Brain Foundation Research Gift 2017: Progress Report

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Title of Project: Brain glutamate imaging in stroke and correlation with post-stroke seizure risk: a pilot 7T MRI study

Summary:

Aims:
We aimed to test the feasibility and utility of 7T Magnetic Resonance Spectroscopy and *GluCEST* imaging to measure cerebral glutamate levels in stroke patients for the purposes of guiding the design of a clinical trial examining whether pharmacological antagonism of glutamate receptors has anti-epileptogenic properties in a high-risk stroke population. We hypothesised that glutamate levels in patients with both ischaemic and haemorrhagic stroke would be higher than in healthy controls and that MRI *GluCEST* and MRS glutamate concentration would correlate with risk of developing post-stroke seizures.

Methods:
Patients were recruited from the inpatient stroke unit at the Royal Melbourne Hospital. The inclusion criteria required radiological confirmation of acute cortical ischaemic stroke or lobar haemorrhage. 7T MRI was performed within 14 days of stroke onset. Patients with a contraindication to 7T MRI, history of previous stroke, or significant risk factors for non-stroke associated epilepsy were excluded. T1-weighted anatomical imaging and diffusion weighted imaging (DWI) was performed. Single voxel (STEAM) MRS was performed within the region of diffusion restriction in the axial slice with the largest DWI lesion and a mirror reference voxel was placed in the contralateral hemisphere. The same axial slice was used for the *GluCEST* acquisition slab. The *GluCEST* images were coregistered with DWI and a region of interest drawn around the area of infarction, from which *GluCEST* contrast was calculated. Water-normalised metabolite concentrations and *GluCEST* contrast were compared between the region of infarction and the contralateral hemisphere across the study population. It was decided to compare glutamate levels within the brain of each patient rather than with healthy controls given the inherent difficulty matching healthy controls with stroke patients.

Patients were followed up for 12 months for occurrence of post-stroke seizures.

Results:

To date, 19 patients, 16 with ischaemic stroke and 3 with haemorrhagic stroke, have undergone 7T MRI imaging and completed the 12 month follow-up. There were 11 males and 8 females with an age range of 31 to 82 years. Scans were performed between 48 hours and 312 hours after onset of stroke. Clinical data for the participants are shown in the table below.

Enrolment No	Age	Sex	Territory	NIHSS	Treatment	Time to scan	MRS ratio	GluCEST ratio
1	63	Male	MCA	17	tPA & ECR	93		0.68
2	56	Male	MCA	10	tPA & ECR	60	0.45	0.78
3	82	Female	MCA	8	tPA	117		0.52
4	60	Male	MCA	19	tPA & ECR	62		
5	67	Female	PCA	0	None	312		0.64
6	46	Male	PCA	1	None	172		0.68
7	45	Female	Haemorrhage	0	None	137		
8	44	Male	MCA	19	tPA & ECR	168		2.46
9	73	Male	Haemorrhage	7	None	142		
10	57	Male	MCA	18	ECR	87	0.38	
11	31	Female	MCA	1	None	312	0.88	0.92
12	64	Female	MCA	2	None	210	0.24	0.10
13	72	Male	MCA	1	None	288	0.74	1.00
14	51	Female	MCA	15	tPA & ECR	48	0.83	0.65
15	63	Male	MCA	10	ECR	82	0.37	
16	57	Male	MCA	17	ECR	158	0.93	2.94
17	66	Female	Haemorrhage	Unknown	None	88		
18	64	Female	MCA	18	tPA & ECR	66	0.48	0.35
19	50	Male	MCA	3	tPA & ECR	50	0.48	0.89

Table 1: Clinical characteristics of the study population. MCA = middle cerebral artery, PCA = posterior cerebral artery, NIHSS = National Institute of Health Stroke Scale, tPA = tissue plasminogen activator, ECR = endovascular clot retrieval. Ratio indicates the glutamate concentration (MRS) or *GluCEST* contrast in the region of stroke divided by in the contralateral hemisphere.

