Citation for published version (APA):
Repeated withdrawal from alcohol is clinically associated with progressive cognitive impairment. Microglial activation occurring during pre-clinical models of alcohol withdrawal is associated with learning deficits. We investigated whether there was microglial activation in recently detoxified alcohol-dependent patients (ADPs), using \(^{[11}C\)PBR28 positron emission tomography (PET), selective for the 18kDa translocator protein (TSPO) highly expressed in activated microglia and astrocytes. We investigated the relationship between microglial activation and cognitive performance. Twenty healthy control (HC) subjects (45 ± 13; M:F 14:6) and nine ADP (45 ± 6; M:F 9:0) were evaluated. Dynamic PET data were acquired for 90 min following an injection of 331 ± 15 MBq \(^{[11}C\)PBR28. Regional volumes of distribution (\(V_T\)) for regions of interest (ROIs) identified \(a\) \(p\riori\) were estimated using a two-tissue compartmental model with metabolite-corrected arterial plasma input function. ADP had an ~ 20% lower \(^{[11}C\)PBR28 \(V_T\) in the hippocampus (F(1,24) 5.694; \(P = 0.025\)), but no difference in \(V_T\) in other ROIs. Hippocampal \(^{[11}C\)PBR28 \(V_T\) was positively correlated with verbal memory performance in a combined group of HC and ADP (\(r = 0.720\), \(P < 0.001\)), an effect seen in HC alone (\(r = 0.738\); \(P = 0.001\)) but not in ADP. We did not find evidence for increased microglial activation in ADP, as seen pre-clinically. Instead, our findings suggest lower glial density or an altered activation state with lower TSPO expression. The correlation between verbal memory and \(^{[11}C\)PBR28 \(V_T\), raises the possibility that abnormalities of glial function may contribute to cognitive impairment in ADP.

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INTRODUCTION

Alcohol dependence can lead to cognitive impairment. Severe forms of alcohol-related brain damage are well recognized, but less often acknowledged is that even apparently functioning alcohol-dependent patients (ADPs) suffer from a range of cognitive deficits.\(^1,2\) These include problems with memory and executive function that persists for months.\(^2\) Though there is improvement after one year of abstinence, these deficits affect the ability of patients to engage with after-care and to remain abstinent.\(^3-6\)

Multiple causal factors contribute to alcohol-related brain damage including nutritional deficiencies, head injury and cirrhosis (reviewed in ref. \(7\)), but cognitive impairment in this population can also occur in their absence.\(^1\) The mechanisms responsible for this cognitive impairment are not understood. Preventative interventions are limited to thiamine supplementation and relapse prevention. Each medicated detoxification is associated with progressively worsening withdrawal symptoms and progressive cognitive impairment.\(^8,9\) Detoxification is thus both a cause for clinical concern, and an important opportunity for intervention.

Microglia are specialized cells of macrophage lineage, highly responsive to their local environment.\(^10\) They become ‘activated’, a histological term describing the retraction of ramifications and assumption of an amoeboid shape, in response to many stimuli, including tissue damage. In the healthy brain, the microglial activation varies regionally. Microglia in the hippocampus express genes that suggest pro-inflammatory priming, even in the absence of disease.\(^12\)

Several functional states of activation are recognized, and two specifically named: an ‘M1’ neurotoxic phenotype that produces pro-inflammatory cytokines, reactive oxygen species\(^13\) and excitotoxins;\(^14\) and a neurotrophic ‘M2’ phenotype which secretes anti-inflammatory cytokines, nerve growth factors and clears debris via phagocytosis.\(^15,16\) It is hypothesized that further functional types of activated microglia exist\(^16\) and that the type of activation is contingent both upon the microenvironment,\(^17\) neuronal regulation\(^17,18\) and systemic inflammation.\(^18\) Either overactivity of the M1 phenotype or suppression of the M2 phenotype could be associated with exacerbation of an acute or chronic neural insult.

