.68)</td>
<td>22</td>
<td>23</td>
</tr>
<tr>
<td>Anterior cingulate*</td>
<td>4.90 (0.69)</td>
<td>5.16 (0.82)</td>
<td>3.98 (0.74)</td>
<td>4.00 (0.71)</td>
<td>19</td>
<td>22</td>
</tr>
<tr>
<td>Posterior temporal</td>
<td>4.36 (0.84)</td>
<td>4.46 (0.85)</td>
<td>3.38 (0.71)</td>
<td>3.52 (0.64)</td>
<td>22</td>
<td>21</td>
</tr>
<tr>
<td>Insula*</td>
<td>5.50 (0.79)</td>
<td>5.42 (0.92)</td>
<td>4.31 (0.71)</td>
<td>4.21 (0.73)</td>
<td>22</td>
<td>22</td>
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<tr>
<td>Anteromedial temporal</td>
<td>4.67 (0.76)</td>
<td>4.70 (0.78)</td>
<td>3.66 (0.71)</td>
<td>3.91 (0.59)</td>
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<td>Anterolateral temporal*</td>
<td>5.08 (0.83)</td>
<td>4.80 (0.84)</td>
<td>4.02 (0.68)</td>
<td>3.77 (0.66)</td>
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<tr>
<td>Inferior frontal*</td>
<td>3.85 (0.55)</td>
<td>3.89 (0.57)</td>
<td>3.04 (0.52)</td>
<td>3.08 (0.49)</td>
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<tr>
<td>Posterior cingulate*</td>
<td>3.33 (0.63)</td>
<td>3.86 (0.63)</td>
<td>2.63 (0.59)</td>
<td>3.08 (0.57)</td>
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<tr>
<td>Occipital</td>
<td>3.16 (0.58)</td>
<td>3.16 (0.58)</td>
<td>2.51 (0.51)</td>
<td>2.52 (0.49)</td>
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<td>20</td>
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<tr>
<td>Parietal</td>
<td>3.97 (0.60)</td>
<td>4.02 (0.63)</td>
<td>3.15 (0.61)</td>
<td>3.22 (0.58)</td>
<td>21</td>
<td>20</td>
</tr>
<tr>
<td>Superior temporal*</td>
<td>4.79 (0.81)</td>
<td>4.88 (0.76)</td>
<td>3.87 (0.71)</td>
<td>3.87 (0.69)</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Medial frontal*</td>
<td>4.05 (0.52)</td>
<td>4.48 (0.64)</td>
<td>3.29 (0.52)</td>
<td>3.56 (0.58)</td>
<td>19</td>
<td>21</td>
</tr>
<tr>
<td>Superior frontal*</td>
<td>3.72 (0.49)</td>
<td>3.66 (0.50)</td>
<td>2.99 (0.54)</td>
<td>2.91 (0.52)</td>
<td>20</td>
<td>20</td>
</tr>
<tr>
<td>Amygdala*</td>
<td>4.90 (0.59)</td>
<td>5.08 (0.85)</td>
<td>3.91 (0.81)</td>
<td>4.14 (0.56)</td>
<td>20</td>
<td>19</td>
</tr>
<tr>
<td>Hippocampus*</td>
<td>5.38 (0.78)</td>
<td>5.65 (0.78)</td>
<td>4.57 (0.75)</td>
<td>4.76 (0.87)</td>
<td>15</td>
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<tr>
<td>Raphe*</td>
<td>3.69 (0.59)</td>
<td>2.90 (0.57)</td>
<td></td>
<td></td>
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<tr>
<td>Global</td>
<td>4.15 (0.62)</td>
<td>3.27 (0.57)</td>
<td></td>
<td></td>
<td>21</td>
<td></td>
</tr>
</tbody>
</table>

*Trend to reduced binding in patients with bulbar signs compared to those without ($P < 0.05$ prior to correction for multiple comparisons).

There were no significant regional bivariate correlations between BP and ALSFRS-R or disease duration.

**SPM analysis**

The SPM of the regional BP of $[11C]$-WAY100635 in the ALS patients compared to controls localized the most highly significant reductions as lying mainly within the temporal and posterior frontal lobes, cingulate and lateral precentral gyri bilaterally (Figure 1).

