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Restoration of blood brain barrier integrity post neurosurgical resection in drug resistant epilepsy

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ABSTRACT

Surgery for temporal lobe epilepsy (TLE) is a well-recognised therapy for drug resistant seizures which occur in more than 50 % of patients with TLE. Blood-brain barrier (BBB) dysfunction is commonly observed in resected brain tissue from patients with treatment resistant epilepsy however, no studies have documented the recovery of BBB function following surgery. We firstly prospectively performed dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) on seven patients scheduled for temporal lobe resections before and after resection. DCE-MRI revealed BBB dysfunction in frontal and temporal brain regions. At 6–24 months post-resection, there was a reduction in the percentage of brain volume with BBB dysfunction in 5/7 patients. We then retrospectively characterised resected brain tissue from 6 further TLE cases (total n = 13) by q-RT-PCR and immunohistochemistry which revealed region-specific changes in markers of BBB integrity and inflammation with changes in *CLDN12* and *TJP1/2* in the hippocampus and CSF1R pathway genes in cortical and hippocampal tissue. BBB dysfunction is a key component of the molecular disruption caused by seizures and in longstanding early onset chronic epilepsy that is refractory to treatment. Here, we demonstrate for the first time the rescue of BBB dysfunction by controlling seizures after surgery.

1. Introduction

The most common treatment intervention for people with epilepsy is the use of anti-seizure medications (ASM), which reliably work to suppress seizures in two thirds of patients but leave as many as 40 % of patients suffering the disabling effects of chronic drug resistant seizures [1,2]. A small proportion of these ASM resistant individuals are suitable for surgical resection of the brain region established to be the source of their epileptic seizures. When chosen carefully, resection can control seizures in just 50–70 % of patients with drug refractory temporal lobe epilepsy (TLE) [3]. Therefore, there is an urgent need to understand the molecular basis of drug resistant epilepsy to inform novel treatments for the remaining uncontrolled cases.

One hypothesis for treatment resistance in people with epilepsy relates to the blood–brain barrier (BBB), which could potentially reduce the bioavailability of ASMs [4]. Homeostasis of the neuronal

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microenvironment is maintained by an intricate network of capillaries that forms the BBB. The BBB is a physiological and functional barrier composed of brain microvascular endothelial cells that controls the exchange of ions and nutrients between the blood and brain and protects neural tissue from potentially damaging blood-borne components such as pathogens, serum proteins, immune cells, and toxins. To limit the diffusion of ions and solutes between cells, brain endothelial cells have well-developed tight junctions, which are integral membrane proteins formed by the oligomerisation of claudin proteins which have 27 family members in mammals [5]. The critical importance of the BBB is highlighted by the severe pathology observed in diseases in which it is broken down. Many studies have shown that breakdown in the integrity of the BBB is associated both with the development of seizures and ongoing epileptic seizures [6–11] while mutations in claudin-5, the most abundant BBB-associated tight junction protein, leads to the development of epilepsy in animal models [12,13]. The limited permeability and the enrichment of tight junction proteins between brain capillary endothelial cells are just two of many specialisations unique to the brain's vascular bed. These specialised properties constitute the BBB.

Accumulating evidence supports the hypothesis that epileptogenic insults increase BBB permeability in both human and experimental animals [8,14]. Measurement of BBB leakage may therefore be a candidate biomarker to predict the likelihood of seizure recurrence following ASM or surgical intervention in patients with epilepsy.

Dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is a non-invasive imaging method to visualise BBB integrity *in vivo*. Here, we performed a pilot study to investigate BBB integrity in 7 patients with drug resistant TLE before and following surgical resection of the established seizure focus. We also retrospectively examined a cohort of 12 TLE cases with preserved surgically resected tissue for markers of BBB integrity and neuroinflammation and found changes to tight junction gene expression and increased neuroinflammation.

2. Materials and methods

2.1. Statement of ethics

Ethical approval was obtained according to the Declaration of Helsinki and from St James' Hospital/Tallaght University Hospital Joint Research Ethics Committee. The subjects gave written, informed consent to participate in the study.

