Decreased carbon-11-flumazenil binding in early Alzheimer’s disease

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Neuronal loss in Alzheimer’s disease, a better correlate of cognitive impairment than amyloid deposition, is currently gauged by the degree of regional atrophy. However, functional markers, such as GABA_A receptor density, a marker of neuronal integrity, could be more sensitive. In post-mortem hippocampus, GABA_A messenger RNA expression is reduced even in mild cognitive impairment. We measured whole-brain GABA_A binding potential in vivo using [11C]-flumazenil positron emission tomography and compared GABA_A binding with metabolic and volumetric measurements. For this purpose, we studied 12 subjects, six patients with early Alzheimer’s disease and six healthy controls, with [11C]-flumazenil and [18F]-fluorodeoxyglucose positron emission tomography, as well as with high-resolution magnetic resonance imaging. Data were evaluated with both voxel-based parametric methods and volume of interest methods. We found that in early Alzheimer’s disease, with voxel-based analysis, [11C]-flumazenil binding was decreased in infero-medial temporal cortex, retrosplenial cortex and posterior perisylvian regions. Inter-group differences reached corrected significance when using an arterial input function. Metabolism measured with positron emission tomography and volumetric measurements obtained with magnetic resonance imaging showed changes in regions affected in early Alzheimer’s disease, but, unlike with [11C]-flumazenil binding and probably due to sample size, the voxel-based findings failed to reach corrected significance in any region of the brain. With volume of interest analysis, hippocampi and posterior cingulate gyrus showed decreased [11C]-flumazenil binding. In addition, [11C]-flumazenil hippocampal binding correlated with memory performance. Remarkably, [11C]-flumazenil binding was decreased precisely in the regions showing the greatest degree of neuronal loss in post-mortem studies of early Alzheimer’s disease. From these data, we conclude that [11C]-flumazenil binding could be a useful marker of neuronal loss in early Alzheimer’s disease.

Keywords: Alzheimer’s disease; flumazenil PET; MRI; GABA_A receptors
Introduction

In brains of patients with Alzheimer’s disease with the mildest clinically detectable dementia (Clinical Dementia Rating scale = 0.5), a 32% loss of neurons has already occurred in the entorhinal cortex (Gomez-Isla et al., 1996). Neuronal loss spreads as the disease progresses and correlates better than amyloid deposition with the degree of clinical worsening (Crystal et al., 1988; Gomez-Isla et al., 1996; Giannakopoulos et al., 2003). Regional neuronal loss can be gauged by the degree of atrophy, currently used as a neuroimaging marker of neuronal loss (Killiany et al., 2003). None of the previous [11C]-flumazenil studies in early Alzheimer’s disease (Lavenex et al., 1997). None of the previous studies in [11C]-flumazenil PET have been reported to be more specific than diffusion-weighted MRI for the detection of neuronal loss in stroke (Heiss et al., 2004). In contrast, a single patient study and two previous series using [11C]-flumazenil in Alzheimer’s disease have been negative (Sedvall et al., 1987; Meyer et al., 1995; Ohyama et al., 1999). The study by Meyer et al. (1995) included five patients with mild or early Alzheimer’s disease (mean Mini-Mental State Examination = 22) and six healthy controls. Ohyama et al. (1999) studied five patients with probable Alzheimer’s disease and five healthy controls. Parity shows lateral temporoparietal association cortex shows the greatest metabolic loss in Alzheimer’s disease, these PET studies focused on a comparison of metabolism or regional cerebral blood flow with [11C]-flumazenil PET, which has been reported to be more specific than diffusion-weighted MRI for the detection of neuronal loss in stroke (Heiss et al., 2004). In contrast, a single patient study and two previous series using [11C]-flumazenil in Alzheimer’s disease have been negative (Sedvall et al., 1987; Meyer et al., 1995; Ohyama et al., 1999). The study by Meyer et al. (1995) included five patients with mild or early Alzheimer’s disease (mean Mini-Mental State Examination = 22) and six healthy controls. Ohyama et al. (1999) studied five patients with probable Alzheimer’s disease and five healthy controls. Parity shows lateral temporoparietal association cortex shows the greatest metabolic loss in Alzheimer’s disease, these PET studies focused on a comparison of metabolism or regional cerebral blood flow with [11C]-flumazenil binding in these regions. However, these are not the regions where the greatest neuronal loss has been detected in post-mortem studies (Brun and Englund, 1981). Additionally, the relationship between atrophy and metabolism is complex, with areas, such as the hippocampus, where there is atrophy but relatively preserved metabolism and others with marked metabolic decline but little atrophy (Chetelat et al., 2008). This is not surprising because metabolism reflects synaptic activity (Rocher et al., 2003); metabolic loss in a region may simply reflect neuronal loss in the region home to the neurons projecting to the metabolically impaired region. Lateral parietotemporal association cortex receives a heavy projection from the medial temporal region (Lavenex et al., 2002; Munoz and Insasuti, 2005), with marked neuronal loss in Alzheimer’s disease (Gomez-Isla et al., 1996, 1997). None of the previous [11C]-flumazenil studies in Alzheimer’s disease were performed using voxel-wise comparisons, partial volume correction or normalization starting with PET co-registration to native MRI. We postulated that more recent imaging methods could facilitate detecting GABA\textsubscript{A}-binding abnormalities in early Alzheimer’s disease. We chose a small sample size to determine whether [11C]-flumazenil PET could separate patients with early Alzheimer’s disease from healthy controls when the power of the voxel-based analysis was too low to separate these two groups based on two Alzheimer’s disease neuroimaging markers, namely grey matter atrophy on MRI or reduced metabolism on [18F]-fluorodeoxyglucose PET (Chen et al., 2011).