Pre-clinical models of alcohol dependence demonstrate microglial activation and expression of inflammatory mediators such as tumor necrosis factor-\(\alpha\) (TNF\(\alpha\)) and interleukin-6 (IL-6) both in the brain and peripheral blood during alcohol withdrawal.\(^19,20\) These changes are associated with neuronal death and learning deficits.\(^19\) There is evidence that similar processes may occur in humans. An increased density of microglia and increased expression of CCL2, an inflammatory chemokine, are found in the anterior cingulate cortex, midbrain and amygdala of ADPs postmortem.\(^21\) Pro-inflammatory cytokines including IL-6 and IL-10 are elevated in plasma (reviewed in ref. \(22\)) and increased CCL2 is found in the cerebrospinal fluid\(^23\) in ADP during withdrawal.
Pre-clinically, hippocampal and piriform cortical microglial activation and proliferation are found during alcohol withdrawal\textsuperscript{19,24,25} and can persist for up to 3 weeks.\textsuperscript{19,26} Microglial activation also is found in the cerebral cortex and cerebellum with chronic alcohol administration in pre-clinical models, and further increased after withdrawal.\textsuperscript{24}

Glia cells, including microglia, can be detected \textit{in vivo} in humans using positron emission tomography (PET) radioligands binding to the 18 kDa mitochondrial translocator protein (TSPO), which is highly expressed in activated glia. Although increased TSPO expression is associated with histological cell changes consistent with activation, it does not distinguish between different activation functions, that is M1 or M2 activation. TSPO is also expressed in other central nervous system cell types, including activated astrocytes, endothelium and blood components such as acute phase proteins.\textsuperscript{27–29} Increased TSPO expression has been demonstrated pre-clinically using \textsuperscript{\[11\]C}PK11195 autoradiography in chronic alcohol intake\textsuperscript{24} and withdrawal models.\textsuperscript{19,26} \textsuperscript{\[11\]C}PK11195 binding was increased in a small clinical cohort with hepatic encephalopathy,\textsuperscript{30} three members of which had a history of alcohol dependence, though they were long abstinent. Activated microglia may therefore represent a target for intervention around the time of alcohol withdrawal.

The aim of this study was therefore to investigate whether increased TSPO radioligand binding suggesting microglial activation is found in ADP who had recently undergone medically assisted withdrawal. A secondary objective was to explore the relationship between this measure of microglial activation and cognitive function. We hypothesized that increased \textsuperscript{\[11\]C}PBR28 PET signal (measure as a volume of distribution, \(V_t\)) would be increased in the brains of ADP particularly in those regions associated with microglial activation in pre-clinical or postmortem samples (cerebellum, hippocampus, midbrain, thalamus and cingulate cortex).\textsuperscript{19,21,24,25} Given that microglial activation has been associated with neuronal damage in pre-clinical models of alcohol withdrawal,\textsuperscript{19} we hypothesized that there would be a negative correlation between TSPO expression and performance on tests of verbal and spatial memory and executive function, which are impaired in alcohol dependence.\textsuperscript{1}

\textbf{MATERIALS AND METHODS}

ADP (DSM-IV) within 1 month of medically assisted withdrawal were recruited through local addiction services, healthy control participants (HC) via local volunteer databases, and HC data obtained from an concurrent study running on the same scanner.\textsuperscript{32} Newly abstinent ADP rather than actively drinking ADP were chosen for inclusion for several reasons. First, the pre-clinical literature predominantly reported increased TSPO expression or microglial activation during and after alcohol withdrawal. Second, scanning actively drinking ADP of the severity encountered in local clinical services is technically challenging, as alcohol intoxication affects PET tracer delivery and the patients are likely to enter withdrawal during scanning, causing tremor, and potentially vomiting and seizures. We scanned people following completion of withdrawal when they were no longer tremulous, no longer taking benzodiazepines and able to tolerate study procedures.

Most ADP had undergone medically assisted detoxification with chlordiazepoxide (\(n = 7\)) or diazepam (\(n = 1\)) before the study. Although none were still taking benzodiazepines at the time of scanning (mean duration since last dose 14 days (range 6–29 days)), three had a positive urine screen for benzodiazepines, reflecting the long metabolite half-life. As metabolites of chlordiazepoxide and diazepam do not bind the TSPO, this was not a concern.\textsuperscript{33} All were prescribed thiamine and vitamin B complex tablets, three acamprosate and one disulfiram.