2.2. DCE-MRI

Imaging was performed using a 3 T Philips Achieva scanner and included T1-weighted anatomical scan (3D gradient echo, TE/TR = 3/6.7 ms, acquisition matrix 268 × 266, voxel size: 0.83 × 0.83 × 0.9 mm), T2-weighted imaging (TE/TR = 80/3000 ms, voxel size: 0.45 × 0.45 × 0.4 mm), FLAIR (TE/TR = 125/11000 ms, voxel size: 0.45 × 0.45 × 4 mm). The variable flip angle method was used for calculation of pre-contrast longitudinal relaxation time (T10) (3D T1w-FFE, TE/TR = 2.78/5.67 ms, acquisition matrix: 240 × 184, voxel size: 0.68 × 0.68 × 5 mm, flip angles: 10, 15, 20, 25 and 30°). Dynamic contrast-enhanced (DCE) sequence was then acquired (Axial, 3D T1w-FFE, TE/TR = 2.78/5.6 ms, acquisition matrix: 240 × 184, voxel size: 0.68 × 0.68 × 5 mm, flip angle: 6°, Δt = 6.5 s, temporal repetitions: 61, total scan length: 22.6 min). An intravenous injection of Gd-BOPTA (Bracco Diagnostics Inc., Milan, Italy) was administered using an automatic injector after the first three DCE repetitions. BBB permeability maps were created using the slope of contrast agent concentration in each voxel over time, calculated by a linear fit model as previously described [10,15,16]. Thresholds of high permeability was defined by the 95th percentile of all slopes in a previously examined control group [17]. Individual heart rate and blood flow variabilities were controlled for by normalising each voxel's leakage rate to that of the superior sagittal sinus. Quantification of BBB disruption was calculated as described previously. In short, image pre-processing involved image segmentation, registration, and normalisation to MNI space using SPM12. To calculate slow accumulation of contrast agent, a linear fit is applied to min 6–22 of the concentration curve of each voxel with normalisation to the venous input function, which is the leakage rate of the superior sagittal sinus. The percent of suprathreshold voxels (slope values exceeding the 95th percentile of controls) was used as a measure reflecting global BBB leakage.

2.3. Immunohistochemistry

Resected brain tissue from TLE patients was frozen on dry ice immediately after surgery. Frozen samples were embedded in optimal cutting temperature (OCT) compound and serial 50 μm sections from the lateral temporal lobe were collected on poly-l-lysine coated slides. Sections were fixed in methanol for 10 min at −20 °C, rehydrated in phosphate buffered saline (PBS) and blocked in 5 % normal goat serum with 0.3 % Triton X-100 in PBS for 30 min at room temperature. Primary antibody incubations were performed overnight at 4 °C. Secondary antibodies were incubated for 1 h at 37 °C. Nuclei were stained with Hoechst for 1 min and slides were coverslipped with Aqua Poly/Mount. Images were acquired on a Zeiss LSM 710 confocal microscope (Carl Zeiss) and Echo Revolution microscope. Primary antibodies used were rabbit anti-claudin-5 (1/500 Life Technologies, #34-1600), rabbit anti-ZO-1 (1/500 Life Technologies, #40-200), mouse anti-collagen IV (1/500 Invitrogen, #14-9871-82), goat anti-human IgG Cy3 (1/500 Abcam, #ab97170), goat anti-rabbit IgG Alexa Fluor 488 (1/500 Abcam, #ab150077), goat anti-mouse Cy3 (1/500 Biosciences, #A10521).

2.4. q-RT-PCR

RNA was isolated from TLE and autopsy control hippocampal tissue by Trizol extraction and cDNA was reverse transcribed from RNA (500 ng) with the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems). Transcript levels were quantified on the StepOne Plus instrument (Applied Biosystems) with FastStart Universal SYBR Green Master (ROX) master mix (Roche). The RT-PCR reaction conditions were as follows: 95 °C × 2 min, (95 °C × 5 s, 60 °C × 30 s) × 40, 95 °C × 15 s, 60 °C x 1 min, 95 °C × 15 s, 60 °C × 15 s. Relative gene expression levels were measured using the comparative CT method (ΔΔCT). Expression levels of target genes were normalized to the housekeeping gene β-actin. Autopsy samples from the hippocampus from the Stanley Medical Research Foundation served as controls [18].

