Increased binding to $5\text{-HT}^{1A}$ and $5\text{-HT}^{2A}$ receptors is associated with large vessel infarction and relative preservation of cognition

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Vascular dementia accounts for ~15–20% of all dementias. In addition, a significant subset of people with Alzheimer’s disease have concurrent cerebrovascular disease. Vascular dementia is caused by different cerebrovascular morphological abnormalities including large artery territory infarction (multi-infarct vascular dementia) and sub-cortical ischaemic vascular dementia. Despite this distinction, there is a lack of studies examining the neurochemistry of individual vascular dementia subtypes. Serotonin is believed to play an important role in cognition, and serotonin receptors may provide a novel target for future anti-dementia therapeutics. This study aimed to determine levels of two serotonin receptors in subtypes of vascular dementia and relate any changes to cognition. We have determined, using saturation radioligand binding, the binding parameters (affinity and maximal binding) of ($^3$H)-WAY 100635 binding to $5\text{-HT}^{1A}$ receptors and ($^3$H)-ketanserin binding to $5\text{-HT}^{2A}$ receptors in post-mortem tissue from the frontal and temporal cortices of patients with either multi-infarct vascular dementia, sub-cortical ischaemic vascular dementia, mixed Alzheimer’s disease/vascular dementia or stroke no dementia (SND). $5\text{-HT}^{1A}$ and $5\text{-HT}^{2A}$ receptor binding was significantly increased in the temporal cortex of patients with either multi-infarct vascular dementia or SND, compared to age-matched controls. $5\text{-HT}^{1A}$ receptor maximal binding in the temporal cortex was also positively correlated with cognition as determined by Mini-Mental State Examination (MMSE) and Cambridge Assessment of Mental Health for the Elderly scores (CAMCOG). These results reveal an important distinction between the neurochemistry of multi-infarct vascular dementia/SND and sub-cortical ischaemic vascular dementia, suggesting that pharmacological manipulation of serotonin offers the possibility to develop novel therapies for stroke and multi-infarct vascular dementia patients. The results also highlight the importance of the cortical $5\text{-HT}^{1A}$ receptor in mediating cognition.

Keywords: vascular dementia; stroke; serotonin; cognition

Abbreviations: CERAD = Consortium to Establish a Registry for Alzheimer’s disease; MIVaD = multi-infarct dementia; MMSE = Mini-Mental State Examination; SIVaD = sub-cortical ischaemic vascular dementia; SND = stroke no dementia; $5\text{-HT}$ = 5-hydroxytryptamine
Introduction

Stroke and dementia individually are common, costly in economic terms to society and personally devastating to patients and their family. One in six older people have a stroke, and 30% of these individuals develop dementia. The costs of care in the UK for stroke and vascular dementia approach £30 billion a year. Vascular dementia is the second most common form of dementia after Alzheimer’s disease, accounting for an estimated 15–20% of the 25 million people worldwide with dementia (Ferri et al., 2005). In addition, up to 40% of individuals with Alzheimer’s disease have significant concurrent cerebrovascular disease (Heyman et al., 1998; Lewis et al., 2006). Despite the clear need, there is a limited evidence base for the treatment of established vascular dementia or the prevention of cognition decline and dementia following large artery territory infarction (Chapman et al., 2004; Areosa et al., 2005; Craig and Birks 2005, 2006). Of particular note, clinical trials have demonstrated more limited efficacy of Alzheimer’s disease therapies in patients with mild to moderate vascular dementia (Kavirajan and Schneider, 2007). Currently there are no therapies licensed for these indications.

Vascular dementia is often thought of as a single disease, but is actually underpinned by a range of different cerebrovascular morphological abnormalities (Roman et al., 1993), the most common of which are large artery territory infarction, usually multiple [multi infarct dementia (MIVaD)] or small vessel disease [sub-cortical ischaemic vascular dementia (SIVaD)] (Roman et al., 1993; Erkinjuntti, 2007). There are no neurochemical studies focusing specifically on patients with MIVaD or SIVaD. Furthermore there has been very limited study of post-mortem brain neurochemistry following large artery territory infarction in the absence of dementia [stroke no dementia (SND)] (Jellinger et al., 1978). Deficits in cholinergic function have, however, been reported in patients with vascular dementia with concurrent Alzheimer’s disease, but not in vascular dementia without concurrent Alzheimer’s disease (Perry et al., 2005), and clinical trials have reported differential treatment responses in vascular dementia with and without concurrent Alzheimer’s disease and in SIVaD and MIVaD (Pantoni et al., 2000; Moretti et al., 2008), further highlighting the priority of understanding the biological basis of impairments in specific types of vascular dementia to inform the development of new treatments and the more effective targeting of existing therapies.