Exclusion criteria applying to both groups included major physical or psychiatric illness as assessed by the Mini-International Psychiatric Interview (M-IDN).\textsuperscript{\textsuperscript{34}} apart from a history of depression or anxiety, 


greater than \(150 \text{U} \text{L}^{-1}\). The participants were required to produce a negative alcohol breath test on the day of the scan and the alcohol-dependent group to score less than 10 on the Clinical Assessment of Withdrawal from Alcohol scale.\textsuperscript{36} Dependence on other drugs, apart from tobacco, was an exclusion criterion for both groups, but recreational use was allowed in ADP. All the participants gave informed consent about data sharing and both studies received approval from local NHS Research Ethics Committees and ARSAC. See Supporting Information for a detailed description of all procedures.

\textbf{Participants and Methods.} The participants completed the Spielberger Trait Anxiety Score, Spielberger State Anxiety Score, Beck Depression Inventory and the Fatigue Severity Scale.\textsuperscript{40} Those controls enrolled in the study investigating alcohol dependence also completed the Obsessive Compulsive Drinking Scale.\textsuperscript{43} Cognitive tests were completed on the day of scanning from a battery previously used by us\textsuperscript{41} including digit span, Trail Making Task A and B,\textsuperscript{44} the Rey-Osterrieth Figure (ROF)\textsuperscript{45} and Weschler Memory Scale paragraph version (WMS).\textsuperscript{40}

Plasma samples were taken at the start of the PET scan in the ADP group for analysis of diazepam, chlordiazepoxide and their common metabolite desmethyldiazepam, using high-performance liquid chromatography (threshold > 4 \(\mu\text{g mL}^{-1}\)) as the ADP group were recently prescribed benzodiazepines and some tested positive for benzodiazepines on urine drug screen. No controls tested positive for benzodiazepines. The serum samples were analysed in the University of Glasgow for 25 cytokines and chemokines via Luminex human multiplex, High-sensitivity C-reactive protein (0.3–500 mg dl\textsuperscript{-1}) was analysed by clinical biochemistry services at the Hammersmith Hospital, London, UK.

The participants were scanned as described previously.\textsuperscript{32} Briefly, each participant received a 90 min \textsuperscript{\[11\]C}PBR28 PET scan following a bolus injection of \textsuperscript{\[11\]C}PBR28 (HC: mean 330.4 MBq, range: 312.4–347.3 MBq; ADP: mean 328.9 MBq, range: 302.7–346.5 MBq), after which dynamic three-dimensional PET data were acquired over 90 min. Continuous arterial blood samples were collected every second from the radial artery for the first 15 min. Discrete blood samples were manually withdrawn at 5, 10, 15, 20, 25, 30, 40, 50, 60, 70, 80 and 90 min after scan start to facilitate measurement of whole-blood and plasma activity. The participants also received a high-resolution T1-weighted magnetic resonance imaging (MRI) scan in a Siemens Verio 3T scanner (Siemens Healthcare, Erlangen, Germany). A consultant neuroradiologist reviewed each MRI scan. Any subjects with clinically significant structural lesions were excluded from the analysis.

The PET images were reconstructed via filtered back projection with attenuation and scatter correction. Dynamic images were separated into 26 frames (\(8 \times 15\) s, \(3 \times 1\) min, \(5 \times 2\) min, \(5 \times 5\) min, \(5 \times 10\) min). A metabolite-corrected plasma input function was generated using a method described previously.\textsuperscript{34} The total plasma time activity curve was calculated by multiplying the whole-blood curve by plasma-over-blood ratio, and the parent fraction data were fitted to a sigmoid model:

\[
   f = \frac{1 - \frac{t^a}{t^a + 10^b}}{1 + c/(1 + c)}
\]

where \(t\) is time and \(a, b, c\) are fitted parameters. The fitted parent fraction profile was multiplied by the total plasma curve and then smoothed post-peak using a tri-exponential fit to derive the required parent plasma input function. A time delay correction was applied to account for delays between blood sample measurement and tomographic time of measurement.