**Discussion**

$[11C]$-WAY100635 PET revealed a striking and widespread decrease in cerebral 5-HT$_{1A}$ binding in ALS patients compared with controls. These results could reflect a reduction of 5-HT$_{1A}$ receptor number, a decrease in the affinity of $[11C]$-WAY100635 for the 5-HT$_{1A}$ receptor, or a loss of the neurones that express these receptors. Based on our VOI and SPM analyses, significant reductions in $[11C]$-WAY100635 binding in ALS patients compared to normal controls were found in both motor and extra-motor regions. This is in keeping with previous pathological (Smith, 1960; Maekawa et al., 2004) and neuroimaging studies (Kew et al., 1993; Pioro et al., 1994; Ellis et al., 2001; Turner et al., 2004). The changes
observed are unlikely to be related to a direct drug effect, as patients were not taking any known serotonergic medication at the time of the scan. Furthermore, riluzole is not known to bind to the 5-HT1A receptor, and significant reductions in binding were still seen in the subgroup of four patients who had never taken riluzole.

The 5-HT1A receptor and depression
The development of a selective ligand for the 5-HT1A receptor, WAY100635, permitted the first in vivo quantitation of the 5-HT1A receptor in humans (Pike et al., 1996; Farde et al., 1998). Furthermore, studies using serotonin manipulation in humans and animal models indicate that [11C]-WAY100635 is relatively insensitive to synaptic serotonin levels, making it suitable for investigating 5-HT1A receptor expression in disease conditions where synaptic serotonin levels might be altered (Hume et al., 2001; Maeda et al., 2001; Rubiner et al., 2002; De Haes et al., 2002). Research into the 5-HT1A receptor has, until recently, centred mainly on the investigation of mood and anxiety disorders (Cowen, 2000). However, the reduction in cortical binding of [11C]-WAY100635 seen in pure cases of major depressive disorder is of the order of 12% (Sargent et al., 2000). In the ALS patient group in this study, there is a consistent and markedly greater reduction in the binding of [11C]-WAY100635, which cannot be explained on the basis of depression. Indeed major affective disorder is relatively rare in ALS compared to the non-ALS population (Moore et al., 1998) and other neurological conditions (Schiffer and Babigian, 1984), suggesting that the marked loss of 5-HT1A

Fig. 1 Statistical parametric map displayed on the generic ‘glass brain’ format and T1 MRI below (left and right hemispheres are labelled accordingly). Using this method, rather than the template VOI analysis, the cluster-corrected regions of greatest reduction (P < 0.05) in the BP of [11C]-WAY100635 in ALS patients compared with controls lie mainly within the temporal and posterior frontal lobes, including the cingulate and lateral precentral gyri. The Z-score is shown as a colour scale.
binding detected in this study is highly unlikely to be related to mood changes. In studies of cortical \(^{11}\text{C}\)-WAY100635 binding in other neurodegenerative conditions, such as non-depressed Parkinson’s disease patients, reductions are only slightly greater (15% cortically) than in depressed patients (Doder et al., 2000).

**Widespread cortical changes and regional variations—physical versus functional**

The decreases in \(^{11}\text{C}\)-WAY100635 binding, using the VOI method, were global rather than predominantly restricted to the primary motor cortex and posterior frontal areas implicated in previous neuroimaging and cognitive studies (Kew et al., 1993; Abrahams et al., 1996; Ellis et al., 2001). Indeed, other imaging studies have confirmed that changes may in fact be much more widespread and not confined to motor and frontal regions (Pioro et al., 1994; Lloyd et al., 2000; Turner et al., 2004; Abrahams et al., 2004). PET research in ALS using the \(\gamma\)-amino butyric acid (GABA)-A receptor radioligand \(^{11}\text{C}\)-flumazenil has revealed some areas of non-motor regional involvement common to the present study (e.g. temporal and parietal regions) (Lloyd et al., 2000). However, \(^{11}\text{C}\)-flumazenil binding reflects changes in a distinct system, most likely inhibitory interneuronal integrity and/or function through GABA-A receptor affinity. The ligand \(^{11}\text{C}\)-WAY100635 offers a new perspective on the cortical lesion within a different receptor system.