Serotonergic [5-hydroxytryptamine (5-HT)] neurones have long been implicated in the process of learning and memory formation. Although the precise mechanism remains unclear, it has been postulated that 5-HT neurones play an important role in regulating the release of other neurotransmitters, including glutamate, acetylcholine and GABA (Barnes and Sharp, 1999; Buhot et al., 2000). In support of this tenet, tryptophan depletion paradigm in healthy adults result in temporary cognitive deficits (Harrison et al., 2004; Schmitt et al., 2006) and both 5-HT1A and particularly 5-HT1A receptors have previously been implicated in learning and memory processes (Harvey, 2003; Ogren et al., 2008). Specifically, partial agonists and antagonists of the 5-HT1A receptor enhance cognition in a variety of animal and human volunteer studies (Ogren et al., 2008). Highly selective 5-HT1A antagonists MDL 100907 and EMD 281014, both developed as anti-psychotics, have also been shown to enhance cognitive function in animal models (Kehne et al., 1996; Terry et al., 2005).

There are few post-mortem studies focusing on the serotonergic system in vascular dementia or SND, and no studies examining the serotonergic system in specific types of vascular dementia. One study reported reduced 5-HT and 5-HT metabolite concentrations in a number of brain regions (e.g. striatum, hippocampus, hypothalamus and caudate nucleus) in vascular dementia patients (Gottfries et al., 1994) and decreases in 5-HT have also been detected in the cerebrospinal fluid of patients with vascular dementia (Tohgi et al., 1995). The only study examining 5-HT receptors in vascular dementia reported no overall change in comparison to controls but did not separately examine SIVaD, MIVaD or SND (Hansson et al., 1996).

The main aim of this study was to determine the binding characteristics of the 5-HT1A and 5-HT2A receptors in human post-mortem tissue from two cortical brain regions in MIVaD, SIVaD and age-matched controls. An additional aim was to determine 5-HT binding in patients with SND, and mixed vascular dementia/Alzheimer’s disease.

Methods

Subjects and brain tissue

Brain specimens from dorsolateral prefrontal cortex [Brodmann area 9—(BA 9)] and inferior/middle temporal cortex (BA 20/21) were obtained from the Newcastle Brain Tissue Resource, Newcastle General Hospital and from the MRC London Neurodegenerative Diseases Brain Bank at the Institute of Psychiatry, King’s College London. These brain tissue resources constitute a large collection of frozen and formalin fixed brains from clinically characterized subjects, a substantial proportion of whom (55%) were from prospective clinical cohort studies with serial standardized evaluations. For individuals who participated in longitudinal clinical studies, standardized cognitive evaluations were completed with Mini-Mental State Examination (MMSE) and the Cambridge Assessment of Mental Health for the Elderly, section b (CAMCOG) at baseline and at annual intervals until death, undertaken by trained psychology assistants or research nurses. The MMSE is a widely used 30-item cognitive screening tool (Folstein et al., 1979). The CAMCOG is a 107-point assessment tool which evaluates a range of cognitive domains and includes a 29-point memory sub-scale (Williams et al., 2003). The CAMCOG was only completed for those participants who were enrolled in prospective clinical studies. Table 1 shows the age range, gender distribution, post-mortem delay, tissue pH, Consortium to Establish a Registry for Alzheimer’s disease (CERAD) description of plaque frequency (Mirra et al., 1991) and Braak Stageing (Braak and Braak, 1995) of the 81 participants (25 controls, eight MIVaD, 11 SIVaD, 29 mixed vascular dementia/Alzheimer’s disease and eight SND). In the majority of cases, bronchopneumonia was recorded as the cause of death. Of the 22 participants who completed CAMCOG assessment, no individuals were taking cholinesterase inhibitors, memantine or anti-parkinsonian medication. Only one patient was prescribed an anti-cholinergic drug. No participants had formal diagnosis of alcohol abuse or drug dependency.