For analysis, we used the PET data analysis and kinetic modelling toolkit, MIAKAT (www.miakat.org),\textsuperscript{37} which also uses software from SPMS (Wellcome Trust Centre for Neuroimaging) and FSL (FMrib, University of Oxford). The PET data were corrected for motion via frame-by-frame co-registration to each participant’s T1 MRI. Anatomical ROIs were delineated by the application of the CIC neuroanatomical atlas,\textsuperscript{46} warped to the participant’s structural MRI scan. The ROIs were applied to the PET data to derive regional time activity curves.

We used a two-tissue compartmental model using a metabolite-corrected input function, with blood volume fixed at 5%, applied to...
was included as a covariate as TSPO expression increases with age. 54
were assessed using unpaired two-tailed
difference between groups.53 Demographic differences between groups
of the relative novelty of the tracer and uncertainty about the anticipated
maximum of 8 mm.
for the movement of [11C]PBR28 from plasma to brain parenchyma and
included as a
where
modulated with the Jacobian determinants of the deformations52 and
structural MRI scans were compared using voxel-based
interaction, corrected for age and genotype, was undertaken. Two-tailed
partial correlations, accounting for age and genotype, were performed
in a secondary analysis. A mass univariate
and GGT signiﬁcant group differences in educational
attainment between ADP and HC. Most ADP had had at least one
previous detoxification (median 1 (range 0–7)) and were
moderately to severely dependent (Severity of Alcohol Dependence
Questionnaire: 29 ± 9). ADP scored higher on measures of
alcohol craving, depression and anxiety, and the Fatigue Severity
Scale. ADP performed less well on tests of verbal and spatial
cognition compared to HCs. Given that the ADP group were
moderately to severely dependent, it was determined that inclusion
of age and TSPO genotype in the model would be appropriate.

RESULTS
Clinical characteristics
The clinical characteristics of the study sample are shown in Table 1. There were no signiﬁcant group differences in educational
attainment between ADP and HC. Most ADP had had at least one
previous detoxiﬁcation (median 1 (range 0–7)) and were
moderately to severely dependent (Severity of Alcohol Dependence
Questionnaire: 29 ± 9). ADP scored higher on measures of
alcohol craving, depression and anxiety, and the Fatigue Severity
Scale. ADP performed less well on tests of verbal and spatial
memory, but there were no differences in performance on tests
related to executive function.

Mean high-sensitivity C-reactive protein was within normal
range in both the groups. Albumin was signiﬁcantly lower in ADP and
GGT signiﬁcantly higher, but other liver function tests were
within normal limits in both the groups (see Table 2). No diazepam
or chlordiazepoxide was detectable in any blood samples, but
desmethyldiazepam was detectable in three ADP (370 ± 148 ng).
There was no signiﬁcant difference (P > 0.05) in concentrations of
any of the cytokines measured (see Table 2).
Group differences in $[^1^C]^PBR28$ $V_T$

Hippocampal $[^1^C]^PBR28$ $V_T$ (corrected for the effects of genotype and age) was 19% lower in ADP relative to HC ($F(1,24) = 5.694; P = 0.025; \phi = 0.192$), while no significant effect of group or an interaction between group and age was found.