Materials and methods

Participants

Twelve participants, divided into two groups (Alzheimer’s disease and healthy controls) of six participants each, were studied with neuropsychological testing, structural MRI and PET with [11C]-flumazenil, a GABA\textsubscript{A} receptor antagonist. Participants in the two groups had similar age, sex and educational level (Table 1). Patients with Alzheimer’s disease met National Institute of Neurological and Communication Disorders and Stroke - Alzheimer Disease and Related Disorders Association (NINCDS-ADRDA) criteria (McKhann et al., 1984) for the diagnosis of probable Alzheimer’s disease but with a mild degree of cognitive impairment (mean Mini-Mental State Examination score was 21.2). Other neurological or psychiatric disorders were excluded in both patients with Alzheimer’s disease and controls by a thorough medical and neurological evaluation, including standard biochemical and haematological studies. Because both groups had served as controls for another study (Pascual et al., 2010), comparing them to patients with microvascular disease, the presence of vascular lesions, including white matter changes visible on T\textsubscript{2}-weighted images or more than punctate hyperintensities on T\textsubscript{2}-weighted images, was carefully excluded by MRI. To facilitate sleep, one patient and one healthy control were taking a similar dose of lorazepam, which in both cases was discontinued 1 week before the PET procedure. Two patients were taking donepezil, also discontinued 1 week before undergoing PET. The study had been approved by the local Ethics Committee following Declaration of Helsinki guidelines for the study of human subjects. All subjects were fully instructed on the experimental procedures and provided written, informed consent.

Neuropsychological testing

The tests performed are listed in Table 2. They were obtained according to standard procedures and were analysed using SPSS Version 17 (2006). Due to the small sample size, comparisons between patients

<table>
<thead>
<tr>
<th>Variable</th>
<th>Alzheimer’s disease</th>
<th>Healthy controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of subjects</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>Gender (female)</td>
<td>3</td>
<td>4</td>
</tr>
<tr>
<td>Age (years)</td>
<td>78.0 (6.1)</td>
<td>78.2 (3.2)</td>
</tr>
<tr>
<td>Years of education</td>
<td>10.0 (3.4)</td>
<td>10.0 (3.4)</td>
</tr>
<tr>
<td>Dementia screening</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clinical dementia rating</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Memory (range: 0–3)</td>
<td>1.1 (0.5)*</td>
<td>0</td>
</tr>
<tr>
<td>Sum of boxes (range: 0–18)</td>
<td>4.2 (3.6)*</td>
<td>0</td>
</tr>
<tr>
<td>Mini-Mental State Examination (range: 0–30)</td>
<td>21.2 (5.9)*</td>
<td>28.8 (0.7)</td>
</tr>
<tr>
<td>DemTect (range 0–19)</td>
<td>8.0 (2.3)*</td>
<td>18.2 (1.2)</td>
</tr>
</tbody>
</table>

