In-vivo NMDA receptor density as assessed via PET during recovery from NMDA receptor encephalitis

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N-methyl-D-aspartate receptor (NMDAR)-autoantibodies are amongst the commonest causes of autoimmune encephalitis.[1] *In vitro* and animal studies indicate that NMDAR internalisation is a major mechanism underlying NMDAR-antibody encephalitis.[2] However, direct support for this in humans is limited to a reduction of NMDAR staining in the *post mortem* hippocampi at autopsy.[2,3]

Methods

We performed a cross-sectional resting-state positron emission tomography (PET) study of NMDARs *in-vivo* using the radioligand [^{18}F]GE-179, currently only available for research purposes, that binds within the ion channel of the open, i.e. activated, NMDAR. We included five patients during recovery from definite NMDAR-antibody encephalitis[1] (see Online Supplement for clinical characteristics) and 29 healthy volunteers who (i) could tolerate a 70-minute PET-MR scan, and (ii) took no medications which interfere with NMDARs. NMDAR-antibody encephalitis cases #1-4 had persistent GluN1-autoantibodies in serum as a marker of recent disease activity, mild symptoms, were scanned 2–8 months after hospital discharge, and were classified as persistently "seropositive". Case #5 had undetectable serum GluN1-antibodies, was scanned 16 months after discharge from hospital and was classified as "seroreverted". The study was reviewed and approved by the local ethical committees and all participants gave written informed consent.

We pre-processed the data as previously described[4] and used [^{18}F]GE-179 total volume of distribution (V_T) to quantify open, activated NMDAR density. We assessed grey matter atrophy using MRI-based voxel-based morphometry and compared regional and voxel-wise data using the general linear model adjusting for age, sex, and site. We reported voxel-wise p-values <0.05 on a cluster-level corrected for multiple comparisons (p_{FWE}). We followed the STROBE reporting guideline.

Results

Seropositive cases #1-4 had lower grey matter V_T (estimated marginal mean 6.2, 95% confidence interval [CI] 4.4–8.0) in comparison to both healthy volunteers (8.8, 95% CI 8.1–9.4, F=6.5, p=0.02) and the seroreverted case #5 (9.7, **Fig. 1**). Voxel-based analysis showed reduced V_T in seropositive cases within bilateral anterior temporal lobes (left, T=4.5, p_{FWE}=0.02; right, T=4.9, p_{FWE}=0.05) and a large cluster involving bilateral superior parietal lobes, paracentral lobules, left posterior cingulate gyrus, and left precuneus (T=5.8, p_{FWE}< 0.001). Volume of interest analyses corroborated regional V_T reductions in seropositive cases in the temporal (34% reduction, F=8.3, p=0.008) and parietal (31% reduction, F=7.3, p=0.01) lobes and the mesial temporal region (40% reduction, F=8.9, p=0.006).

There were non-significant trends towards lower grey matter V_T with higher serum GluN1 immunoglobulin G levels and shorter time from hospital discharge and from episode onset. Grey matter V_T did not correlate with cognition (ACE-III questionnaire), at the time of scanning nor symptom severity at discharge (CASE score), but there was little variability between patients in these data.

There was no overlap between areas of decreased grey matter volume in the cerebellar hemispheres and regions with significantly reduced V_T.

Discussion

We report a large (mean 30%) regional reduction in the density of open, active NMDA receptors, most prominently in the anterior temporal and superior parietal cortices, in a small series of patients with persisting serum GluN1-autoantibodies during recovery from NMDAR-antibody encephalitis. The patients had only mild cognitive symptoms, pointing to the considerable compensatory capacity of the human brain. In contrast, a clinically

completely recovered, now seroreverted, patient had slightly elevated NMDA receptor density, which points towards normalisation or "rebound" of NMDAR function.

NMDAR hypofunction has also been observed, albeit not to such a marked degree, in patients with depression,[5] or with first episode psychosis[6] and correlating with psychotic and depressive symptoms. Our results are not explained by brain atrophy or brain perfusion.[4] The potential of [^{18}F]GE-179 PET as a clinical biomarker for NMDAR-antibody encephalitis severity and recovery should be evaluated by future studies.

Limitations include a small sample size, lack of intrathecal GluN1-autoantibody titres on scanning day, a different age and sex distribution of healthy volunteers and patients, lack of detailed cognitive testing, and lack of a comparison group with other inflammatory or encephalopathic central nervous system diseases. The findings may be more prominent in the acute phase than during disease recovery but there are logistical barriers to scanning severely affected cases. Nevertheless, our in-vivo human study supports the hypothesis of NMDA receptor internalisation and indicates the involvement of large cortical areas beyond the limbic system.

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Conflicts of interest of named authors

MG reports fees from Advisis, Arvelle, Bial, Eisai, Nestlé Health Science, and UCB outside the submitted work. SRI is a coapplicant and receives royalties on patent application WO/210/046716 (U.K. patent no., PCT/GB2009/051441) entitled 'Neurological Autoimmune Disorders' (licensed for the development of assays for LGI1 and other VGKC-complex antibodies) and ‘Diagnostic Strategy to improve specificity of CASPR2 antibody detection. (Ref. JA94536P.GBA; PCT/G82019 /051257). SRI has received honoraria/consultancy/research support from UCB, Immunovant, MedImmun, Janssen, ADC therapeutics, CSL Behring, and ONO Pharma. MCW reports a grant from Vitaflo and personal fees from UCB Pharma, Eisai, Sage and Marinus outside the submitted work.