Microglial activation and cognitive performance

Table 2. Blood results in ADP and HC

<table>
<thead>
<tr>
<th></th>
<th>Alcohol-dependent</th>
<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Standard clinical blood tests</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Haemoglobin</td>
<td>14.8 ± 1.2</td>
<td>14.6 ± 1.2</td>
<td>0.731</td>
</tr>
<tr>
<td>Mean cell volume</td>
<td>96.1 ± 6.1</td>
<td>84.9 ± 13.6</td>
<td>0.007**</td>
</tr>
<tr>
<td>White cell count</td>
<td>9.3 ± 2.0</td>
<td>6.5 ± 1.3</td>
<td>0.002**</td>
</tr>
<tr>
<td>Bilirubin</td>
<td>8 ± 5</td>
<td>12 ± 8</td>
<td>0.161</td>
</tr>
<tr>
<td>Alanine transaminase</td>
<td>56 ± 20</td>
<td>26 ± 8</td>
<td>0.110</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>71 ± 50</td>
<td>68 ± 19</td>
<td>0.702</td>
</tr>
<tr>
<td>Gamma glutamyl transferase</td>
<td>134 (range 15–466)</td>
<td>30 ± 20</td>
<td>0.064</td>
</tr>
<tr>
<td>Albumin</td>
<td>40 ± 3</td>
<td>44 ± 3</td>
<td>0.004**</td>
</tr>
<tr>
<td>Adjusted partial thromboplastin time</td>
<td>26.6 ± 2.3</td>
<td>28 ± 2</td>
<td>0.086</td>
</tr>
<tr>
<td>C-reactive protein</td>
<td>3.0 ± 2.39</td>
<td>2.6 ± 3.63</td>
<td>0.775</td>
</tr>
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</table>

**Pro- and anti-inflammatory cytokines**

<table>
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<th>Control</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumour-necrosis factor $\alpha$</td>
<td>0 (0–225.29)</td>
<td>0 (0–859.03)</td>
<td>0.776</td>
</tr>
<tr>
<td>Interleukin 1$\beta$</td>
<td>0 (0–414.77)</td>
<td>0 (0–1288.16)</td>
<td>0.776</td>
</tr>
<tr>
<td>Interleukin 1 receptor antibody</td>
<td>0 (0–518.83)</td>
<td>0 (0–1696.38)</td>
<td>0.776</td>
</tr>
<tr>
<td>Interleukin 6</td>
<td>0 (0–285.06)</td>
<td>0 (0–902.75)</td>
<td>0.882</td>
</tr>
<tr>
<td>Interleukin 10</td>
<td>130.31 (60.68–537.95)</td>
<td>74.00 (42.50–1283.45)</td>
<td>0.412</td>
</tr>
<tr>
<td>GM-CSF</td>
<td>61.64 (26.99–387.18)</td>
<td>41.69 (11.77–1173.56)</td>
<td>0.370</td>
</tr>
</tbody>
</table>

**Type 1 interferons**

<table>
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</tr>
</thead>
<tbody>
<tr>
<td>Interferon $\alpha$</td>
<td>0 (0–305.73)</td>
<td>0 (0–902.75)</td>
<td>0.824</td>
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</table>

**Cytokines related to T-cell activation**

<table>
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</thead>
<tbody>
<tr>
<td>IL-2</td>
<td>0 (0–292.86)</td>
<td>0 (0–866.97)</td>
<td>0.710</td>
</tr>
<tr>
<td>IL-7</td>
<td>32.89 (23.84–64.53)</td>
<td>36.60 (17.88–359.04)</td>
<td>0.552</td>
</tr>
<tr>
<td>IL-15</td>
<td>0 (0–916.53)</td>
<td>0 (0–2933.43)</td>
<td>0.766</td>
</tr>
<tr>
<td>IL-2R</td>
<td>21.87 (2.28–227.93)</td>
<td>12.15 (4.84–595.21)</td>
<td>1.000</td>
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**Th1 cytokines**

<table>
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<tbody>
<tr>
<td>IL-12</td>
<td>10 019 (4770–46 685)</td>
<td>10 205 (8258–29 077)</td>
<td>0.412</td>
</tr>
<tr>
<td>IFN-7</td>
<td>0 (0–225.29)</td>
<td>0 (0–859.03)</td>
<td>0.766</td>
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**Th2 cytokines**

<table>
<thead>
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<tbody>
<tr>
<td>IL-5</td>
<td>5.06 (3.51–560.75)</td>
<td>5.93 (3.1–1667.44)</td>
<td>1.000</td>
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<tr>
<td>IL-13</td>
<td>39.53 (0–1357.48)</td>
<td>26.59 (0–4083.75)</td>
<td>0.766</td>
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**Th17 cytokines**