10/19 patients underwent Magnetic Resonance Spectroscopy. Across the sample glutamate concentrations were lower in the region of stroke compared with the contralateral hemisphere ($p = 0.000$) as were concentrations of N-acetylaspartate ($p = 0.004$) and myoinositol ($p = 0.001$). There was no statistically significant difference between glutathione concentrations, and lactate concentrations were higher ($p = 0.001$). There was no correlation between glutamate concentration ratio and either stroke severity or time to MRI acquisition.

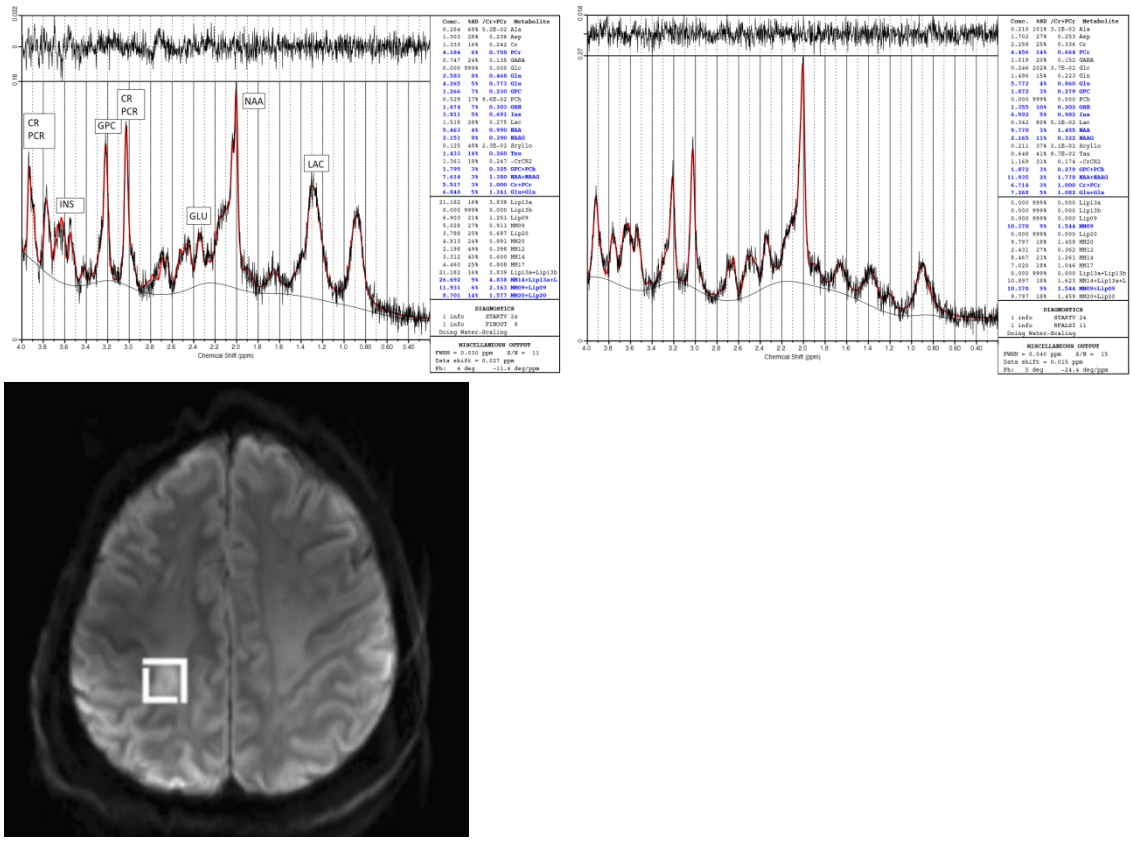

Figure 1: MRS spectra from the region of infarction (left) and contralateral hemisphere (right) in patient 13. The image below demonstrates the region of MRS voxel placement within the infarct. NAA = N-acetylaspartate, LAC = lactate, GLU = glutamate, CR = creatine, PCR = phosphocreatine, GPC = glycerophosphocholine, INS = myoinositol.

Of the 19 patients undergoing *GluCEST* imaging, the three patients with haemorrhagic stroke were excluded from the *GluCEST* contrast calculation due to the presence of artefact from blood products. Of note however, patient 17's scan demonstrated an increase in *GluCEST* contrast throughout the cerebral cortex in the same hemisphere as the haemorrhage (see figure 3). This patient presented with a seizure at the time of stroke onset and this finding is postulated to be a post-seizure effect.