Data are presented as means, with standard deviations in parentheses. *Worse performance than healthy controls, P < 0.05.
Flumazenil PET in early Alzheimer's disease

Magnetic resonance imaging data acquisition and processing

MRI was obtained for all participants on the same 3T Siemens Trio scanner (Siemens Medical Solutions), including T₁-weighted structural images acquired using a 3D MP-RAGE sequence, with imaging parameters: resolution = 1 x 1 x 1 mm³, field of view = 240 x 256 mm², 160 sagittal slices, repetition time/echo time/inversion time = 2250/900/2.96 ms, flip angle = 9°, bandwidth = 230 Hz/pixel. Whole-brain cortical thickness and hippocampal volume were determined using FreeSurfer software (Althouna Martinos Centre for Biomedical Imaging). Voxel-based morphometry was performed with SPM8 software (Wellcome Department of Imaging Neuroscience, University College of London), using a fast diffeomorphic registration algorithm (DARTEL; Ashburner 2007). Details of the MRI methods are provided in the online Supplementary material.

[¹⁸F]-Fluorodeoxyglucose positron emission tomography data acquisition and processing

Brain metabolism was measured with [¹⁸F]-fluorodeoxyglucose PET using standard acquisition and processing methods (Supplementary material).

[¹¹C]-Flumazenil positron emission tomography data acquisition and processing

GABA_A binding was measured with [¹¹C]-flumazenil PET using an ECAT EXACT HR+ (Siemens/CTI) scanner with a nominal resolution of 4.5 mm but a measured resolution of 7.3 mm (Prieur et al., 2010). Subject motion was minimized with individually fitted thermoplastic masks. Following a transmission scan obtained using three ⁶⁸Ge sources and after the bolus injection of 659.71 ± 136.16 MBq (17.83 ± 3.68 mCi) of [¹¹C]-flumazenil, a set of 19 sequential 3D PET frames of the entire brain were obtained over a period of 60 min, according to the following protocol: 4 x 30 s, 3 x 60 s, 2 x 150 s and 10 x 300 s. Arterial blood samples were drawn from the radial artery during the dynamic emission scans, for a total of 21 blood samples (intervals: 8 x 15 s, 3 x 60, 2 x 150 and 8 x 300 s). The sampled blood was processed to obtain arterial plasma input function for modelling. Using a procedure that provides reliable findings for [¹¹C]-flumazenil PET (Okazawa et al., 2004), metabolites were not measured but were calculated as described by Okazawa et al. (2004), and plasma input function corrected accordingly. The radioactivity measured in the PET scans and blood samples was corrected for decay to the starting point of each scan.

Voxel-based parametric images of [¹¹C]-flumazenil volume of distribution (Vr) were computed with PMOD software (PMOD Technologies) using the graphic plot method of Logan. The slope of the graphical plot was calculated from 15 to 60 min, representing the volume of distribution (Innis et al., 2007), which is here simply

Table 2 Neuropsychological testing performance and its correlation with [¹¹C]-flumazenil hippocampal binding