Macroscopic and microscopic pathological assessment was undertaken by three of the authors (RK, RP and TH) at the Newcastle and London sites, using standardized protocols. Tissue blocks were
fixed in 10% formalin and processed into paraffin wax. Blocks were taken as defined by CERAD from the middle frontal gyrus (BA 8/9), superior and middle temporal gyri (BA 21/22) and the anterior hippocampus and parahippocampal gyrus (BA 28, 36), intraparietal sulcus (BA 40) and midbrain; as well as the occipital lobe with the calcarine sulcus (BA 17–19), cingulated gyrus (BA 24, 32), frontal and occipital lobe deep white matter with the ‘watershed’ areas of the blood supply territories of major arteries, striatum, amygdala, thalamus, cerebellum, pons and medulla oblongata. Haematoxylin & eosin, Luxol fast-blue/ Nissl stains, modified Bielschowsky’s silver impregnation and immunohistochemistry with antibody raised against phosphorylated tau (AT8, Autogen Bioclear, Wiltshire, UK), ubiquitin (Dako) and glial fibrillary acidic protein (GFAP) (Dako) were applied according standardized protocols.

Haematoxylin & eosin was used as the standard stain for general neuropathological assessment of the cytoarchitecture, cellularity, composition and integrity of neuroanatomical structures represented on the sections; it also visualized cells with acute and chronic ischaemic damage and infarcts. Luxol fast blue combined with Nissl staining (LFB/Nissl) showed the cellular pattern and, along with the Loyez stain, highlighted myelin loss in areas of white matter rarefaction and infarcts. Bielschowsky’s silver impregnation was applied to assess neuritic plaques and neurofibrillary tangles for ‘CERAD’ plaque, and ‘Braak & Braak’ neurofibrillary tangle staging, respectively. The density of plaques and tangles were determined by semi-quantitative scoring as described in the staging protocols. The respective antibodies were applied to label hyperphosphorylated tau, β-amyloid, α-synuclein, ubiquitin and GFAP to ascertain the neuropathological diagnosis, and exclude diseases other than Alzheimer’s disease, vascular dementia and stroke.

Operationalized clinical diagnosis of vascular dementia was made according to the NINDS-ARIEN criteria (Roman et al., 1993). Patients with a stroke according to World Health Organisation criteria (1988), who did not meet NINDS-ARIEN criteria for vascular dementia or DSM IIIIR criteria for dementia were diagnosed as SND. Pathological diagnosis of vascular dementia was defined by the presence of multiple or cystic infarcts involving both cortical and sub-cortical structures, border-zone infarcts, lacunae (<15 mm), micro-infarcts (visible by microscopy only) and small vessel disease in sub-cortical structures in the general absence of neurofibrillary tangles (Kalaria et al., 2004). Clinical evidence of dementia along with the same type or a combination of these lesions at three different coronal levels was considered diagnostic for vascular dementia (Gold et al., 2002). None of the ‘pure’ vascular dementia cases exhibited tangle burden above Braak & Braak stage III (Braak and Braak, 1995) or more than ‘sparse’ neuritic plaques as defined by CERAD (Mirra et al., 1991). Concurrent neuropathological features of Alzheimer’s disease were characterized by the presence of neuritic plaques in cortical lobes, midbrain and hippocampal formation according to the CERAD criteria. Tangle burden was graded according to the method of Braak and Braak (1995) and neuritic plaque density following the CERAD criteria. Patients were designated as mixed vascular dementia/Alzheimer’s disease if they met criteria for vascular dementia and had either a Braak & Braak stage above III or were classified as CERAD probable or definite Alzheimer’s disease. Control subjects had no evidence of dementia as determined by retrospective assessments with absence of neurological or psychiatric disorder and confirmed by neuropathological examination.

### Tissue pH determination

Samples of ~50 mg of brain tissue from the grey matter of BA 9 were homogenized in 1 ml distilled water using a glass-Teflon homogenizer (Jencons, UK). pH values were measured at room temperature (20°C) in 1.5 ml centrifuge tubes using an Orion 3-Star Benchtop pH meter fitted with an Orion PerpHecT Ross microcombination pH micro-electrode (Thermo Electron Corp., USA).

