RESEARCH PAPER

NMDA receptor binding in focal epilepsies

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ABSTRACT

Objective To demonstrate altered N-methyl-D-aspartate (NMDA) receptor availability in patients with focal epilepsies using positron emission tomography (PET) and [18F]GE-179, a ligand that selectively binds to the open NMDA receptor ion channel, which is thought to be overactive in epilepsy.

Methods Eleven patients (median age 33 years, 6 males) with known frequent interictal epileptiform discharges had an [18F]GE-179 PET scan, in a cross-sectional study. MRI showed a focal lesion but discordant EEG changes in two, was non-localising with multifocal EEG abnormalities in two, and was normal in the remaining seven patients who all had multifocal EEG changes. Individual patient [18F]GE-179 volume-of-distribution (V1) images were compared between individual patients and a group of 10 healthy controls (47 years, 7 males) using Statistical Parametric Mapping.

Results Individual analyses revealed a single cluster of focal V1 increase in four patients; one with a single and one with multifocal MRI lesions, and two with normal MRIs. Post hoc analysis revealed that, relative to controls, patients not taking antidepressants had globally increased [18F]GE-179 V1 (±28%; p<0.002), and the three patients taking an antidepressant drug had globally reduced [18F]GE-179 V1 (−29%; p<0.002). There were no focal abnormalities common to the epilepsy group.

Conclusions In patients with focal epilepsies, we detected primarily global increases of [18F]GE-179 V1 consistent with increased NMDA channel activation, but reduced availability in those taking antidepressant drugs, consistent with a possible mode of action of this class of drugs. [18F]GE-179 PET showed focal accentuations of NMDA binding in 4 out of 11 patients, with difficult to localise and treat focal epilepsy.

INTRODUCTION

N-methyl-D-aspartate (NMDA) receptors are ligand-gated and voltage-gated ion channels that mediate fast excitatory neurotransmission in the central nervous system (CNS). NMDA receptor-mediated neurotransmission is necessary for cognition, memory and neuronal survival, but excessive NMDA receptor activation mediates excitotoxic neuronal injury following acute cerebral insults, is associated with cell death and is thought to contribute to disorders of neuronal hyperexcitability, such as epilepsy and neuropathic pain, and chronic neurodegenerative diseases, depression and schizophrenia. In chemical models, administration of agonists of either the NMDA or α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid (AMPA)/kainate receptors induces convulsions in vivo either by directly mediating an epileptic depolarisation through the NMDA calcium channels or by indirectly activating NMDA channels subsequent to AMPA/kainate receptor activation for review: ref. 12). Blockade of NMDA receptors is neuroprotective, prevents paroxysmal depolarisation shifts, which are the intracellular correlate of interictal epileptiform discharges (IEDs), and blocks the development of kindling. Several but not all kindling model studies have shown the presence of increased NMDA receptor availability in the hippocampus and cerebral cortex of epileptic animals. Autoradiography of human epileptogenic temporal lobe tissue has revealed increased NMDA receptor availability in the parahippocampal gyrus, in contrast to decreased availability in the hippocampi, particularly in sclerotic regions. In vitro studies in tissue dissected from patients with epilepsy have associated NMDA receptor-mediated neurotransmission with epileptic activity.

Receptor activation, however, can only be shown in vivo. Human microdialysis studies have revealed marked elevations in extracellular glutamate concentrations preceding and during seizures, which would be expected to result in increased NMDA receptor activation. Hence, there is interest in the development of radioligands that allow assessment of NMDA receptor function in humans in vivo.