<table>
<thead>
<tr>
<th>Cognitive domains</th>
<th>Test</th>
<th>Performance</th>
<th>Correlation (r) with [¹¹C]-flumazenil hippocampal binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cognitive domains</td>
<td></td>
<td>Alzheimer disease patients</td>
<td>Healthy controls</td>
</tr>
<tr>
<td>Memory</td>
<td>RAVLT</td>
<td>22.6 (7.2)*</td>
<td>40.3 (9.1)</td>
</tr>
<tr>
<td></td>
<td>First five trials</td>
<td>2.4 (1.7)</td>
<td>8.7 (5.1)</td>
</tr>
<tr>
<td></td>
<td>Immediate recall</td>
<td>1.2 (1.8)*</td>
<td>8.3 (5.1)</td>
</tr>
<tr>
<td></td>
<td>Delayed recall</td>
<td>8.8 (5.8)</td>
<td>13.3 (1.5)</td>
</tr>
<tr>
<td></td>
<td>Recognition</td>
<td>44.0 (25.0)*</td>
<td>83.3 (4.5)</td>
</tr>
<tr>
<td>Logical memory</td>
<td>Immediate recall</td>
<td>10.0 (6.0)*</td>
<td>28.5 (9.0)</td>
</tr>
<tr>
<td></td>
<td>Delayed recall</td>
<td>2.4 (2.8)</td>
<td>15.3 (9.9)</td>
</tr>
<tr>
<td></td>
<td>Recognition</td>
<td>10.4 (6.5)</td>
<td>19.0 (7.0)</td>
</tr>
<tr>
<td></td>
<td>Rey complex figure, recall</td>
<td>4.2 (4.0)*</td>
<td>18.4 (5.7)</td>
</tr>
<tr>
<td>Language</td>
<td>Boston naming test</td>
<td>39.2 (9.6)</td>
<td>50.2 (5.6)</td>
</tr>
<tr>
<td></td>
<td>Verbal fluency (named in 1')</td>
<td>11.0 (3.6)*</td>
<td>18.0 (4.0)</td>
</tr>
<tr>
<td></td>
<td>Animals</td>
<td>12.8 (6.3)</td>
<td>16.8 (6.2)</td>
</tr>
<tr>
<td></td>
<td>Words starting with letter 'P'</td>
<td>17.2 (3.6)</td>
<td>19.5 (0.5)</td>
</tr>
<tr>
<td></td>
<td>Token test</td>
<td>22.7 (13.0)*</td>
<td>33.7 (2.1)</td>
</tr>
<tr>
<td>Visuospatial function</td>
<td>Rey complex figure, copy</td>
<td>4.0 (1.9)</td>
<td>6.8 (2.6)</td>
</tr>
<tr>
<td></td>
<td>Visual span backwards</td>
<td>10.0 (3.4)</td>
<td>14.7 (3.4)</td>
</tr>
<tr>
<td></td>
<td>Executive function, attention and speed of processing</td>
<td>19.2 (6.8)*</td>
<td>8.3 (0.8)</td>
</tr>
<tr>
<td>Other domains</td>
<td>GERIATRIC depression scale</td>
<td>12.4 (4.1)</td>
<td>5.2 (4.4)</td>
</tr>
<tr>
<td></td>
<td>Neuropsychiatric inventory</td>
<td>3.6 (2.9)</td>
<td>1.7 (1.9)</td>
</tr>
</tbody>
</table>

Performance data are presented as means, with standard deviations in parentheses.

FCSRT = free and cued selective reminding test, picture version;
IADL = instrumental activities of daily living scale; RAVLT = Rey auditory verbal learning test; WAIS-R = Wechsler adult intelligence scale-revised; WC = word-color.

*P < 0.05; **P < 0.01.
called ‘binding’. For the voxel-based analysis, PET studies were spatially normalized with SPM8 after individual PET and structural MRI studies had been co-registered in native space. Using Statistical Parametric Mapping 8 (SPM8), we analysed $[^{11}C]$-flumazenil binding, both for the whole brain and for a volume of interest including the entire temporal lobes of both hemispheres as the medial temporal regions have previously been identified as bearing marked neuronal loss in early Alzheimer’s disease (Gomez-Isla et al., 1996).