Patients with ischaemic stroke with secondary haemorrhage (3 patients) were excluded from the analysis for the same reason.

Of the remaining patients, *GluCEST* contrast was higher in the region of stroke in 2/13, lower in 8/13, and similar in 3/13, although there was no significant difference across the sample (p = 0.164). In the 8 patients who had glutamate quantification by both MRS and *GluCEST*, there was a significant discrepancy between MRS glutamate concentration and *GluCEST* contrast in 1, possibly due to the small lesion

volume and its location at a junction between grey matter and white matter structures.

Although patient 17 had a seizure at stroke onset, none of the patients in the study population suffered seizures during the 12 month follow-up period.

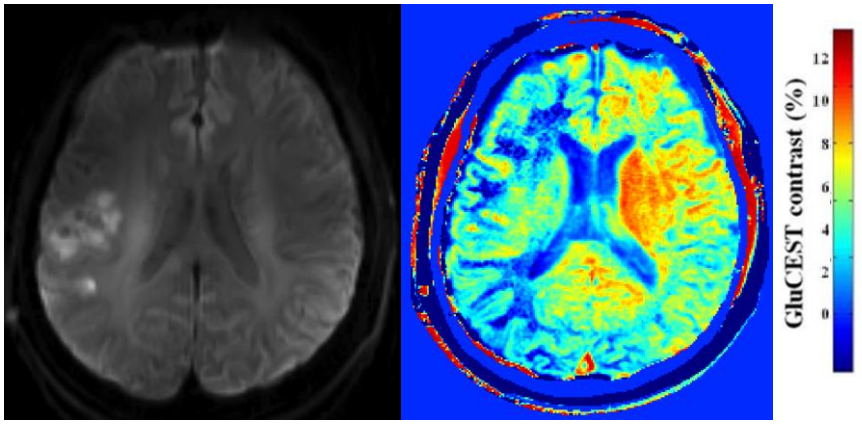

Figure 2: Diffusion Weighted (left) and *GluCEST* (centre) images from patient 4, demonstrating regionally decreased *GluCEST* contrast in the region of infarction.

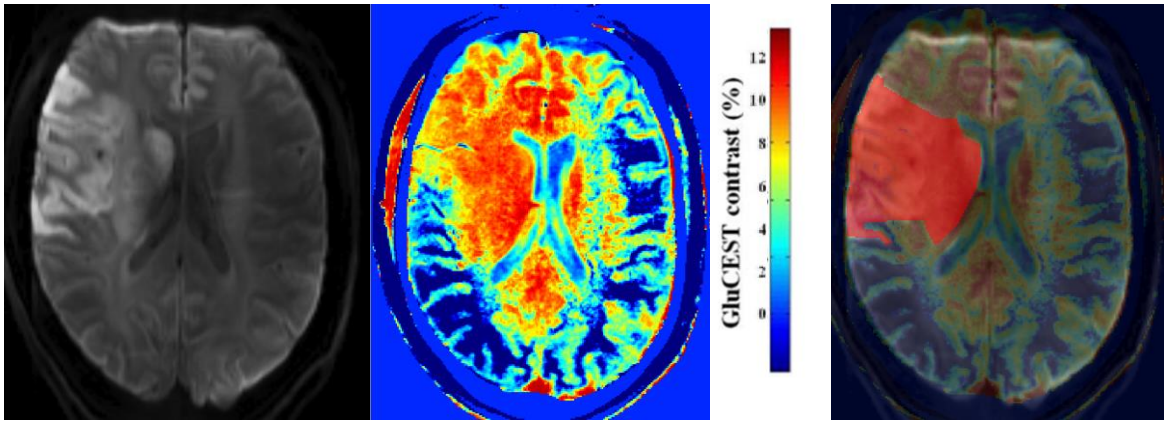

Figure 3: Diffusion Weighted (left) and *GluCEST* (centre) images from patient 7, demonstrating regionally increased *GluCEST* contrast in the region of infarction. On the right is a combined *GluCEST*-DWI image showing the segmented region of infarction in red.