We have previously observed good brain penetration, moderately heterogeneous distribution in grey matter and suitable rapid washout of the novel NMDA positron emission tomography (PET) tracer [18F]GE-179 in healthy controls. This ligand binds at the phencyclidine (PCP) recognition site and blocks the NMDA/kainate receptors induces convulsions in vivo either by directly mediating an epileptic depolarisation through the NMDA calcium channels or by indirectly activating NMDA channels subsequent to AMPA/kainate receptor activation for review: ref. 12). Blockade of NMDA receptors is neuroprotective, prevents paroxysmal depolarisation shifts, which are the intracellular correlate of interictal epileptiform discharges (IEDs), and blocks the development of kindling. Several but not all kindling model studies have shown the presence of increased NMDA receptor availability in the hippocampus and cerebral cortex of epileptic animals. Autoradiography of human epileptogenic temporal lobe tissue has revealed increased NMDA receptor availability in the parahippocampal gyrus, in contrast to decreased availability in the hippocampi, particularly in sclerotic regions. In vitro studies in tissue dissected from patients with epilepsy have associated NMDA receptor-mediated neurotransmission with epileptic activity.

We report the first use of [18F]GE-179 PET in focal epilepsies of different focal and multifocal onset. The objective of this proof-of-principle study was to demonstrate in vivo a hypothesised increased NMDA receptor activation in patients with drug-resistant epilepsy.

METHODS

The study was approved by the Research Ethics Committee of the Royal Marsden Hospital, Imperial College Healthcare NHS Trust and University College London Hospitals NHS
Epilepsy and control populations
This was a proof-of-principle, cross-sectional pilot study with targets of 12 participants per group. Eleven patients with refractory focal epilepsies (median age 33 years; range 20–50 years; 6 males) were recruited from the outpatient clinics at the National Hospital for Neurology and Neurosurgery. Demographics and clinical details are listed in Table 1. Their diagnoses were based on history, seizure semiology, prolonged and repeated interictal and ictal EEG recordings (where available), and MRI data. Patients were chosen who had frequent interictal spikes on previous EEG recordings, which we hypothesised would maximise our chances to detect increased binding to open NMDA receptors. None of the patients were taking an antiepileptic drug (AED) known to act at the NMDA receptor. Exclusion criteria included inability to provide informed consent, claustrophobia, standard MR contraindications, a positive urinary pregnancy test on the day of the PET scan and history of drug abuse. Patient 4, whose seizures consisted of a sustained fluctuation of perception of brightness with pupillary hippus, has been presented in a detailed case report.30

The control group, 9 of whom have been described previously38 comprised 10 healthy volunteers without history of neurological or psychiatric illness (median age 46 years; range 25–62 years; 7 males). Additional exclusion criteria were as described for the patients above. A further three seemingly healthy individuals were subsequently excluded; one due to excessive movement throughout the PET scan acquisition, one whose MRI revealed evidence of a cerebral infarct; and one who was discovered to have a history of benzodiazepine abuse. The original control group data were used, rather than repeat imaging; the patient data were acquired at approximately the same time as those of the control group (i.e. within 12 months) using the same imaging protocol.

Median age and body mass index (BMI) were compared between patients and control groups by Mann-Whitney U statistic in SPSS. Gender balance was compared between groups with the (Pearson) $\chi^2$ test.

MRI data acquisition
Three-dimensional volumetric T1-weighted coronal MRI sequences were acquired at Epilepsy Society (Chalfont St. Peter, UK), as previously described.38 MRIs were reviewed by an experienced neuroradiologist (CM). MRIs were not available for one control participant, in whom 3.0 T MRI was contraindicated.

EEG
All patients had an EEG during the PET scan using a Trackit 18/8 (Lifelines Limited, Hants, UK) ambulatory EEG recorder and an ECI E1 Cap (Electro-Cap International, Eaton, Ohio, USA) with 19 electrodes placed according to the International 10–20 system. An additional reference electrode (Fpz) was sited just anterior to Fz. The O1 and O2 electrodes were removed from the cap prior to scanning for several patients in order to minimise discomfort. The participants were closely observed for evidence of seizures throughout the scan. EEGs were reviewed by an experienced clinical neurophysiologist (RMP). The number of IEDs during the first 30 min of scan acquisition was quantified and correlated with $^{[18F]}$GE-179 global volume-of-distribution ($V_T$) using Spearman’s $r$ correlation coefficient.