For volume of interest analysis, $[^{11}C]$-flumazenil parametric images of each subject were processed and corrected for partial volume effects using the method of Rousset et al. (1998) implemented in PVElab (pvelab@nru.dk) (Quarantelli et al., 2004). The point-spread function to perform partial volume correction was calculated by determining the true resolution of the PET camera (Prieto et al., 2010). Thus, a full-width at half-maximum of 7.3 mm was used for this purpose. Individual PET images were co-registered to high-resolution native-space $T_1$-weighted MRI images using the mutual information method of SPM8. Then, the $T_1$-weighted images were segmented into grey matter, white matter and CSF. The segmented grey matter images were spatially normalized into the Montreal Neurological Institute (MNI) grey matter provided by SPM8, and the inverse transformation matrix was used to label the segmented grey matter voxels in the patient’s MRI-PET space according to a template of volumes of interest defined in the MNI space (Supplementary Fig. 1) (Berkouk et al., 2006). For each volume of interest, partial volume effects-corrected, mean tracer concentrations were calculated for each participant. Briefly, segmented grey matter volumes of interest were corrected for the percentage of activity spilling from/to white matter whereas the CSF was constrained to zero because CSF is supposed to not have significant activity (Quarantelli et al., 2004). Because the size of the sample was small, comparisons between Alzheimer’s disease and healthy controls were performed using the Mann–Whitney U test. Alpha was set at 0.05 for all analyses.

In addition to using the arterial input function, we analysed the $[^{11}C]$-flumazenil PET data and compared the two groups using the pons as a reference region (Supplementary material).

Results

Neuropsychological performance

Patients with Alzheimer’s disease had impaired memory. In addition, they performed significantly worse than healthy controls in some language tasks, but not on executive function tasks (Table 2).

Volumetric magnetic resonance imaging

On volume of interest analysis, hippocampal volumes were smaller in the Alzheimer’s disease group (Fig. 2, Supplementary Table 1 and Supplementary Fig. 2). Cortical thickness and voxel-based morphometry yielded several regions, including the left medial temporal area, with decreased volume in Alzheimer’s disease at uncorrected $P < 0.005$, but none which survived corrected significance (Supplementary Table 2 and Supplementary Figs 3 and 4). All the voxel-based, parametric contrasts, are displayed at the same $P$-value, to facilitate comparison across imaging modalities.

$[^{18}F]$-Fluorodeoxyglucose positron emission tomography

Left parietal association cortex and both precunei showed lower metabolism in Alzheimer’s disease ($P < 0.005$ uncorrected), but none of these comparisons survived corrected significance (Supplementary Table 3 and Supplementary Fig. 5).

$[^{11}C]$-Flumazenil positron emission tomography

With voxel-based parametric analysis using arterial input function, flumazenil binding in patients with Alzheimer’s disease was decreased in the left medial temporal region, including entorhinal cortex (whole brain analysis, cluster-level FWE-corrected $P = 0.048$; volume of interest, voxel-level FWE-corrected $P = 0.023$); and with uncorrected significance (voxel level $P < 0.005$), there was decreased binding in the right inferomedial temporal region, left precuneus and supramarginal gyri of both hemispheres (Fig. 1 and Table 3). With volume of interest analysis, $[^{11}C]$-flumazenil binding was decreased ($P < 0.05$) bilaterally in hippocampi and in the posterior cingulate gyrus (Supplementary Table 4 and Supplementary Fig. 6). Unlike with volumetric MRI, no false positives were recorded with $[^{11}C]$-flumazenil binding (Fig. 2). Hippocampal $[^{11}C]$-flumazenil binding correlated positively with measures of memory and other cognitive functions (Table 2 and Supplementary Fig. 7).

With the analysis using the pons as reference region, no regions were identified reaching corrected significance on voxel-based testing (Supplementary Table 5) or in volume of interest analysis (Supplementary Table 6). However, in voxel-based testing, regions that differed across groups were detected at the uncorrected $P < 0.005$ voxel level, largest in the left medial temporal region (Supplementary Table 5 and Supplementary Fig. 8) that were very similar to the ones detected with the arterial input method.