### Tissue homogenate preparation

For each sample, grey tissue matter was dissected from white matter and ~500 mg grey matter was homogenized in 10 volumes ice-cold phosphate buffer (pH 7.4). The homogenates were transferred to centrifuge tubes and centrifuged at 29 000×g for 20 min at 4°C using a Beckman Allegra 64R centrifuge and Beckman F0850 rotor. The supernatant was discarded and the pellet was washed by re-suspension in 50 volumes ice-cold 50 mM Tris–HCl, pH 7.4 using an Ultra-Turrax T25 homogenizer (IKA Laboratories) and centrifugation using the same conditions as detailed above. This wash step was repeated twice more before the pellet was finally re-suspended in 50 mM Tris–HCl, pH 7.4, aliquoted and stored at ~70°C. Prior to use in the binding assay, aliquots were thawed and washed a further time in assay buffer using the above procedure before being re-suspended using an Ultra-Turrax T25 homogenizer in an appropriate volume of assay buffer.

### Table 1 Patient demographics showing the mean ± SEM (number of determinants in brackets) of age at death, post-mortem delay and tissue pH and median with inter-quartile range (IQR) of CERAD score and Braak stage

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Infarct VaD</th>
<th>SIVaD</th>
<th>Mixed VaD/AD</th>
<th>SND</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td>14M, 11F</td>
<td>3M, 5F</td>
<td>9M, 2F</td>
<td>11M, 18F</td>
<td>5M, 3F</td>
</tr>
<tr>
<td>Age at death (years)</td>
<td>75.68 ± 2.29 (25)</td>
<td>84.88 ± 3.69 (8)</td>
<td>80.00 ± 4.58 (11)</td>
<td>85.21 ± 1.12 (29)</td>
<td>81.00 ± 0.85 (8)</td>
</tr>
<tr>
<td>Post-mortem delay (h)</td>
<td>38.96 ± 5.21 (24)</td>
<td>47.00 ± 8.52 (8)</td>
<td>55.40 ± 10.34 (10)</td>
<td>38.62 ± 4.78 (29)</td>
<td>30.88 ± 4.12 (8)</td>
</tr>
<tr>
<td>Tissue pH</td>
<td>6.35 ± 0.07 (21)</td>
<td>6.31 ± 0.10 (8)</td>
<td>6.19 ± 0.05 (11)</td>
<td>6.28 ± 0.05 (27)</td>
<td>6.30 ± 0.08 (8)</td>
</tr>
<tr>
<td>CERAD (median, IQR, n)</td>
<td>No plaques (no plaques-sparse, 8)</td>
<td>Sparse (sparse-sparse, 10)</td>
<td>Moderate (moderate-frequent, 27)</td>
<td>None (none-sparse, 8)</td>
<td></td>
</tr>
<tr>
<td>Braak (median, IQR, n)</td>
<td>1 (0–2, 8)</td>
<td>2 (1–2, 10)</td>
<td>5 (4–6, 27)</td>
<td>2 (1–2, 8)</td>
<td></td>
</tr>
</tbody>
</table>

* The mean age of the mixed VaD/AD group was significantly higher (*P*<0.01) than that of the control group. There were no significant differences in post-mortem delay or tissue pH in any patient group compared with control. All comparisons between groups were conducted using one-way ANOVA and Dunnett’s post hoc test to compare patient groups with disease groups.

VaD = vascular dementia; AD = Alzheimer’s disease.
5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor saturation binding

Brain homogenate (20–60 μg) was incubated with increasing concentrations of radioligand [0.01–3 nM (3H)-WAY 100635 for 5-HT<sub>1A</sub> receptors, or 0.3–3.5 nM (3H)-ketanserin for 5-HT<sub>2A</sub> receptors] in 50 mM Tris–HCl, pH 7.4 buffer for 1 h at 25°C [(3H)-WAY 100635] or 37°C [(3H)-ketanserin]. Prazosin of 20 nM was included in each tube in the 5-HT<sub>2A</sub> assays to prevent (3H)-ketanserin binding to α<sub>1</sub>-adrenoceptors. Non-specific binding was defined by 1 μM pindolol for 5-HT<sub>1A</sub> receptors or 5 μM mianserin for 5-HT<sub>2A</sub> receptors. All total and non-specific binding was done in triplicate for each sample. Pindolol was selected as it possesses a nanomolar affinity for the 5-HT<sub>1A</sub> receptor and it is an antagonist that demonstrates some partial agonist activity (Milligan et al., 2001). The protein concentration added to each tube was later determined using a Bio-Rad DC protein assay (Bio-Rad, UK).