There are several possible explanations for the widespread reductions seen in this study. First, it is possible that loss (or dysfunction) of cortical pyramidal neurones is indeed generalized or widespread, despite the predominance of motor symptoms and signs in ALS. As a corollary to this, the lack of correlation with ALSFRS-R score, a predominantly motor functional measure, is therefore not surprising. Previous neuropathological studies have shown neurodegeneration extending beyond the motor cortex (Smith, 1960), and in our own quantitative neuropathological studies, we have found loss of cortical pyramidal neurones in the dorsolateral posterior frontal and anterior cingulate cortex, in addition to the primary motor cortex (Maekawa et al., 2004). There are no other systematic quantitative studies of neuronal loss in extra-motor cortex in ALS.

Statistical parametric mapping was used as a complementary, but distinct, method of regional analysis in view of the global changes found using the VOI method. SPM performs approximately 500 independent tests, with spatial resolution not confined by manually defined anatomical borders, but is also limited by the image normalization and smoothing processes inherent to this type of analysis. This was an attempt to characterize just those regions of particularly significant BP reduction perhaps ‘driving’ the global cortical differences. These seem to be predominantly frontotemporal which raise the possibility that the cortical changes we are observing are associated in some way with the well-described cognitive changes in ALS (discussed later).

The parahippocampal and fusiform gyri, and inferior medial temporal regions demonstrated particularly large BP reductions in this study. Pathological changes in similar areas have been identified from neuropathological studies in ALS patients without overt cognitive dysfunction (Kew et al., 1993; Anderson et al., 1995; Kawashima et al., 2001; Wakabayashi et al., 2001; Tsuchiya et al., 2002; Piao et al., 2003). Some neuroimaging studies have shown widespread decreases in regional cerebral blood flow (Dalakas et al., 1987; Hatazawa et al., 1988; Ludolph et al., 1992), and electrophysiological evidence suggests that sensory, as well as motor, pathways are abnormal in ALS (Munte et al., 1998, 1999; Paulus et al., 2002; Pekkonen et al., 2004). Thus, there is much evidence that cerebral involvement in ALS is more widespread than has been appreciated in the past, and the widespread changes in this study should not be discounted because they are not predominantly within, or confined to, motor regions. More extensive quantitative morphometric studies on cortical cell loss are still needed, however, to further support these findings.

Alternatively, our observations could reflect a change in the distribution or binding affinity of the 5-HT\(_{1A}\) receptor on cortical astrocytes (Azmitia et al., 1996). Astrocytosis is observed in ALS (Schiffer and Fiano, 2004), but would be expected to lead to increased, rather than decreased, 5HT\(_{1A}\) binding. Finally, the changes in \(^{11}\text{C}\)-WAY100635 binding could be due to functional changes in 5-HT\(_{1A}\) receptor binding, apparently unrelated to anxiety or depression, and related instead to alterations in cortical excitatory and inhibitory pathways (see later). This would have significant potential as a target for future molecular biological research with the potential to develop improved animal models of disease.

**The cerebral localization of 5-HT\(_{1A}\) receptors**

There are serotonergic projections to most CNS regions (Fuxe, 1965). In the raphe, 5-HT\(_{1A}\) receptors function as somatodendritic autoreceptors, with inhibitory influence over cortical neuronal projections (Sharp and Hjorth, 1990). Reductions in raphe receptor numbers or affinity could, therefore, parallel the widespread cortical receptor losses as part of the ALS disease process, as observed in this study. Alternatively, this might be a response to cortical receptor binding decreases (Zimmer et al., 2004). 5-HT\(_{1A}\) receptors are expressed throughout the brain with the highest density within the limbic regions and on pyramidal cell neurones (Pompeiano et al., 1992; Francis et al., 1992; Burnet et al., 1995; Pasqualetti et al., 1996; DeFelipe et al., 2001; Varnas et al., 2004). The receptor is also located on astrocyte cell bodies and other non-neuronal cells (Azmitia et al., 1996). The basal ganglia and substantia nigra contain low densities of 5-HT\(_{1A}\) receptors and there is very little expression in the
cerebellum (De Vos et al., 1991; Chalmers and Watson, 1991).