Discussion

We found $[^{11}C]$-flumazenil binding to be decreased in regions which, on extensive histological sampling, have shown the greatest degree of neuronal loss in early Alzheimer’s disease. In a classical study, Brun and Englund (1981) found most marked neuronal loss in the medial temporal region, where $[^{11}C]$-flumazenil binding was most decreased in our study, but also in the posterior cingulate gyrus and in the supramarginal gyri of both hemispheres, regions with a strong trend for decreased $[^{11}C]$-flumazenil binding on voxel-based imaging (Supplementary Fig. 9), and in the case of the posterior cingulate, significantly lower for Alzheimer’s disease in volume of interest analysis (Supplementary Table 4 and Supplementary Fig. 6).

In a study comparing $[^{11}C]$-flumazenil binding in older participants, two potential confounders were vascular disease and depression, both of which have been reported to lower $[^{11}C]$-flumazenil binding (Heiss et al., 2004; Klumpers et al., 2010). Vascular disease was carefully excluded from the Alzheimer’s disease group, which served as a control for a different
[18F]-fluorodeoxyglucose PET study (Pascual et al., 2010), where Alzheimer’s disease was compared with microvascular disease, the type of vascular disease most likely to be a confounder for this study. The depression rating scale score was higher in the group with Alzheimer’s disease, although not significantly so and [11C]-flumazenil binding correlated with depression scores (Table 2). However, we used the standard Geriatric Depression Scale to measure depression. Some of the items in this scale will be checked as positive not only in patients with depression, but also in those with cognitive impairment. For instance, the item that reads: ‘Is your mind as clear as it used to be?’ None of the patients with Alzheimer’s disease had clinical depression.

We did not find significantly decreased binding in the posterolateral parietotemporal association cortex, where decreased metabolism is regularly seen in Alzheimer’s disease (Chetelat et al., 2008) and where an intermediate degree of neuronal loss was found by Brun and Englund (1981). Preserved [11C]-flumazenil binding in this region may result from a delicate balance of neuronal loss versus increased expression of the GABA<sub>A</sub> receptor in the remaining neurons. Receptor plasticity has been documented after lesions of projection pathways. In rodents, lesions of the perforant hippocampal pathway resulted in an acute loss of GABA<sub>A</sub> receptors, but similar levels to controls were recorded 30 days after the acute lesion (Mizukami et al., 1997). A similar compensatory plasticity has been shown in Alzheimer’s disease using post-mortem tissue. In the hippocampal formation, immunohistochemical, biochemical and in situ hybridization methods have shown an increased expression of GABA<sub>A</sub> receptors in the surviving neurons (Armstrong et al., 2003; Iwakiri et al., 2009). Similar data are not available for the posterior parietotemporal association cortex. While marked neuronal loss in medial temporal regions (Brun and Englund, 1981; Gomez-Isla et al., 1996, 1997) would dictate that the total GABA<sub>A</sub> activity be reduced here, a milder loss in posterior parieto-temporal association cortex (Brun and Englund, 1981) may be compensated by an increase of GABA<sub>A</sub> receptors in the remaining neurons, leaving regional [11C]-flumazenil binding unaltered, as we found in this region.

Several methodological differences may explain the positive finding in our study while previous studies of [11C]-flumazenil in Alzheimer’s disease, with a number of patients similar to ours, were negative (Meyer et al., 1995; Ohyama et al., 1999). First, we used voxel-based comparisons. By comparing all the voxels in the brain of patients with Alzheimer’s disease and healthy controls, decreased binding can be found in areas that, when averaged with preserved areas in a volume of interest methodology, may pass undetected. In our study, apart from extensive changes in the medial temporal regions, other areas of decreased binding were small and likely to disappear when averaged with tissue containing normal binding values. For instance, despite decreased binding in both supramarginal gyri and the left precuneus (Fig. 1), overall binding in parietal lobes was not decreased in our volume of interest analysis (Supplementary Table 4 and Supplementary Fig. 6). Secondly, we used native space MRI to co-register it with native PET data and bring PET data to standard space. This technique not only allowed us to correct for partial volume effects, but also improved the quality of PET data in standard space and therefore the yield of all the comparisons performed across subjects and groups of subjects (Shan et al., 2011). In addition, our subject samples were better matched by sex (Meyer et al., 1995) and by age (Meyer et al., 1995; Ohyama et al., 1999).