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Conception or design of the work: MG, AAD, SRI, MJK

Data collection: MG, AAD, UV, SRI

Data analysis and interpretation: MG, SRI, AAD, MCW

Study coordination: MJK, SRI

Drafting the article: MG, AAD, UV, SRI, MCW, MJK

Critical revision of the article: all authors

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Figures

Figure 1: *[^{18}F]GE-179 uptake in autoantibody "seropositive" or "seroreverted" patients with NMDAR-antibody encephalitis and healthy volunteers.*

The figure shows the spatial distribution of [^{18}F]GE-179 total volume of distribution (V_T) on brain slices and surface projections. **Panel A** shows mean uptake in healthy volunteers (HV, n=29, mean age 41 ± 13 years, 8 [28%] female). **Panel B** displays individual V_T distributions in persistently autoantibody "seropositive" NMDAR-antibody encephalitis cases (n=4, mean age 28 ± 6 years, all female) that were scanned 2-8 months after discharge and had elevated serum GluN1-autoantibodies (1:160 – 1:320). **Panel C** displays one "seroreverted" NMDAR-antibody encephalitis case (age-range 25-30 years, female) scanned 16 months after discharge with undetectable GluN1-autoantibodies on the day of scanning.

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Study coordination: MJK, SRI, CJM, AH, JPC
Drafting the article: MG, AAD, UV, SRI, MCW, MJK
Critical revision of the article: all authors

Full conflicts of interest

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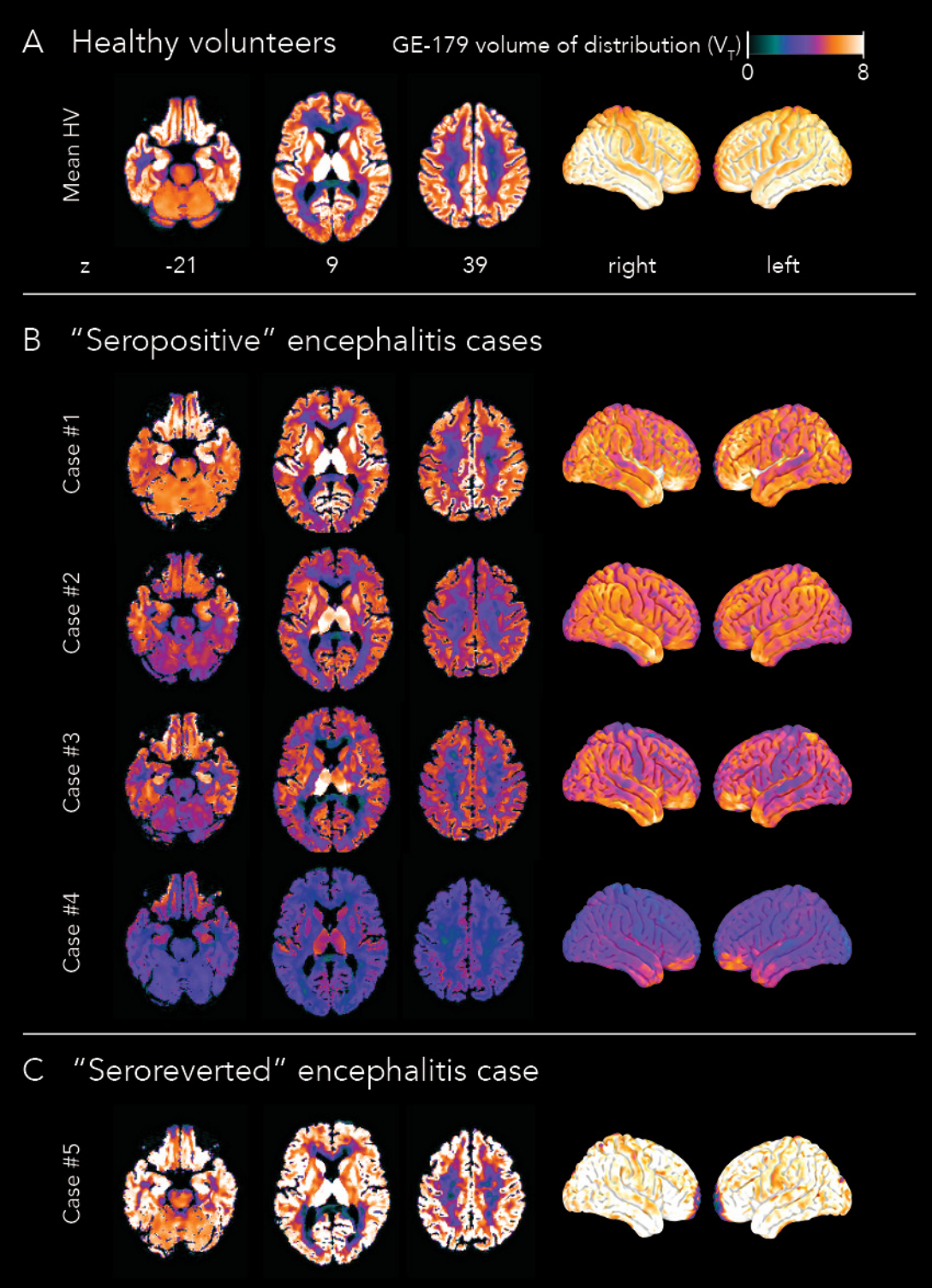
A Healthy volunteers
GE-179 volume of distribution (V_T)
0
8
Mean HV
z
-21
9
39
right
left
B "Seropositive" encephalitis cases
Case #1
Case #2
Case #3
Case #4
C "Seroreverted" encephalitis case
Case #5