<table>
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</thead>
<tbody>
<tr>
<td>IL-17</td>
<td>22.89 (15.64–663.44)</td>
<td>19.39 (15.64–2174.24)</td>
<td>0.552</td>
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</tbody>
</table>

**Chemokines**

<table>
<thead>
<tr>
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<th>Alcohol-dependent</th>
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<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>CXCL-8 (IL-8)</td>
<td>20.16 (10.68–1439.79)</td>
<td>11.6 (2.91–2808.70)</td>
<td>0.131</td>
</tr>
<tr>
<td>CXCL-9 (MIG)</td>
<td>0 (0–1698.11)</td>
<td>0 (0–5066.90)</td>
<td>0.766</td>
</tr>
<tr>
<td>CXCL-10 (IP-10)</td>
<td>170.46 (60.82–1088.97)</td>
<td>82.56 (3133.54)</td>
<td>0.131</td>
</tr>
<tr>
<td>CCL-2 (MCP-1)</td>
<td>0 (0–196.47)</td>
<td>0 (0–663.28)</td>
<td>0.710</td>
</tr>
<tr>
<td>CCL-3 (MIP-1a)</td>
<td>0.29 (0.19–595.03)</td>
<td>0.29 (0.19–2204.86)</td>
<td>0.824</td>
</tr>
<tr>
<td>CCL-4 (MIP-1b)</td>
<td>855.78 (218.01–2023.38)</td>
<td>399.59 (160.48–3672.07)</td>
<td>0.201</td>
</tr>
<tr>
<td>CCL-5 (RANTES)</td>
<td>189.59 (48.06–637.74)</td>
<td>105.53 (43.23–608.39)</td>
<td>0.295</td>
</tr>
<tr>
<td>CCL-11 (Eotaxin)</td>
<td>19.39 (10.56–839.90)</td>
<td>22.11 (7.41–2334.10)</td>
<td>0.656</td>
</tr>
</tbody>
</table>

Abbreviations: ADP, alcohol-dependent patients; GM-CSF, gray matter-cerebrospinal fluid; HC, healthy controls; IFN, interferon; IL, interleukin. For parametrically distributed data, mean ± s.d. is displayed. For non-parametrically distributed data, median (range) is displayed. **P < 0.01.

There were no differences between the groups (HAB HC: 2219 ± 334.6; HAB ADP: 2523.8 ± 686.8; MAB HC: 2436 ± 551.9; MAB ADP: 2475 ± 928.5; P = 0.566) or in free fraction (HAB HC: 0.0247 ± 0.0161; HAB ADP: 0.0115 ± 0.0046; MAB HC: 0.0176 ± 0.0115; MAB ADP: 0.0191 ± 0.0054, P = 0.424).

The exploratory correlates of $[^1^C]^PBR28$ $V_T$ were no significant correlations between peripheral cytokine concentrations and $[^1^C]^PBR28$ $V_T$. Age and genotype were significantly associated with variation in $[^1^C]^PBR28$ $V_T$ in all
the brain regions tested (for example, in the hippocampus: genotype $F(1,24) = 8.190; P = 0.009$; age: $F(1,24) = 5.370; P = 0.029$). There was no patient group × genotype interaction ($P > 0.05$).

Voxel-based morphometry conducted using the MRI structural images to establish whether there was atrophy in ADP revealed no significant differences between the groups (family-wise error corrected $P > 0.05$; t-statistic threshold $= 6.01$). The peak t-statistics for the ROIs identified a priori were as follows: anterior cingulate cortex: 1.805; left hippocampus 1.680; right hippocampus: 1.447; midbrain 2.885; left thalamus: 2.031; right thalamus: 2.840; cerebellum: 2.312.