PET image acquisition and data analysis
PET image acquisition has been described previously.38 Briefly, images were acquired using a Siemens/CTI ECAT EXACT3D 962 HR+PET camera (Siemens, Erlangen, Germany) at Hammersmith Imanet Limited. Each participant had a 90 min dynamic emission scan with a smooth bolus intravenous injection of median 187 MBq (range 173–192 MBq) $^{[18F]}$GE-179 administered 30 seconds after starting image acquisition. For calculation of continuous decay-corrected and metabolite-corrected parent plasma input functions, discrete arterial blood samples were taken throughout the scan, with continuous arterial blood sampling for the first 15 minutes.

The area under the metabolite model curves ($AUC_{\text{metabs}}$) was used as a measure of the rate of metabolism for each individual. The $AUC_{\text{metabs}}$ over $t=0–30$ minutes and $t=0–90.5$ minutes was compared between groups by multivariate general linear model (GLM), with gender as a fixed factor, and age and BMI as covariates. The residual sum of squares ($\text{RSS}_{\text{metabs}}$) for the metabolite model curve was compared between groups by univariate GLM. The threshold for statistical significance was $p=0.05$.

The $V_T$ of $^{[18F]}$GE-179 was computed at the voxel level for each participant by exponential spectral analysis, as described previously.38 Each participant’s $V_T$ image was spatially normalised using a scanner-specific template. To enable group-wise comparisons, images acquired from patients with an epileptogenic zone in the right hemisphere (3 patients) were left-right flipped prior to normalisation; three control participants’ images were also left-right flipped prior to group-wise comparison (controls 1, 4 and 6, selected at random). For individual (1 patient vs 10 controls) comparisons, the native (unflipped) patient and control images were used.

The primary outcome measure, global $V_R$, was computed as an overall mean over the entire matrix, thresholded at 1/8 of that value to create a brain mask, and averaged again within this mask. The global $V_T$ values were compared between groups using a univariate GLM, with gender as a fixed factor, and age and BMI as covariates.

In order to identify changes in the regional distribution of activated NMDA receptors, group-wise SPM8 analyses based on the smoothed (12 mm Full Width at Half Maximum (FWHM) isotropic Gaussian kernel), transformed parametric $V_T$ images, were performed, comparing patients with focal epilepsy against the controls. The images were compared on a voxel-by-voxel basis using a two-sample t test, assuming unequal variances, with global $V_T$ taken into account via an analysis of covariance (ANCOVA) by group. The images were grey and white matter (explicit) masked, with relative threshold masking at 0.4. We report differences in $^{[18F]}$GE-179 $V_T$ at $p<0.001$ (uncorrected), clusters at $p<0.05$ (uncorrected) and an extent threshold of 15 voxels.

In order to identify participant-specific changes in the regional distribution of activated NMDA receptors, individual SPM8 analyses based on the smoothed transformed parametric $V_T$ images were also performed for each of the patients with frequent IEDs against the 10 controls. Equal variances were assumed and global $V_T$ taken into account via an ANCOVA by group. The unflipped $V_T$ image for each control participant was compared with those of the nine other controls in an identical fashion.
Table 1  Patients with focal epilepsy and frequent IED—clinical details