2.5. Statistical analysis

Statistical analyses were performed using GraphPad Prism version 9.0 for Windows. Differences between groups are reported as exact P-values and were considered statically significant at $p < 0.05$. Normality of data was assessed using the Shapiro-Wilk normality test. Pre- and post-surgery DCE-MRI was compared with a Wilcoxon signed-rank test. Gene expression changes were compared between groups using a two-way ANOVA followed by Tukey post hoc test. Spearman's correlation was performed to determine association between gene expression changes and clinical factors.

3. Results

3.1. DCE-MRI imaging before and after neurosurgical resection revealed restoration of BBB integrity

We recruited 7 participants with drug resistant TLE for DCE-MRI imaging pre- and post-resection of the epileptic brain tissue. Age of onset, seizure type, ASMs and other phenotypic details are provided in Table 1. 3T MRI revealed left hippocampal atrophy (HA) with associated neocortical atrophy in 3 patients; isolated left HA in one and Right HA in

Table 1
Patient demographics.

ID	Age	Sex	Age of Onset	Years with epil	3 T MRI	Type seizure	Seizure frequency pre-surgery	Seizure frequency post-surgery	ASM	Psychiatry comorbidity	Type surgery	Surgery-Scan interval (years)	Pathology
1	62	M	26	38	Left HA, neocortex: bilateral atrophy, ROB: mild generalised atrophy	FIA	>1/month	Seizure free	CLOB, LEV, ZNS	Post-ictal psychosis	LAT lobectomy (amygdalo hippocampectomy)	2.32	No evidence of focal cortical dysplasia, Hippocampus (fragmented) with mild pyramidal neuronal loss, CA1., No evidence of inflammation or neoplasia.
2	35	M	13	24	Left HA, neocortex: mild left sided atrophy, ROB: normal	FA, FIA, FBTCS	>1/month	Seizure free	LTG, LEV	Depression	LAT lobectomy (amygdalo hippocampectomy)	2.24	Chaslin's subpial gliosis, no evidence of focal cortical dysplasia, hippocampus with granular neuronal depletion and dispersion, marked and pyramidal neuronal loss, CA4 and CA1.
3	29	M	23	8	Left HA neocortex: normal ROB: normal	FA, FIA, FBTCS	>1/week	Nocturnal events > 1/ year	CLOB, ESL, PER, ZNS	None	L selective amygdalo hippocampectomy	5.18	No evidence of focal cortical dysplasia, neuronal depletion + dispersion, hippocampal dentate fascia and marked neuronal loss CA1, no evidence of inflammation or neoplasia
4	20	F	13	9	Hippocampus normal, neocortex: L temporal normal, left hemisphere atrophy, ROB: normal	FA, FIA, FBTCS	>1/month	>1/month	CLOB, LTG, ZNS	None	LAT lobectomy	2.02	Chaslin's subpial gliosis, gliosis in the white matter, no evidence of inflammation, no evidence of tumour
5	39	M	27	12	Hippocampus normal, neocortex normal, ROB: normal	FA, FIA, FBTCS	>1/week	Seizure free	BRIV, VPA, CBZ, ZNS	None	Right temporal lobectomy	0.65	No evidence of focal cortical dysplasia, hippocampus confirmed with granular neuronal dispersion, no evidence of inflammation, vascular malformation or neoplasm
6	46	M	2	45	Left HA, neocortex: Left temporal atrophy, ROB: mild generalised atrophy affecting left hemisphere more than right	FIA, FBTCS	>1/month	Seizure free	BRIV, CBZ, CLOB, LCM, TOP	Mild ID	Left temporal lobectomy and amygdalo hippocampectomy	0.25	No evidence of focal cortical dysplasia, Hippocampal neuronal loss and severe gliosis CA1, evidence of inflammation, vascular malformation or neoplasm
7	21	F	8	12	Right HS, neocortex: normal, ROB: normal	FIA	>1/week	Seizure free	LTG, LCM, LEV	None	Right temporal lobectomy and amygdalo hippocampectomy	0.43	Hippocampal sclerosis (severe) ILAE type1 with dentate gyrus thinning and dispersion, mild and focal oligodendroglia hyperplasia, temporal neocortex, mild subpial gliosis in temporal neocortex, mild, focal hyalinised vasculopathy with perivascular haemorrhage