Hippocampal $[^{11}C]PBR28 V_T$ and cognitive performance measures

There was a positive correlation (after controlling for the effects of age and genotype) between hippocampal $V_T$ and both WMS and ROCF performance ($WMS: r = 0.720; P < 0.001$; age: $r(1,24) = 5.370; P = 0.029$). There was no patient group × genotype interaction ($P > 0.05$). $V_T$ was not associated with duration of abstinence in alcohol-dependent group in any ROI. Voxel-based morphometry conducted using the MRI structural images to establish whether there was atrophy in ADP revealed no significant differences between the groups (family-wise error corrected $P > 0.05$; t-statistic threshold $= 6.01$). The peak t-statistics for the ROIs identified a priori were as follows: anterior cingulate cortex: 1.805; left hippocampus 1.680; right hippocampus: 1.447; midbrain 2.885; left thalamus: 2.031; right thalamus: 2.840; cerebellum: 2.312.

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the distribution of HC in the combined analysis but the correlation with verbal memory was not significant in the ADP group alone ($r = 0.331; P = 0.468$). There were no associations between hippocampal $V_T$ and performance on digit span or the trail making task, nor any associations between the frontal cortex $V_T$ and performance on digit span or the trail making task.

**DISCUSSION**

To the best of our knowledge, this is the first study to report changes in $^{[11]}$C$^{2}$PBR28 $V_T$ in a cohort of otherwise healthy ADPs within the first few weeks of abstinence. Refuting our initial hypothesis, we found that $^{[11]}$C$^{2}$PBR28 $V_T$ is decreased in the hippocampus in recently abstinent ADPs relative to healthy participants with no significant changes in other brain ROIs identified a priori. In an exploratory analysis, we also found a positive relationship between hippocampal $^{[11]}$C$^{2}$PBR28 $V_T$ and verbal memory in the healthy participants. When the patient and healthy control group were combined, the correlation remained and a significant positive correlation was found between hippocampal $^{[11]}$C$^{2}$PBR28 $V_T$ and both verbal and spatial memory.

We hypothesized that $^{[11]}$C$^{2}$PBR28 binding would be increased in alcohol withdrawal, based on pre-clinical evidence. However, our findings of a decrease are more consistent with either a decrease in expression of the protein or a loss of cells expressing the protein. TSPO is a mitochondrial protein and would therefore be expected to decrease with a reduction of mitochondrial density. Slight decreases in mitochondrial enzymes and uncoupling with production of potentially damaging free radicals have been reported in a pre-clinical chronic alcohol model.

Alternatively, decreases in the activation state, or absolute numbers of several populations of cells could explain the reduction seen. First, loss, or altered activation, of microglia or astrocytes could explain the change. The one existing stereological human postmortem study of microglia and astrocytes in ADP, which included data on the hippocampus supports this as there was selective loss of both astrocytes and microglia.

Prior human magnetic resonance spectroscopic results showed no decreases in myo-inositol, a metabolic marker for activated astrocytes, in recently abstinent alcoholics making astrocytic decreases around the time of withdrawal less likely than microglial changes. Pre-clinically, microglial activation during alcohol withdrawal has been reported to be M2 activation, raising the possibility that loss of such activation may hamper repair. Second, lower $^{[11]}$C$^{2}$PBR28 binding could relate to suppressed neurogenesis, as TSPO is also expressed by hippocampal neural stem cells. Pre-clinical findings regarding the effect of alcohol on neurogenesis have yet to be replicated in human postmortem samples, which showed no differences in numbers of neural progenitors between ADP and HC in the subventricular zone and olfactory bulb. Absolute numbers of neural progenitors are low in adult humans, making this unlikely. Finally, TSPO is also expressed in the endothelial cells, so microvascular changes could contribute to our findings. Changes in the hippocampal capillary lumen diameter and decrease in the length and density has been described in at least one pre-clinical chronic alcohol model.

The difference between pre-clinical findings and the outcome of this clinical study may relate to differences between pre-clinical models of alcohol dependence and clinical populations. Pre-clinical studies are performed in adolescent rodents, and periods of heavy alcohol exposure are relatively short ($< 2$ weeks). For logistical reasons, we were not able to scan patients during the first week of withdrawal. Some pre-clinical models show only very short-lived microglial activation, but others show activation at 3 weeks of abstinence. There was no relationship between duration of abstinence and $^{[11]}$C$^{2}$PBR28 $V_T$ in our study, though it was not designed to investigate this. Of interest is that while pre-clinical studies in cocaine dependence have reported pro-inflammatory gene expression, clinical imaging has shown no difference in TSPO expression, suggesting there may be similar translational challenges across addictions.