Our findings agree with binding studies on Alzheimer’s disease post-mortem tissue showing a 40% loss of benzodiazepine...
binding in the entorhinal cortex (Jansen et al., 1990) and variable losses of benzodiazepine or GABA\textsubscript{A} receptor study performed with PET and were cautious given the small sample size. Yet, the regions showing a trend for decreased volume were smaller but anatomically similar to areas showing decreased \([11C]\)-flumazenil binding (Supplementary Fig. 4). This finding supports the notion that decreased GABA\textsubscript{A} binding reflects neuronal loss (La Fougere et al., 2011), which will also cause atrophy. However, the effect size for volume loss, both in the voxel-based analysis and the volume of interest analysis of volume (compare \(r\) values in Supplementary Tables 1 and 4), was smaller than for \([11C]\)-flumazenil binding loss.

\([18F]\)-Fluorodeoxyglucose PET showed a trend for decreased metabolism in the regions where this finding has been classically described in Alzheimer’s disease (Chetelat et al., 2008). A greater magnitude of neuronal loss, measured as decreased \([11C]\)-flumazenil binding, in the left medial temporal region (Fig. 1 and Table 3), corresponded to a greater loss of metabolism in the ipsilateral angular gyrus and retrosplenial region (Supplementary Fig. 5). This finding supports the concept that loss of metabolic activity in parietal association cortex may result, at least in part, from a reduction in the rich projections reaching this region from inferomedial temporal cortex (Lavenex et al., 2002; Munoz and Insauti, 2005). It also highlights the dissociation between neuronal loss and loss of metabolism that has been reflected in other studies by a regional dissociation between atrophy and metabolic loss (Chetelat et al., 2008).

The applicability of \([11C]\)-flumazenil PET imaging to the study of large samples of patients with Alzheimer’s disease and controls would be greatly limited by the need to obtain arterial sampling, which is an invasive procedure. Fortunately, reference region methods have been described that obviate the need for arterial sampling in order to obtain an input function (Hammers et al., 2008; Mourik et al., 2008; Klumpers et al., 2008). In our study, using the pons as a reference region yielded intergroup differences in medial temporal regions and prefrontal regions that were significant at the uncorrected \(P < 0.005\) level, which compares favourably to studies of \([11C]\)-flumazenil PET binding in epilepsy (Hammers et al., 2008) or depression (Klumpers et al., 2012) using the pons as reference region. However, at the same threshold, the arterial input method provided larger and more significant regions on voxel-based analyses and a greater intergroup effect on volume of interest analyses (Fig. 1 and Table 3; Supplementary Fig. 8 and Supplementary Tables 4–6). This finding agrees with the dictum that the ideal input function should not contain specific binding. Although in smaller amounts than in the cortex, GABA\textsubscript{A} receptors are present in the pons, and therefore the presence of some specific binding in this region makes it less than ideal as a reference region (Delforge et al., 1995). It is possible that using carotid pixel density as an input function may yield...
findings even more similar to the arterial-input-function method, yet without the need for arterial sampling (Mourik et al., 2008). Another potential drawback of using [11C]-flumazenil is the short half-life of 11C, which dictates that a cyclotron be available at the imaging site. To address this issue, flumazenil labelled with 18F, an isotope with a longer half-life, is being readied for clinical use (Massaweh et al., 2009; la Fougere et al., 2009). [11C]-Flumazenil is not the only agent available to image GABA\textsubscript{A} receptors in vivo. [123I]-Iomazenil, a partial agonist, has also been used to image GABA\textsubscript{A} receptors in Alzheimer’s disease by means of single photon emission computed tomography. The two [123I]-iomazenil studies in Alzheimer’s disease were positive, but data on medial temporal binding, the region with the greatest neuronal loss, were not available (Soricelli et al., 1996; Fukuchi et al., 1997). The patients in both studies had a lower Mini-Mental State Examination score than in our study, 14.7 ± 2.3 in Soricelli et al. (1996), suggesting more advanced Alzheimer’s disease than in our sample (Student’s t-test, two-tailed, \(P = 0.02\)). A study of [123I]-iomazenil in mild cognitive impairment has been negative (Pappata et al., 2010). Our findings may encourage more research with this compound. However, because the methodology differ substantially, it is outside the scope of this paper to discuss investigations performed with [123I]-iomazenil.