Abbreviations: epil = epilepsy, ASM = anti seizure medication, F = female; M = male; HA = hippocampal Atrophy ROB = rest of brain; FA = focal aware; FIA = focal impaired awareness; FBTCS = focal to bilateral tonic-clonic seizure; 3 T = 3 Tesla; CLOB = clobazam; LEV = levetiracetam; ZNS = zonisamide; LTG = lamotrigine; ESL = eslicarbazepine; PER = perampanel; BRIV = brivaracetam; VPA = valproic acid; CBZ = carbamazepine; LCM = lacosamide; TOP = topiramate; LAT = left anterior temporal.

one; left hemispheric atrophy in one and the final case appeared normal on imaging. All underwent standard anterior temporal lobectomy consisting of resection of the lateral temporal and mesial temporal structures (Table 1). 5/7 patients were subsequently seizure free post-op. The pathology of the persistent seizure cases showed isolated Left hippocampal sclerosis in one and isolated gliosis of white matter with a normal hippocampus in the other as suggested by imaging. DCE-MRI imaging revealed numerous hotspots of gadolinium extravasation prominently in the temporal lobes contralateral and ipsilateral to the seizure zone (Fig. 1a). Follow-up DCE-MRI between 4–24 months (Table 1) after neurosurgical resection revealed a reduction in BBB disruption in TLE patients as determined by a decrease in the percentage of brain volume with leaky blood vessels (Fig. 1a, c). In two patients with breakthrough seizures following surgical resection, there was no change in the extent of BBB disruption (Fig. 1b).

3.2. Region-specific changes in BBB integrity and neuroinflammation in resected TLE brain samples

Following imaging, patients underwent lateral temporal lobectomy and hippocampal and cortical tissue was collected for neuropathology. We performed immunohistochemistry on these samples for markers of BBB integrity. We included additional surgical samples from patients who did not undergo DCE-MRI to increase the numbers to $n = 13$. We performed qPCR for markers of BBB integrity and inflammation (Fig. 2a, b). qPCR revealed region-specific changes in markers of BBB integrity with an increase in expression of tight junction components specifically in hippocampal tissue of epileptic patients including *OCLN*, *TJP1* and *TJP2*, as well as regulators of tight junction gene expression such as *CTNNB1* (Fig. 2b). There was also upregulation of the glucose transporter *SLC2A1*. Inflammatory markers were upregulated in both cortex and hippocampus and included markers involved in microglial homeostasis such as *CSF1R* and *IL34* as well as pro-inflammatory markers such as *TNF*, *IL1B* and *IL8* (Fig. 2b). There was also downregulation of *CLDN12* and *VEGFA*. Spearman correlation analysis revealed a

