***What is GluCEST?*** Measuring neuronal integrity and associated neurochemical dysregulations *in vivo* is typically accomplished using high-field magnetic resonance imaging (MRI). Due to its molecular structure, Glu gives rise to a complex proton nuclear magnetic resonance spectrum characterized by the coupled spins of the C2-C4 hydrogen nuclei.132 As one of few neurotransmitters that can be detected with non-invasive neuroimaging, Glu has been intensely studied in early development,133 neurological and neuropsychiatric conditions,116 and to some extent in healthy aging.117 1HMRS is the most common technique used (e.g.120) and has high sensitivity and specificity for Glu, particularly at high MRI field strength (e.g. 3T). Yet, using 1HMRS to measure Glu across the brain is challenging due to limited spatial coverage, diffuse cortical morphology, partial volume effects, and confounding with other neurochemicals, namely glutamine (Gln).134 Here, we propose to overcome many of these limitations by using **ultra-high field 7T GluCEST imaging.29** GluCEST (**Fig. 4**) is an *imaging technique*, pioneered by Dr. Ravinder Reddy (Co-I), that selectively measures Glu in the brain using magnetization transfer. GluCEST offers distinct advantages over 1HMRS including: 1) improved spatial resolution, as simultaneous measurement of brain Glu is feasible across multiple brain regions (**Fig. 4**), 2) higher sensitivity,29 and 3) the ability to assess Glu independent of Gln.

Figure 4. Schematic representation of the GluCEST acquisition and analysis. An optimized within-subject acquisition and analysis pipeline is implemented. Participants undergo A. structural (UNI image) and GluCEST (CEST) at 7T MRI. GluCEST, B0 and B1 maps are collected. B. Structural data is segmented and C. GluCEST contrast images corrected and aligned to structural MRI (UNI). D. GluCEST values are then extracted from regions of interest and tabulated off-line.5,6 GluCEST values can be extracted from standard anatomical parcellation or from data-driven components. Medial temporal slice through the hippocampus is shown as an illustration.



Macintosh HD:Users:roalf:Dropbox:Grants:Roalf_Glutamate_Psychosis:June_05_Submission:Sections:Figures:GluCEST_description.pdfCEST imaging uses contrast from endogenous compounds containing exchangeable protons (e.g. -OH, -NH2, -NH groups).135-139 In the case of Glu (as a solute) exchangeable protons are the amine group on Glu that *resonate at 3 ppm from down field of water*, which is different from the resonant frequency of bulk water protons. The exchangeable protons on Glu are saturated using a low power, frequency selective, RF pulse. Due to chemical exchange, this saturated magnetization of solute pool is transferred to the bulk water (solvent pool) and the water signal becomes slightly attenuated. Since the bulk water pool is much larger than the saturated solute proton pool, each saturated solute proton is subsequently replaced by a non-saturated water proton, which is then again saturated. This process is repeated many times. If the solute protons have a sufficiently fast exchange rate (residence time in millisecond range) and the saturation time is sufficiently long (seconds range), prolonged collection leads to substantial enhancement of this saturation effect, which becomes visible on the water signal (**Fig. 5A-C**). Difference of an image of water taken while saturating +3 ppm and another image of water taken on the other side of the water peak at the same frequency (-3 ppm) gives the CEST signal that is proportional to the concentration of solute pool. Due to repeated exchange during the saturation process, CEST methods typically provide significantly higher sensitivity compared to the standard 1HMRS measurement of the metabolite29. This high sensitivity allows the presence of low-concentration solutes, like Glu, to be imaged indirectly. Importantly, Gln does not show a CEST effect (**Fig. 5D)** due to its faster exchange rate than Glu, thus unlike 1HMRS, there is no contamination of the Glu signal from Gln.140 To date, GluCEST has been used to estimate changes in Glu in vitro,29 in mouse models of Parkinson’s Disease,141 Alzheimer’s disease,142,143 Huntington’s disease,144 in the brain145 and spinal cord of healthy subjects,135 and clinically in epileptic patients146 and in patients with psychosis.5 Importantly, the MRI-based technique does not require the use of ligands like PET imaging. Recent advances have improved acquisition approaches allowing for better spatial resolution using a 3D acquisition (See **Fig. 9**),147 which we will use here.

Thus, using 3D GluCEST we aim to characterize the influence of altered brain Glu in HOA, MCI and SCD **(Aim 2).** Furthermore, we will relate brain Glu to age-related changes in clinical and neurocognitive performance at baseline, which will be collected using a standardized comprehensive adaptive computerized assessment **(Aim 1).** Finally, we will leverage an accelerated longitudinal design that will allow us to measure change in clinical and cognitive features associated with aging and at-risk dementia while associating this change with baseline Glu levels**(Aim 3)***. We will test the underlying hypothesis that glutamatergic hypofunction underlies clinical and cognitive dysfunction.* We believe that a comprehensive multimodal approach is necessary to identify risk. Without sensitive tools and insight into the biological mechanisms underlying incipient dementia, both early and accurate detection and appropriate use of interventional treatment will remain challenging. We propose to address some of these gaps by measuring brain Glu levels, which will offer a nuanced and uniquely individualized view of the brain and provide insight into potential mechanism underling the development of dementia.

Figure 5. The GluCEST technique. (A) When magnetization from the exchangeable protons (blue H+) of a metabolite (e.g., glutamate) are saturated with a frequency-selective radiofrequency pulse, (B) a proportional decrease of the water signal results from the exchange mediated accumulation of saturated protons (red H+s) in the bulk water pool. (C) The difference between these two water signals obtained with and without saturation of the metabolite pool is measured as the CEST effect. Thus, the change in free water magnetization, while selectively saturating the exchangeable protons of the metabolite, represents a measure of the metabolite content. (D) Importantly, not all metabolites show a CEST effect. Glutamine (Gln) does not show a CEST effect, while glutamate (Glu) does.