Following incubation, bound radioligand was separated from free by rapid vacuum filtration using a cell harvester (Skatron, Norway) onto Whatman GF/B glass fibre filters (Whatman, UK) pre-soaked in 0.1% (v/v) polyethylenimine. Filters were placed into vials and 3 ml Optiphase Hisafe 2 scintillation fluid (Perkin-Elmer, UK) was added. Following over-night equilibration, bound radioactivity was quantified in a Wallac Wisspectral 1414 liquid scintillation counter.

Radioligands and materials

(3H)-WAY 100635 (74 Ci/mmol) was purchased from GE Healthcare (UK) and (3H)-ketanserin (67 Ci/mmol) was purchased from Perkin Elmer (UK). Pindolol and mianserin were purchased from Tocris (UK). All other materials and chemicals were purchased from either Sigma (UK) or VWR (UK).

Data analysis

Saturation isotherms were fitted to a one site binding model described by the equation \( Y = B_{\text{max}} X/(K_d + X) \) using Graphpad Prism version 4.03 software (San Diego, USA). Statistically significant differences in 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor binding parameters in the frontal and temporal cortices were determined using a one-way ANOVA followed by Dunnett’s post hoc test to compare disease groups with the age-matched control group. The clinical relevance of alterations in 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> binding was examined by undertaking correlations of receptor binding and MMSE score, total CAMCOG score and CAMCOG memory score using Spearman’s non-parametric correlations. Additional evaluations were undertaken to examine the impact of potential confounding factors. All statistical tests were conducted using SPSS version 15.0 software (Chicago, USA).

Results

5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor binding

Both the binding of (3H)-WAY 100635 to 5-HT<sub>1A</sub> receptors and (3H)-ketanserin to 5-HT<sub>2A</sub> receptors best fit a one-site binding model. Examples of saturation isotherms for each ligand are shown in Fig. 1A and B.

Serotonin receptor binding: specific comparison of MIVaD and SIVaD with controls

There were significant increases in the maximal binding (\( B_{\text{max}} \)) of (3H)-WAY 100635 binding to 5-HT<sub>1A</sub> receptors (\( P<0.01 \)) and (3H)-ketanserin binding to 5-HT<sub>2A</sub> receptors (\( P<0.05 \)) in the MIVaD group compared with controls (Fig. 2A and B). The \( B_{\text{max}} \) of (3H)-WAY 100635 or (3H)-ketanserin in the SIVaD group were not significantly different from controls (Fig. 2A and B).

Serotonin receptor binding in mixed VaD/AD, SND and controls

Temporal cortex (BA 20/21)

There was a significant increase (\( P<0.01 \)) in the maximal binding of both (3H)-WAY 100635 and (3H)-ketanserin binding in the SND group compared to the control group in BA 20/21 (Fig. 2A and B). No significant differences were found in the maximal binding of either (3H)-WAY 100635 or (3H)-ketanserin binding in the mixed vascular dementia/Alzheimer’s disease group compared to control in BA 20/21 (Fig. 2A and B). As the mixed vascular dementia/Alzheimer’s disease group were significantly older than the controls, additional analyses were undertaken to examine the
potential impact of age on the results. There was no correlation of either maximal binding for 5-HT\(_{1A}\) or maximal binding for 5-HT\(_{2A}\) with age at death (\(P>0.05\)), furthermore, when age was included as a covariate in ANOVA age was not a significant influence on the results (5-HT\(_{1A}\), \(F=1.33, P=0.253\); 5-HT\(_{2A}\), \(F=0.075, P=0.786\)).

When combining the specific vascular dementia subtypes as a generic vascular dementia group there were no significant differences between this ‘pooled group’ and controls (data not shown).

There were no significant changes in the affinity (\(K_d\)) of either (3H)-WAY100635 or (3H)-ketanserin between the control group and any of the disease groups in the temporal cortex (Table 2).

**Frontal cortex (BA 9)**

There were no significant changes in either the affinity (\(K_d\)) (Table 2) or \(B_{max}\) (Fig. 3A and B) of either (3H)-WAY 100635 binding to 5-HT\(_{1A}\) receptors or (3H)-ketanserin binding to 5-HT\(_{2A}\) receptors between the control group and any of the disease groups in the frontal cortex.