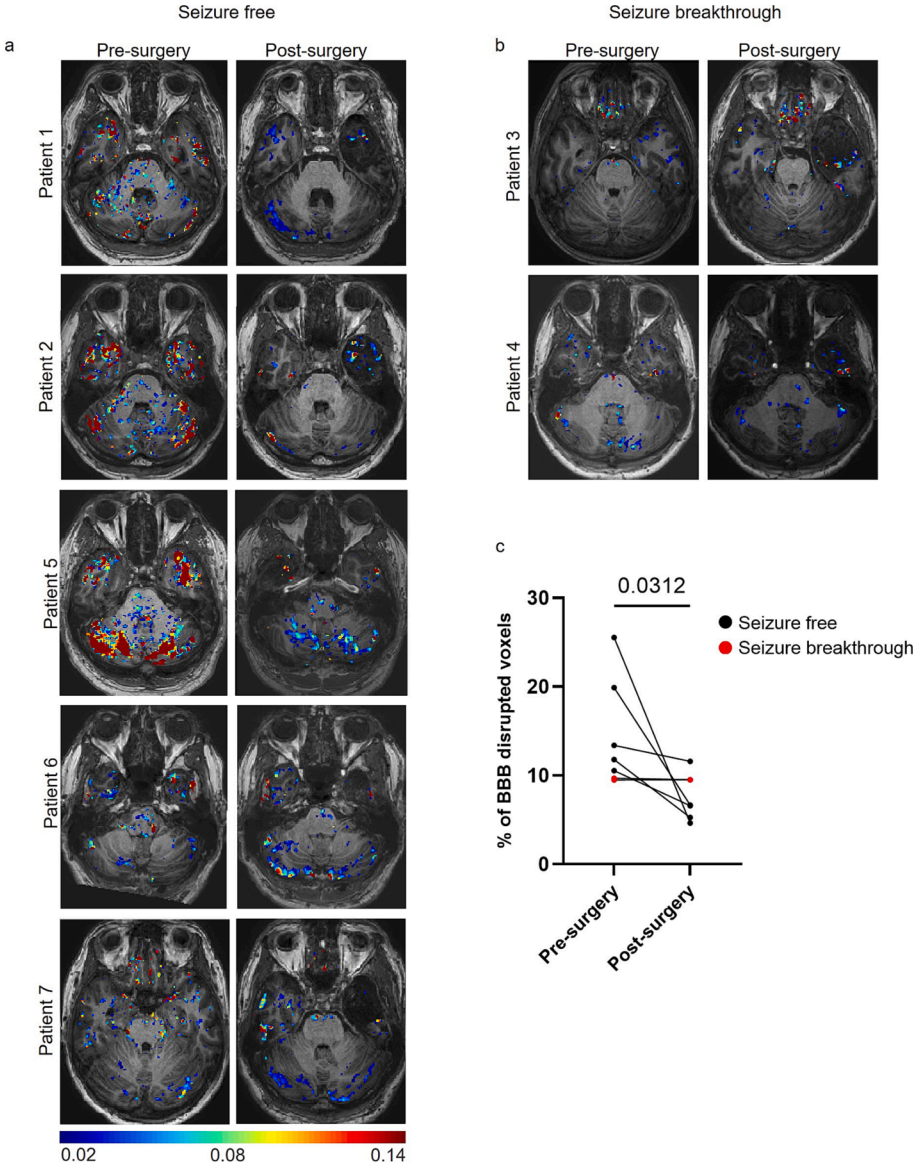

Fig. 1. Blood-brain barrier integrity is restored following resection of epileptic tissue. a) BBB permeability maps from 5 patients pre- and post-surgical resection. Colour bar represents suprathreshold slope values. **b)** BBB permeability maps from 2 patients with breakthrough seizures post-surgical resection. **c)** Quantification of whole brain BBB permeability. BBB permeability was compared between groups with a Wilcoxon matched pairs signed rank test. *p < 0.05.