| ID | Age/sex/handedness | Probable localisation MRI/EEG/MEG | Onset/ duration (years) | Postictal interval Treatment | Seizures | EEG | MEG | MRI | \[^{18}F\] FDG-PET | Global \[^{18}F\] GE-179 \(V\_\text{r}\) | Approximately N of observed IEDs (t=0–30 min) | \[^{18}F\]GE-179 \(V\_\text{r}\) increases | \[^{18}F\]GE-179 \(V\_\text{r}\) decreases |
|----|------------------|-----------------------------------|-------------------------|----------------------------|----------|-----|-----|-----|----------------|-----------------|-----------------------------------|----------------|----------------|----------------|
| 1  | 41/4/M/R         | L frontal                         | 14.5/26.5               | 6.0 h                      | CBZ, LEV, LTG, ZNS           | SPS, CPS, SGS | L frontotemporal and R temporal | NA             | R IFG lesion | L temporal | 6.16             | 62               | L frontal                  |
| 2  | 22/2/M/R         | L temporal                        | 4/18                    | 7.5 h                      | CBZ, LEV, LAC               | SPS, CPS, SGS | L frontotemporal and R temporal | NA             | L HS          | L hemisphere | 7.20             | 43               |                        |
| 3  | 38/M/L           | Multifocal MRI, EEG/MEG           | 2.5/36.5                | 45 min                     | CBZ, CLB, PHF, TPM, oxcarbazepine | SPS, CPS, SGS | R frontotemporal and R temporal | R parieto-occipital | Bilateral tubers: F, P, L-O, periventricular calc | L frontal | 8.16 | 0 |
| 4  | 28/F/R           | Multifocal MRI, EEG/MEG           | 10/18                   | 20.5 h                     | LEV, sertraline, amiodipine | SPS (pupillary hippus) | R temporoparietal and R temporal | L and R temporal | Bilateral tubers: L and R frontal, L temporal, R parieto-occipital, L occipital | R parietal | 4.53 | EEG data corrupted |
| 5  | 50/F/R           | MRI negative Multifocal EEG/MEG   | 11/39                   | 39 days                    | LEV, PH, levetiracetam      | CPS SGS | L=R temporal and R temporal | L and R temporal | Neg | L temporal | 5.32 | 26 | R frontal |
| 6  | 33/M/L           | MRI negative Unifocal EEG         | 19/14                   | NA                         | CLN, RUF, fluoxetine        | CPS | L temporal | L frontotemporal | Neg | Neg | L frontal; R frontal | 3.90 | 168 |
| 7  | 23/M/L           | MRI negative Unifocal EEG         | 16/7                    | 3 days                     | CBZ, VAL                    | SPS, SGS | R frontal | NA             | Neg | Neg | L frontal; R frontal | 7.72 | 86 | L frontal |
| 8  | 40/F/L           | MRI negative PET/MEG L Frontal    | 12.5/28                 | 11 days                    | CLB, LAC, OX, OX              | SGS | L=R frontal | L F             | Neg | L frontal | 8.40 | 42 | R frontal |
| 9  | 24/M/R           | MRI negative Multifocal EEG/MEG   | 7/17                    | 6.5 h                      | LAC, LEV, LTG, OX, CLB      | SPS, CPS, SGS | L fronto-occipital and R temporal | R fronto-occipital | L frontal | 8.44 | 9 |
| 10 | 50/F/R           | MRI negative Multifocal EEG/MEG   | 13/37                   | 10+years                   | LEV, LAC, LTG               | CPS | L=R temporal and R temporal | L and R T | Neg | NA | 8.17 | Cont epileptiform activity* |
| 11 | 20/F/R           | MRI negative Multifocal EEG       | 14/6                    | 39.5 h                     | CLB, OX, OX, OX              | CPS, SGS | R=L temporal | NA             | Neg | Neg | 8.88 | 3 |

Antidepressant drugs (patients 4–6) are displayed in bold font.

*EEG revealed continuous ongoing focal epileptiform activity in patient 10, who had not shown clinically evident seizure activity within the preceding 10 years. Underline indicates concordance between the cluster of increase and the location of the presumed epileptogenic zone, where known.

Calc, calcification; CBZ, carbamazepine; CLB, clozapine; CLN, clonazepam; CPS, complex partial seizures; EEG, electroencephalography; F, frontal lobe; F/M, female/male; \[^{18}F\]FDG-PET, \[^{18}F\]fluorodeoxyglucose positron emission tomography; HS, hippocampal sclerosis; ID, identifying number; IED, interictal epileptiform discharges; IFG, inferior frontal gyrus; IR, left/right; LAC, lacosamide; LEV, levetiracetam; LTG, lamotrigine; MEG, magnetoencephalography; MRI, magnetic resonance imaging; NA, not available; Neg, negative, that is, no significant findings; O, occipital; OX, oxcarbazepine; P, parietal lobe; PHF, phenytoin; RUF, rufinamide; SGS, secondary generalised seizures; SPS, simple partial seizures; TPM, topiramate; VAL, valproate; \(V\_\text{r}\), volume-of-distribution; ZNS, zonisamide.
RESULTS

There was no difference between patient and control group in terms of age (p=1.00), BMI (p=1.00) or gender mix (p=0.47).