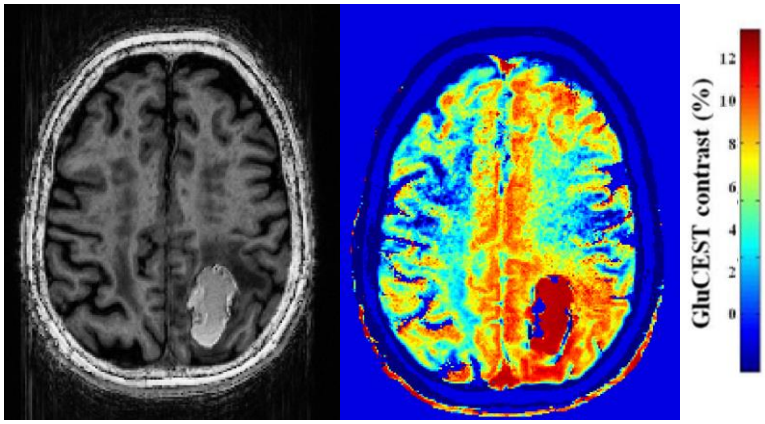

Figure 4: T1 (left) and *GluCEST* (right) images from patient 17. Despite the artefact in the region of haemorrhage there is globally increased cortical CEST contrast in the same hemisphere as the haemorrhage.

Conclusions:

Our interim results demonstrate the feasibility of performing 7T MRI for quantification of cerebral glutamate levels in the setting of stroke. Compared with the contralateral hemisphere, glutamate levels appeared lower in the region of stroke compared with MRS, while GluCEST contrast ranged from decreased to increased in the affected hemisphere. This technique may be employed in future studies to determine whether cerebral glutamate levels might be a biomarker of post-stroke epilepsy.

Hypothesis vs Findings:

Contrary to our predictions, we found decreased rather than increased glutamate concentrations in the region of stroke, both when measured by Magnetic Resonance Spectroscopy and by *GluCEST*. This may relate in part to the timing of scan acquisition in reference to stroke onset; it may be that the rise in glutamate is limited to the very early phase of stroke, after which glutamate levels fall due to a failure of various cellular functions. We didn't however demonstrate a correlation between time to MRI acquisition and either MRS glutamate concentration ratio or GluCEST ratio. Another explanation is that there is a shift in glutamate from the intracellular to extracellular space, which cannot be detected by our imaging methods.

A small proportion of the population did however have increased *GluCEST* contrast in the region of stroke. As no patients experienced seizures over the 1 year follow-up period we haven't yet identified whether increased *GluCEST* contrast is a marker of increased seizure risk. However this analysis is limited by the small sample size.

Unfortunately the artefacts created by blood products on both MRS and *GluCEST* sequences preclude reliable measurement of cerebral glutamate levels in haemorrhagic stroke using this current technique.

Unanswered Questions:

Given no patients suffered seizures during the 12 month follow-up period, we cannot yet evaluate the hypothesis that high cerebral glutamate levels following stroke are a biomarker for seizure risk.

It is unclear why, across the study population, *GluCEST* contrast ranged from decreased to increased in the region of stroke. Possible explanations include stroke severity, timing from stroke onset to MRI, and reperfusion therapy, although we haven't yet identified associations between these variables, acknowledging the limitation of our small sample size.

Finally, we observed a discrepancy between MRS glutamate concentration and *GluCEST* glutamate concentration in 1 patient. This may reflect a technical error due to the location of the stroke and small stroke volume, but we also need to consider other factors known to affect CEST contrast, such as pH changes and a contributing effect from other metabolites.

What these research outcomes mean:

We have demonstrated the feasibility of using 7T MRI to measure and compare cerebral glutamate levels in patients with ischaemic stroke but we haven't established whether increased glutamate levels are predictive of seizure risk. We have continued recruiting patients for our study at a variety of time points following stroke onset, and we will follow up our existing patients over an extended time period to identify the development of seizures beyond 12 months. Using this research data we have devised an imaging protocol for a clinical trial in which patients with stroke are treated with a glutamate receptor antagonist to see if this lowers seizure risk.