**Pathological studies in ALS**

Little information exists on serotonergic neurotransmission in ALS (Whitehouse et al., 1983) and studies have been limited to postmortem tissue. There have been no published quantitative studies of neuronal loss in the raphe nuclei in ALS. Published pathological studies have not commented on cell loss in this area, and involvement cannot be excluded without detailed morphometric studies. Neuropathological studies in ALS tissue of changes in the levels of 5-HT metabolites and 5-HT\textsubscript{1A} receptor binding have been variable (Bertel et al., 1991; Ohsumi et al., 1987; Manaker et al., 1988; Forrest et al., 1996). Overall, they suggested some alteration in 5-HT\textsubscript{1A} receptor numbers in the spinal cord.

**Serotonergic and motoneuronal excitability**

The interaction between serotonin, pyramidal motor neurones, and the excitatory neurotransmitter glutamate is complex (Rekling et al., 2000). Serotonin depresses glutamatergic transmission in spinal and cranial motoneurones (Shupliakov et al., 1995; Ladewig et al., 2004), and has also been shown to have an inhibitory effect on cortical pyramidal cell firing (McCormick and Williamson, 1989; Singer et al., 1996; Bouryi and Lewis, 2003). A reduction in serotonergic input could therefore contribute to glutamate-driven excitotoxic mechanisms which have been proposed in a variety of neurodegenerative disorders including ALS (Plaitakis and Carosci, 1987; Rothstein et al., 1990; Leigh and Meldrum, 1996; Doble, 1999). Pyramidal neurones are also postulated to be under the influence of serotonin via 5-HT\textsubscript{1A} receptors present on inhibitory interneurones (DeFelipe et al., 2001; Czyrak et al., 2003; Aznar et al., 2003). Transcranial magnetic stimulation studies suggest increased cortical excitability in ALS (Ziemann et al., 1997; Hanajima and Ugawa, 1998; Eisen and Weber, 2000), and interneuronal inhibitory influences might have a role in modulating this in certain phenotypes of ALS with prolonged survival (Weber et al., 2000). The loss of such interneurones, demonstrated neuropathologically in motor and extra-motor brain regions in ALS (Maekawa et al., 2004) supports this concept.

**Neurotrophic influences of serotonin in ALS**

Agonists at the 5-HT\textsubscript{1A} receptor may have a neurotrophic role (Banasr et al., 2004), which could have therapeutic implications, although this is highly speculative on the basis of our results. Cultures of rat septal cholinergic neurones demonstrated increases in choline acetyltransferase activity and augmentation of the dendritic branching after treatment with 5-HT\textsubscript{1A} receptor agonists, which have included buspirone (Riad et al., 1994; Yan et al., 1997; Lechtzin et al., 2001). In a mouse model of spinal cord transection, 5-HT\textsubscript{1A} receptors were central to the long-term recovery of motor function (Antri et al., 2003), and 5-hydroxytryptophan (a precursor for serotonin) delayed the development of neuromuscular disease in a mouse model of ALS (Turner et al., 2003). On the basis of neurotrophic activity in cultured motoneurones (Iwasaki et al., 1998; Labie et al., 1999), a 5-HT\textsubscript{1A} receptor agonist Xaliproden has been studied in a large double-blind, placebo-controlled clinical trial looking at survival in ALS patients (Meininger et al., 2004; Lacambiez et al., 2004). Although no clinically significant benefit has been reported, there were trends towards improved survival and slower functional deterioration. We believe that these findings, coupled with results from the present study, warrant further study of drugs with 5-HT\textsubscript{1A} receptor affinity and could be prioritized in high-throughput screening models.

**Conclusions**

Using the PET ligand ([\textsuperscript{11}C]-WAY100635, we have identified a marked global decrease in cerebral 5-HT\textsubscript{1A} receptor binding in ALS. SPM analysis indicates that the most marked changes within this global decrease include frontotemporal regions. There is no evidence that the reduction in binding is related to age, gender, drugs (including riluzole) or major affective disorder. Our hypothesis is that these findings reflect
widespread damage to cortical pyramidal neurones that express 5-HT1A receptors, although a purely functional change in receptor binding cannot be excluded. Our observations are unlikely to be explained by technical or other artefacts. The changes reported here may provide a useful tool for detecting early or presymptomatic disease. Further study is needed to clarify the relationships between altered serotonergic mechanisms, phenotype, genotype and progression in ALS.

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