**Relationship of serotonin receptor binding and cognition**

MMSE and CAMCOG scores were available for 24 and 22 participants, respectively. The \(B_{max}\) of (3H)-WAY 100635 binding to 5-HT\(_{1A}\) receptors in the temporal cortex was significantly correlated with higher MMSE score (Spearman correlation co-efficient (\(R_s\)) = 0.614, \(P=0.001, n=24\), Fig. 4A) and a higher total CAMCOG score (\(R_s=0.654, P=0.001, n=22\) Fig. 4B). In addition, there was a specific correlation of 5-HT\(_{1A}\) receptor binding in the temporal cortex with a higher CAMCOG memory score (\(R_s=0.641, P=0.001, n=22\) Fig. 4C). There were no significant correlations of 5-HT\(_{2A}\) receptor binding with cognition in either the frontal or temporal cortices.

**Discussion**

The key finding of the current study is the significantly increased binding capacity of 5-HT\(_{1A}\) and 5-HT\(_{2A}\) receptors in the temporal cortex of patients with MIVaD or SND in comparison to controls.

**Table 2** Equilibrium dissociation constants (\(K_d\)) of [3H]-WAY 100635 and [3H]-ketanserin binding to 5-HT\(_{1A}\) and 5-HT\(_{2A}\) receptors, respectively in the temporal (BA 20) and frontal (BA 9) cortices

<table>
<thead>
<tr>
<th>BA 20/21</th>
<th>BA 9</th>
</tr>
</thead>
<tbody>
<tr>
<td>5-HT(_{1A}) (3H)-WAY 100635</td>
<td>5-HT(_{2A}) (3H)-Ketanserin</td>
</tr>
<tr>
<td>Control</td>
<td>0.096 ± 0.010 (16)</td>
</tr>
<tr>
<td>Infarct VaD</td>
<td>0.083 ± 0.008 (8)</td>
</tr>
<tr>
<td>SIVaD</td>
<td>0.073 ± 0.008 (10)</td>
</tr>
<tr>
<td>Mixed VaD/AD</td>
<td>0.099 ± 0.010 (25)</td>
</tr>
<tr>
<td>SND</td>
<td>0.100 ± 0.017 (7)</td>
</tr>
</tbody>
</table>

Values shown in nano molaras mean ± SEM (number of determinants shown in brackets). There was no significant difference between the control group and any disease group in either the temporal or frontal cortices for binding to 5-HT\(_{1A}\) or 5-HT\(_{2A}\) receptors.
There was also a highly significant positive correlation between 5-HT1A binding and preserved global cognitive function and memory. There was no evidence of increased 5-HT1A or 5-HT2A binding in patients with SIVaD or mixed vascular dementia/Alzheimer’s disease. This highlights a specific change in 5-HT binding after large artery territory infarction, but not following other major types of cerebrovascular morphological abnormalities. The findings also raise the exciting possibility that upregulation of 5-HT1A receptors may be associated with preserved cognitive function and may therefore provide a novel treatment target in these patients.

The only previous study examining serotonergic receptors in vascular dementia reported no changes in binding to 5-HT1A, 5-HT2A receptors or the serotonin transporter protein (SERT) in either the frontal or temporal cortices in comparison with controls (Hansson et al., 1996). The study, however, included both cases with large artery territory infarction and SIVaD in the vascular dementia group. Consistent with this observation, if in the current study the MIVaD and SIVaD groups are combined, the significant differences in 5-HT1A and 5-HT2A receptor binding from controls are lost.

Figure 3  \(B_{\text{max}}\) (mean denoted by line) in the frontal cortex BA 9 of (A) \(^{3}\text{H}\)-WAY 100635 binding to 5-HT1A receptors (control \(n = 22\), MIVaD \(n = 6\), SIVaD \(n = 10\), SND \(n = 8\), mixed vascular dementia/Alzheimer’s disease \(n = 25\)), and (B) \(^{3}\text{H}\)-ketanserin binding to 5-HT2A receptors (control \(n = 21\), MIVaD \(n = 6\), SIVaD \(n = 10\), SND \(n = 8\), mixed vascular dementia/Alzheimer’s disease \(n = 24\)).