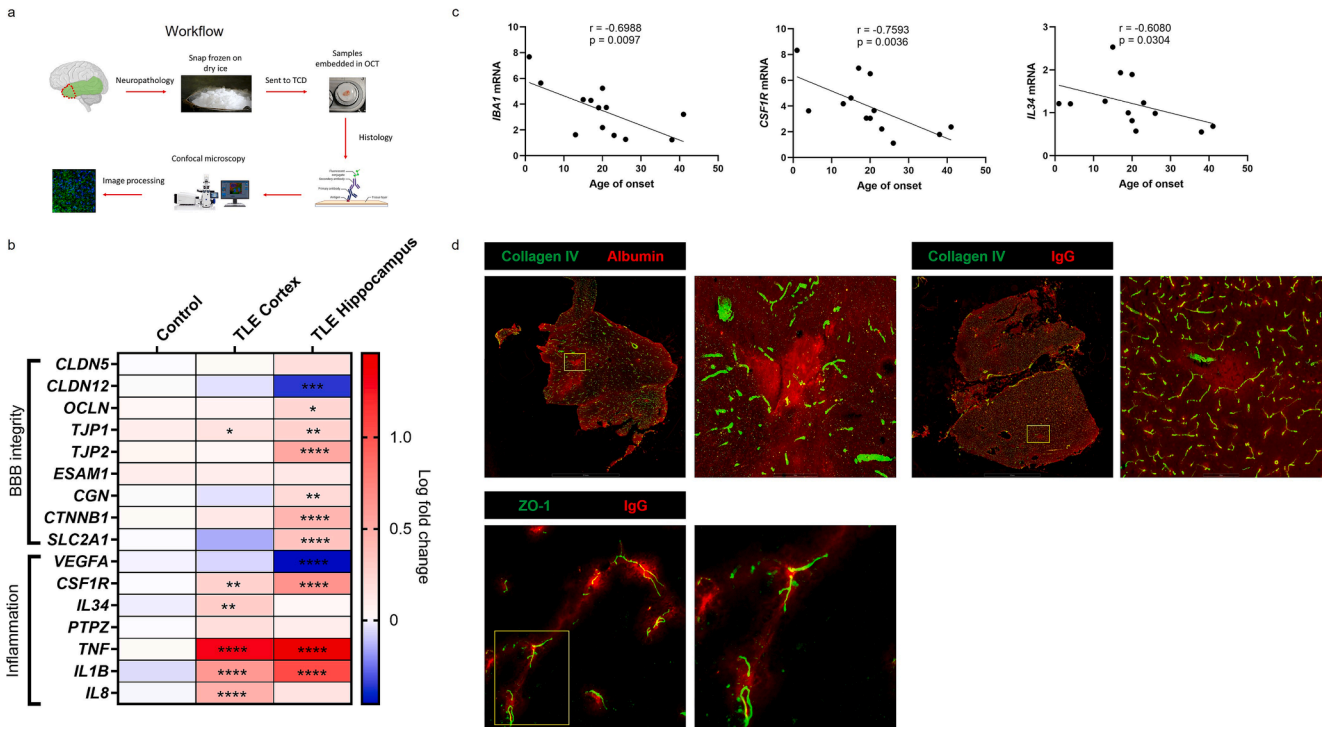

Fig. 2. Blood-brain barrier dysfunction in resected brain tissue. a) Experimental workflow. **b)** Gene expression changes in markers of BBB integrity and inflammation in cortical (n = 11) and hippocampal (n = 13) tissue from TLE patients compared to autopsy control samples from the hippocampus (n = 11). **c)** Correlation between markers of inflammation in the hippocampus (n = 13) and the age of onset of epilepsy. **d)** Immunohistochemical analysis of collagen IV, ZO-1, albumin, and IgG in resected hippocampus. Gene expression changes were compared with a Two-Way ANOVA with Tukey post-hoc test. Correlation analysis was performed with Spearman rho. *p < 0.05, **p < 0.01, ***p < 0.001, ****p < 0.0001.

significant negative association between the age of onset of TLE with *IBA1*, *CSF1R* and *IL34* (Fig. 2c).

Finally, we performed immunohistochemistry on resected brain tissue for albumin, immunoglobulin (IgG) and the tight junction associated protein ZO-1, to confirm BBB disruption. Immunohistochemical analysis of sections revealed numerous hotspots of albumin and IgG extravasation. Several regions also showed discordant ZO-1 immunoreactivity which was prominent in areas with extravasated IgG (Fig. 2d).

4. Discussion

This is the first study to show the effect of neurosurgical resection of the epileptic focus on lasting BBB integrity months and years after surgery. We found that restoration of BBB integrity was observed at the time of imaging post operatively in the 5 of 7 patients who became seizure free post operatively. The remaining two had breakthrough seizures. Among the cohort, the greatest reduction in BBB leakage was observed in two patients with differing epilepsy phenotypes and clinical characteristics. Given the variability and small sample size, no clear relationship between epilepsy phenotype or surgical type and BBB leakage reduction could be established.