Using a sample size similar to those in previous studies of [11C]-flumazenil binding, but updated methodology, we were able to separate patients with early Alzheimer’s disease from healthy controls. As the findings on [11C]-flumazenil PET reached statistical significance in precisely the areas of the brain known to have greatest neuronal loss, this report may encourage larger studies, which could confirm the usefulness of [11C]-flumazenil PET for the detection of neuronal loss in early clinical or even preclinical stages.

Acknowledgements

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Funding


Supplementary material

Supplementary material is available at Brain online.

References


Table 3  Regions of lower [11C]-flumazenil binding in patients with Alzheimer’s disease compared with healthy controls on the voxel-based SPM analysis (sorted by cluster-level FWE-corrected significance)

<table>
<thead>
<tr>
<th>Area of between-group difference</th>
<th>Brodmann areas(s)</th>
<th>Side</th>
<th>k</th>
<th>t</th>
<th>Z</th>
<th>P-value</th>
<th>Maxima voxel MNI coordinates</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medial temporal lobe</td>
<td>28, 35, 36</td>
<td>L</td>
<td>1980</td>
<td>6.60</td>
<td>4.01</td>
<td>0.048</td>
<td>0.000**</td>
<td>-42, -64, -4</td>
</tr>
<tr>
<td>Supramarginal gyrus</td>
<td>39, 40</td>
<td>L</td>
<td>352</td>
<td>4.50</td>
<td>3.25</td>
<td>0.758</td>
<td>0.001</td>
<td>-46, -28, 20</td>
</tr>
<tr>
<td>Infero-medial temporal lobe</td>
<td>28, 36</td>
<td>R</td>
<td>242</td>
<td>4.24</td>
<td>3.14</td>
<td>0.865</td>
<td>0.001</td>
<td>40, -26, 18</td>
</tr>
<tr>
<td>Precuneus cortex</td>
<td>31</td>
<td>L</td>
<td>190</td>
<td>4.45</td>
<td>3.23</td>
<td>0.909</td>
<td>0.001</td>
<td>-14, -72, 30</td>
</tr>
<tr>
<td>Supramarginal gyrus</td>
<td>39, 40</td>
<td>R</td>
<td>181</td>
<td>4.21</td>
<td>3.12</td>
<td>0.916</td>
<td>0.001</td>
<td>48, -34, 22</td>
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<tr>
<td>Posterior parahippocampal gyrus</td>
<td>35, 36</td>
<td>L</td>
<td>60</td>
<td>3.86</td>
<td>2.95</td>
<td>0.985</td>
<td>0.002</td>
<td>12, -40, 6</td>
</tr>
<tr>
<td>Anterior insular cortex</td>
<td>44</td>
<td>L</td>
<td>51</td>
<td>4.50</td>
<td>3.25</td>
<td>0.988</td>
<td>0.001</td>
<td>-34, 26, 8</td>
</tr>
<tr>
<td>Angular gyrus</td>
<td>39</td>
<td>R</td>
<td>27</td>
<td>3.68</td>
<td>2.86</td>
<td>0.994</td>
<td>0.002</td>
<td>46, -68, 48</td>
</tr>
</tbody>
</table>

*Voxel-level uncorrected P-values are provided as is customary and to facilitate comparison with other techniques (Supplementary material) as most of the intergroup comparisons do not reach corrected significance.

**Corrected for a volume encompassing both temporal lobes, known to be the cortical regions with earliest neuronal loss.

FWE = family-wise error; SPM = Statistical Parametric Mapping.

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Rissman RA, Bennett DA, Armstrong DM. Subregional analysis of GABA(A) receptor subunit mRNAs in the hippocampus of older