There were no significant differences in the AUCmetabs or the RSSmetabs between the groups (p=0.19 and p=0.47, respectively). Age, BMI and gender also did not significantly influence the AUCmetabs (all p>0.09).

Global changes in VT

Global VT was higher in the focal epilepsy group (median 7.51, range 3.77–8.66) than in controls (median 6.21, range 5.37–7.56), although this did not reach statistical significance (p=0.40). There were two distinct subgroups of patients with higher (n=8) and lower (n=3) than normal global VT. To explore this distribution in patients further, post hoc analysis revealed large and significant differences in global VT between patients with focal epilepsy who were not taking antidepressant drugs (median 7.97, range 6.04–8.66; +28% relative to controls), and those who were taking antidepressant drugs (median 4.40, range 3.77–5.15; −29% relative to controls), and controls (6.21, range 5.37–7.56; all p<0.002; figure 1).

There were no differences in gender, age, AUCmetabs, BMI, number of IEDs as well as one, as was the second largest cluster of a further two patients.

DISCUSSION

Using [18F]GE-179 PET, we identified global increases in NMDA receptor ion channel availability for patients with focal epilepsies who were not taking antidepressants, whereas tracer binding was globally decreased in those patients with epilepsy who were also taking antidepressant drugs. Increases in [18F]GE-179 VT are consistent with an increase in activated/open NMDA receptors in actively discharging cortex as shown in preclinical work.21 22 We suggest that increased NMDA activation in patients with chronic focal epilepsy extends beyond the presumed epileptogenic zone and is a global phenomenon, as reflected in the increased global VT.

Our group analyses did not identify any focal redistribution of activated NMDA receptor availability in this group of heterogeneous patients with focal epilepsies arising from different cortical locations; this is perhaps not surprising given the heterogeneity and small sample size. For this pilot study, patients were selected based on the frequency of IEDs on previous EEGs, to maximise our chances of detecting increases in NMDA binding. Concordance of [18F]GE-179 foci with EEG, structural and functional imaging in individual patients is difficult to assess

Figure 1 [18F]GE-179 VT by subgroup. The top row (A) depicts the mean [18F]GE-179 VT patients with focal epilepsy who were taking an antidepressant drug; the middle row (B) for the controls; and the bottom row (C) for the patients with focal epilepsy who were not taking an antidepressant drug. Images are displayed according to radiological convention (ie, ‘left is right’). L, left; R, right; VT, volume-of-distribution.
given that some of our cohort had normal or EEG-discordant MRI and poorly defined epileptogenic zones: the epileptogenic zone could not be localised using high-resolution MRI, ictal EEG recordings, magnetoencephalography and fluoro-deoxyglucose PET (FDG-PET) in four patients, and could only be localised to one lobe in four of the remaining seven patients. In contrast, our individual [18F]GE-179 PET analysis identified focal clusters of increased NMDA receptor activation in four patients with focal epilepsy, and these were concordant with the location of the presumed epileptogenic zone in two of those three patients, in whom this could be localised to one lobe. Patient 1 was one of the best-localised cases in our cohort, having concordant scalp EEG, [18F]FDG-PET and ictal single-photon emission CT findings. An intracranial EEG recording over the left temporal lobe revealed a diffuse ictal onset, consistent with spread from a nearby lobe. Consistent with these data, a large cluster of increased VT was observed in the left frontal lobe for this participant.

Interictal regional cerebral blood flow (rCBF) studies usually show reduced perfusion in focal epilepsy, and significant increases in rCBF scans were only seen with prolonged discharges of 8–105 seconds after electrostimulation. Our findings are unlikely to reflect changes in cerebral blood flow (ie, altered [18F]GE-179 availability) as decreased perfusion in the epileptogenic zone would likely result in decreased [18F]GE-179 availability and thus VT. While NMDA receptor ion channel opening and the excitatory postsynaptic current are extremely rapid events, grey matter uptake and the metabolism of [18F]GE-179 occurs over minutes. Hence, we interpret the VT data as indicative of the integrated extent of NMDA receptor activation during the tracer uptake phase (first 30 min) of the scan.