Clinical populations in the United Kingdom are medicated with benzodiazepines during withdrawal. Suppression of microglial activation via GABA(A) transmission has been reported raising the possibility that microglial suppression by benzodiazepines explains the decrease. A direct effect of benzodiazepines binding the TSPO is however unlikely as only diazepam at high dose binds the TSPO and the only benzodiazepine present in the ADP plasma was the metabolite desmethylzolpidem, which does not bind the TSPO.

We found that hippocampal $^{[11]}$C$^{2}$PBR28 binding was positively associated with verbal memory in the sample considered as a whole and in healthy controls. Although ADPs clustered towards the lower end of the distribution, the relationship was not maintained in ADPs alone—probably because of small numbers, particularly small numbers of HABs, who have a higher signal-to-noise ratio. To detect a correlation of the strength of that seen in HC with 80% power, 11 HABs would have been needed.

The positive correlation between TSPO expression and memory could be attributed to the function of the protein itself or the cells which express it. TSPO over-expression has been found pre-clinically to protect against lipopolysaccharide induced memory dysfunction. The relationship between mitochondrial function, TSPO expression and memory function under healthy conditions has not been explored. Microglia and astrocytes are both involved in memory function in the healthy hippocampus: while microglia integrate neural progenitors into hippocampal circuits and undertake activity-related synaptic remodelling (reviewed in ref. 68), astrocytic release glutamate, adenosine triphosphate and cytokines helps to consolidate nascent synaptic connections. The positive correlation seen in healthy participants supports the importance of microglia and astrocytes, and perhaps TSPO itself, in the healthy functioning of the hippocampus.

Although we did not find a correlation in the ADPs alone, the finding of a positive relationship between memory performance and TSPO expression is intriguing. Verbal and spatial memory deficits, which relate to hippocampal function, are well described in alcohol dependence. We did not find brain volume changes in our small ADP sample, but larger volumetric studies have shown hippocampal atrophy in alcohol-dependent cohorts. No relationship between volume loss and memory has been demonstrated, raising the possibility that loss or dysfunction of cell subsets such as microglia or astrocytes is a more important determinant.

The moderately to severely dependent drinkers that we studied were challenging to recruit and retain as they were relatively unstable and at high risk of disengagement from treatment. Recruitment challenges affected both statistical power to detect the group differences and stringency of inclusion and exclusion criteria. The study is therefore affected by limitations mainly around size and design. Our patient sample is small, was scanned on average 3 weeks from cessation of alcohol, were receiving relapse prevention medications and were more likely to be smokers. The small sample meant we were unable to interrogate the possible contribution of these factors. Another limitation is that the majority of the patient group had undergone medicated detoxification, raising the possibility that changes are seen related to medications taken during detoxification or other non-specific effects.

We found a statistically significant decrease in $^{[11]}$C$^{2}$PBR28 $V_T$ in the hippocampus of ADPs shortly following alcohol withdrawal consistent with microglial or astrocytic loss or functional change, or changes in TSPO expression related to oxidative stress or mitochondrial pathology. This finding is supported by a previous postmortem study showing hippocampal microglial and astrocytic
loss in ADP. Hippocampal [11C]PBR28 V1, indicative of TSPO expression was positively correlated with performance on a delayed memory task, suggesting this may be of clinical relevance. This relates to pre-clinical research suggesting that microglial activation may be related to homeostatic functions rather than inflammatory functions in the hippocampus under healthy conditions. Binding was not higher in the alcohol-dependent participants, raising questions about microglial and mitochondrial function in this context and how this may translate into treatment targets. It remains to be established whether an increase in TSPO expression occurs in humans during chronic drinking, or in binge alcohol exposure, which more closely resembles animal models.

CONFLICT OF INTEREST

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