Previous work has shown that loss of BBB integrity is associated with the development of seizures and epilepsy while therapies targeting microvascular stability have anti-seizure effects [8]. This suggests that restoring BBB integrity may be essential for seizure control and future studies will be required with larger patient cohorts to determine if restoration of BBB integrity is associated with positive surgery outcome and seizure suppression. Furthermore, it will be important to investigate if BBB integrity measured by DCE-MRI can be used as a biomarker of surgery outcome. Interestingly, we found widespread contrast agent accumulation in the brains of TLE patients in brain regions including the ipsilateral frontal and temporal lobes. However, following successful surgical resection, BBB disruption in these regions was reduced or completely absent suggesting a degree of cerebrovascular plasticity associated with barrier restoration in these regions. These changes in distal regions may also result from increased inflammation as we found evidence of neuroinflammation in surrounding cortical regions in our TLE cohort with an upregulation in *IBA1*, *CSF1R*, *TNF* and *IL1B* in otherwise healthy cortical tissue.

Numerous central nervous system pathologies have demonstrated BBB dysfunction, with evidence that this can play a significant role in specific disease aetiologies including Alzheimer's disease [19] schizophrenia [20] and depression [21] and long COVID. This study along with others, supports the hypothesis that the BBB is disrupted after seizures and, therefore, a possible target for drug therapy [22]. Furthermore, this work suggests that imaging-based assessment of BBB dysfunction is a potential biomarker of chronic seizure activity and its repair a marker of improvement.

Medical imaging-based assessment of epilepsy patients provides unparalleled insight into the long-term status of BBB integrity and neurophysiology. However, the high associated costs of running MRI and CT scanners, as well as the ethical considerations of repeated administration of gadolinium-based contrast agents or radiation, can prohibit their use of infrequent patient assessment. Similarly, the use of albumin CSF-to-serum ratio has limited utility in the routine measurement of BBB integrity due to their invasive procedures.

We cannot exclude the possibility that persisting seizure activity in 2 of the 7 patients for reasons which we have not determined may have given rise to the persisting breakdown in the BBB. The pathological substrate was different in each case. We were unable to detect any difference in either the radiologic or pathologic marker of BBB incompetency between the 5 patients whose seizures resolved and the two patients who have been left with persisting post resection seizure activity.

CRediT authorship contribution statement

Claire Behan: Writing – original draft, Project administration, Methodology, Investigation, Formal analysis, Data curation. **Chris Greene:** Writing – review & editing, Writing – original draft, Methodology, Formal analysis. **Nicole Hanley:** Methodology, Formal analysis. **Carme Vila Salla:** Investigation. **Declan Brennan:** Investigation. **Ruairi Connolly:** Investigation. **Kieron Sweeney:** Investigation, Data curation. **Donncha O'Brien:** Investigation, Data curation. **Michael Farrell:** Resources, Data curation. **James Meaney:** Resources. **David C. Henshall:** Resources. **Matthew Campbell:** Writing – review & editing, Supervision, Resources, Methodology, Funding acquisition, Conceptualization. **Colin P. Doherty:** Writing – review & editing, Supervision, Methodology, Funding acquisition, Conceptualization.

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2.6. Data Availability

Data supporting the findings of this study are available from the corresponding authors upon request.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