Figure 4 Scatter plots demonstrating the correlation of \(^{3}\text{H}\)-WAY 100635 binding to 5-HT1A receptors in BA 20/21 and (A) MMSE score \((R_s = 0.614, P = 0.001, n = 24\)), (B) CAMCOG score \((R_s = 0.654, P = 0.001, n = 22\)) and (C) CAMCOG combined memory score \((R_s = 0.641, P = 0.001, n = 22\)).
The finding of increased 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptor binding following large artery territory infarction is novel and the mechanism has not yet been determined. One potential mechanism would involve upregulation of these receptors in response to reduced serotonin concentrations. The lack of a suitable pre-synaptic marker in the present study represents a limitation and further work addressing this hypothesis is indicated. In the present study, antagonist radioligands were used as an index of the total number of receptors in a given sample. It could be argued that the balance of high- and low-affinity receptors would require both an agonist and an antagonist. A future study might usefully address this point.

The current study has demonstrated a positive correlation between 5-HT<sub>1A</sub> receptor binding in the temporal cortex and preserved cognition as defined by the MMSE score, CAMCOG total score and the memory score of the CAMCOG. These data are consistent with previous experimental studies indicating that the 5-HT<sub>1A</sub> receptor plays a role in the ability to form new memories (Bert et al., 2008; Ogren et al., 2008). We, therefore, interpret the current findings as indicating that the up-regulation of 5-HT<sub>1A</sub> receptor binding in the temporal cortex is likely to have a ‘pro-cognitive’ effect, although clearly not of sufficient magnitude to prevent the development of dementia. Nevertheless, the activity of 5-HT at the 5-HT<sub>1A</sub> receptor may be a novel and exciting therapeutic target to improve cognition in people with large artery territory infarction in the absence of dementia and those with MIVaD. The results of the present study suggest that 5-HT<sub>1A</sub> receptor agonism is likely to be the preferred treatment approach and there is emerging evidence from animal studies that 5-HT<sub>1A</sub> receptor agonism has a neuroprotective effect against transient global cerebral ischaemia in the gerbil hippocampus by preventing phosphorylation of the NR1 NMDA receptor subunit and increasing the expression of brain-derived neurotrophic factor (BDNF) (Salazar-Colocho et al., 2008a,b). However, it should be noted that the 5-HT<sub>1A</sub> receptor antagonist lecozotan (SRA-333), has also been shown to enhance cognition in primates and is now being tested in people with Alzheimer’s disease (Schechter et al., 2005; Raje et al., 2008). Further work is needed to directly examine the impact of 5-HT<sub>1A</sub> agonism and antagonism upon receptor upregulation and to quantify any consequent benefits on cognition following large artery territory infarction.

While similar in magnitude to that of 5-HT<sub>2A</sub>, the clinical significance of the increase in 5-HT<sub>2A</sub> receptors in the temporal cortex is difficult to interpret as the increased binding of 5-HT<sub>2A</sub> receptors in the temporal cortex was not correlated with any cognitive benefits in the current study. 5-HT<sub>2A</sub> Receptors are generally excitatory on target cells via activation of phospholipase C and are located on both pyramidal neurones and inter-neurones in the cerebral cortex (Burnet et al., 1995). Previous studies have reported increases in 5-HT<sub>2A</sub> receptors in both schizophrenia and people who have committed suicide, in frontal, but not temporal, brain regions (Arango et al., 1995; Burnet et al., 1996). In this context, it will be important to determine whether upregulation of 5-HT<sub>1A</sub> or 5-HT<sub>2A</sub> receptors is associated with key non-cognitive symptoms such as depression, anxiety and psychosis after large artery territory infarction. Vascular depression may be of particular interest given the frequency after stroke, the reduced responsiveness to antidepressants in these individuals and the overlap with subtle cognitive deficits (Roman, 2006).

In summary, we have identified an increased binding of both 5-HT<sub>1A</sub> and 5-HT<sub>2A</sub> receptors in the temporal cortex related to infarct injury. We have also demonstrated a correlation of 5-HT<sub>1A</sub> receptor binding with cognitive function. This study has demonstrated a difference in the neurochemistry of MIVaD and SIVaD, which may be an important determinant of treatment response in these patients. The results suggest that pharmacological manipulation of the serotonergic system offers the possibility to develop novel therapies for large artery territory infarction with and without dementia.

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Serotonergic receptors in vascular dementia


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