The lack of focal increases in VT for 7 of the 11 patients with focal epilepsy did not appear to relate to the frequency of scalp-detected IEDs. Global [18F]GE-179 VT was not significantly correlated with the number of IEDs in the first 30 min following injection. The true extent of epileptic activity will not be detectable on scalp EEG, which may explain the lack of correlation between focal spike activity and global [18F]GE-179 uptake. Moreover, it would be oversimplistic to assume a linear relation between global [18F]GE-179 VT and IED frequency. We expect that NMDA receptors involved in generation of the IED would rapidly internalise or desensitise following their activation. Individual short IEDs would be very difficult to visualise over a 90 min PET scan with [18F]GE-179. We assume any observed increase of [18F]GE-179 binding to be related to underlying generalised baseline overactivity, rather than to transient NMDA channel opening over a few milliseconds.

In the other only in vivo PET study of NMDA ion channel activity in epilepsy, Kumlien et al did not detect focal increases in receptor availability using (S)-[N-methyl-13C]ketamine.

### Table 2 Focal decreases in [18F]GE-179 VT—individual patients versus 10 controls

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Probable localisation (EEG)</th>
<th>MRI</th>
<th>[18F]GE-179 VT decreases</th>
<th>Cluster size (mm3/voxels)</th>
<th>Peak voxel coordinates (x, y, z; mm)</th>
<th>Zmax</th>
<th>Cluster level p (uncorrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>Multifocal</td>
<td>R parietal</td>
<td>4684/608</td>
<td>62–32 38</td>
<td>3.62</td>
<td>0.002</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>R frontal</td>
<td>L temporal</td>
<td>3968/496</td>
<td>–58–20–22</td>
<td>4.32</td>
<td>0.003</td>
<td></td>
</tr>
</tbody>
</table>

The cluster(s) reaching significance at p<0.05 uncorrected are listed. The contribution of global VT to variance was removed by an ANCOVA by group. ANCOVA, analysis of covariance; EEG, electroencephalography; ID, identifying number; L/R, left/right; mm, millimeters; MRI, magnetic resonance imaging; VT, volume-of-distribution.

### Table 3 Focal increases in [18F]GE-179 VT—individual patients versus 10 controls

<table>
<thead>
<tr>
<th>Patient ID</th>
<th>Probable localisation (EEG)</th>
<th>MRI</th>
<th>[18F]GE-179 VT increases</th>
<th>Cluster size (mm3/voxels)</th>
<th>Peak voxel coordinates (x, y, z; mm)</th>
<th>Zmax</th>
<th>Cluster level p (uncorrected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>L frontotemporal</td>
<td>L frontal</td>
<td>7000/875</td>
<td>–323 234</td>
<td>4.46</td>
<td>&lt;0.001</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Multifocal</td>
<td>Brainstem</td>
<td>5152/644</td>
<td>06–28–38</td>
<td>4.58</td>
<td>0.001</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>Bilateral temporal L&gt;R</td>
<td>R frontal</td>
<td>3360/420</td>
<td>381 024</td>
<td>4.43</td>
<td>0.006</td>
<td></td>
</tr>
</tbody>
</table>

The cluster(s) reaching significance at p<0.05 uncorrected are listed. The contribution of global VT to variance was removed by an ANCOVA by group. Underline indicates concordance between the cluster of increase and the location of the presumed epileptogenic zone, where known. ANCOVA, analysis of covariance; EEG, electroencephalography; ID, identifying number; IFG, inferior frontal gyrus; L/R, left/right; mm, millimeters; MRI, magnetic resonance imaging; VT, volume-of-distribution.
Interestingly, each participant in that temporal lobe epilepsy (TLE) cohort showed temporal hypometabolism on $^{18}$F FDG-PET, so the results could have been significantly confounded by cerebral hypoperfusion. Four of our patients had normal $^{18}$F FDG-PET, one of whom had a focal increase in $^{18}$F GE-179 V$_F$. The absence of hypometabolism may have facilitated visualisation of activated NMDA receptors in our cohort. Alternatively, our cohort might have had more actively spiking cortex, and thus possibly greater NMDA receptor activation.