References

[1] Epilepsy. World Health Organisation. Accessed 27/11, 2017. http://www.who.int/mediacentre/factsheets/fs999/en/.
[2] Kwan P, Schachter SC, Brodie MJ. Drug-resistant epilepsy. *N Engl J Med* 2011;365(10):919–26. https://doi.org/10.1056/NEJMra1004418.
[3] Spencer S, Huh L. Outcomes of epilepsy surgery in adults and children. *The Lancet Neurology* 2008;7(6):525–37. https://doi.org/10.1016/s1474-4422(08)70109-1.
[4] Loscher W, Potschka H, Sisodiya SM, Vezzani A. Drug resistance in epilepsy: clinical impact, potential mechanisms, and new innovative treatment options. *Pharmacol Rev* 2020;72(3):606–38. https://doi.org/10.1124/pr.120.019539.
[5] Greene C, Hanley N, Campbell M. Claudin-5: gatekeeper of neurological function. *Fluids Barriers CNS* 2019;16(1):3. https://doi.org/10.1186/s12987-019-0123-z.
[6] Liu JY, Thom M, Catarino CB, et al. Neuropathology of the blood-brain barrier and pharmaco-resistance in human epilepsy. *Brain* 2012;135(Pt 10):3115–33. https://doi.org/10.1093/brain/aws147.
[7] Löscher W, Friedman A. Structural, molecular, and functional alterations of the blood-brain barrier during epileptogenesis and epilepsy: a cause, consequence, or both? *Int J Mol Sci* 2020;21(2):591. https://doi.org/10.3390/ijms21020591.
[8] Greene C, Hanley N, Reschke CR, et al. Microvascular stabilization via blood-brain barrier regulation prevents seizure activity. *Nat Commun* 2022;13(1):2003. https://doi.org/10.1038/s41467-022-29657-y.
[9] Marchi N, Angelov L, Masaryk T, et al. Seizure-promoting effect of blood-brain barrier disruption. *Epilepsia* 2007;48(4):732–42. https://doi.org/10.1111/j.1528-1167.2007.00988.x.
[10] Rüber T, David B, Lüchters G, et al. Evidence for peri-ictal blood–brain barrier dysfunction in patients with epilepsy. *Brain* 2018;141(10):2952–65. https://doi.org/10.1093/brain/awy242.
[11] Seiffert E, Dreier JP, Ivens S, et al. Lasting blood-brain barrier disruption induces epileptic focus in the rat somatosensory cortex. *J Neurosci* 2004;24(36):7829. https://doi.org/10.1523/JNEUROSCI.1751-04.2004.
[12] Deshwar AR, Cytrynbaum C, Murthy H, et al. Variants in CLDN5 cause a syndrome characterized by seizures, microcephaly and brain calcifications. *Brain* 2022. https://doi.org/10.1093/brain/awac461.
[13] Hashimoto Y, Poirier K, Boddaert N, et al. Recurrent de novo mutations in CLDN5 induce an anion-selective blood–brain barrier and alternating hemiplegia. *Brain* 2022;145(10):3374–82. https://doi.org/10.1093/brain/awac215.
[14] Rempe RG, Hartz AMS, Soldner ELB, et al. Matrix metalloproteinase-mediated blood-brain barrier dysfunction in epilepsy. *J Neurosci* 2018;38(18):4301–15. https://doi.org/10.1523/JNEUROSCI.2751-17.2018.
[15] O'Keeffe E, Kelly E, Liu Y, et al. Dynamic blood–brain barrier regulation in mild traumatic brain injury. *J Neurotrauma* 2020;37(2):347–56. https://doi.org/10.1089/neu.2019.6483.
[16] Greene C, Connolly R, Brennan D, et al. Blood–brain barrier disruption and sustained systemic inflammation in individuals with long COVID-associated cognitive impairment. *Nat Neurosci* 2024;27(3):421–32. https://doi.org/10.1038/s41593-024-01576-9.
[17] Weissberg I, Veksler R, Kamintsky L, et al. Imaging blood-brain barrier dysfunction in football players. *JAMA Neurol* 2014;71(11):1453–5. https://doi.org/10.1001/jamaneurol.2014.2682.
[18] Greene C, Hanley N, Campbell M. Blood-brain barrier associated tight junction disruption is a hallmark feature of major psychiatric disorders. *Transl Psychiatry* 2020;10(1):373. https://doi.org/10.1038/s41398-020-01054-3.
[19] Montagne A, Barnes SR, Sweeney MD, et al. Blood-brain barrier breakdown in the aging human hippocampus. *Neuron* 2015;85(2):296–302. https://doi.org/10.1016/j.neuron.2014.12.032.
[20] Greene C, Kealy J, Humphries MM, et al. Dose-dependent expression of claudin-5 is a modifying factor in schizophrenia. *Mol Psychiatry* 2018;23(11):2156–66. https://doi.org/10.1038/mp.2017.156.
[21] Menard C, Pfau ML, Hodes GE, et al. Social stress induces neurovascular pathology promoting depression. *Nat Neurosci* 2017;20(12):1752–60. https://doi.org/10.1038/s41593-017-0010-3.
[22] van Vliet EA, Aronica E, Gorter JA. Blood-brain barrier dysfunction, seizures and epilepsy. *Semin Cell Dev Biol* 2015;38:26–34. https://doi.org/10.1016/j.semcdb.2014.10.003.