A striking and unanticipated finding of our study was reduced V$_T$ in the small number of patients with focal epilepsies who were taking antidepressants, suggesting that either depression or the use of antidepressant drug constituted an additional confounder. Our finding is in keeping with the mounting evidence for the action of antidepressant drugs at NMDA receptors including tricyclics$^{52-56}$ and serotonin-selective reuptake inhibitors$^{55,57,58}$ such as fluoxetine,$^{57,59-61}$ the two classes used by patients in this study. While our finding may represent the first in vivo evidence of an NMDA-mediated mechanism of action of antidepressants (the extent of which is likely to vary between drugs), caution is warranted as, given the sample size, the result could be due to random chance. Hence, in order to better understand $^{18}$F GE-179 binding, a larger study is required, which would compare $^{18}$F GE-179 binding between unmedicated patients with depression and those taking antidepressants. It will also be of interest to ascertain whether AEDs that are characterised by use-dependent inhibition of NMDA receptor function (such as felbamate$^{52,62}$), or certain combinations of AEDs, affect NMDA receptor binding.

A recent multicentre review concluded that administration of the NMDA receptor antagonist ketamine ‘likely’ or ‘possibly’ contributed to the achievement of control in 32% of (19 of 60) episodes, whereas treatment was discontinued due to adverse events in approximately 8%.$^{64}$ Smaller series have reported greater success.$^{65,66}$ Similarly to $^{18}$F GE-179,$^{38}$ ketamine binds to the PCP site in the NMDA ion channel pore.$^{67}$ Hence, the demonstration of increased NMDA receptor activation via $^{18}$F GE-179 PET might aid the stratification of patients with refractory status epilepticus.

Limitations of this proof-of-principle study include the small and heterogeneous population. While $^{18}$F GE-179 has a low affinity for other CNS receptors in vitro,$^{38}$ we cannot exclude the possibility that non-specific binding confounded the analyses. Further studies are needed to confirm our findings, and to quantify reproducibility and specificity of $^{18}$F GE-179 binding in vivo.

In conclusion, our results provide in vivo evidence for widespread increases in activated NMDA receptor availability in patients with focal epilepsies. A PET radioligand that reliably demonstrates focal increases in NMDA receptor activity in humans in vivo would hold potential as a method to investigate epileptogenesis in vivo after brain injury, to investigate the role of activated NMDA receptor availability in other conditions, and possibly in the presurgical investigation of patients with refractory focal epilepsy.

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Contributors. CJM contributed to the design of the study, was primarily responsible for data acquisition and analysis, and prepared the manuscript drafts. MJK contributed to conceptualisation and design of the study, data review and manuscript revision. AH contributed to data analysis and review, and manuscript revision. DARB contributed to PET data acquisition and manuscript revision. RMP contributed to conceptualisation and design of the study, and facilitated production of the radioligand at Hammersmith Imanet Ltd. PAJ and WT contributed to conceptualisation and design of the study, data review and manuscript revision. They both also facilitated production of the radioligand at Hammersmith Imanet Ltd. CM reported on the MRIs. MRS assisted with MRI acquisition, and co-registration of 3T and PET data sets. DJB contributed to conceptualisation and design of the study, and data review and manuscript revision. They were both also facilitators of the production of the radioligand at Hammersmith Imanet Ltd. CM reported on the MRIs. MRS assisted with MRI acquisition, and co-registration of 3T and PET data sets. DJB contributed to conceptualisation and design of the study, and data review and manuscript revision. JSD was primarily responsible for identification of the patients with focal epilepsy.

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Data sharing statement. The authors have submitted all major results from the study for publication. Unpublished data consist of individual imaging data sets and are only available to the investigators at present. Any request for data can be addressed to the corresponding author; any data sharing will be subject to restrictions based on anonymisation rules.

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