Investigating the potential contribution of vesicular glutamate to gluCEST signal

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INTRODUCTION: Glutamate-weighted CEST, or gluCEST, allows for high-resolution imaging of glutamate[1], one of the main neurotransmitter of the adult brain. However, the exact origin of gluCEST signal is not well understood and its specificity debated. Simulation studies based on in vitro data suggest that more than 60% of in vivo gluCEST signal could be contaminated by other endogenous agents[2], like creatine or amides, and it has been reported in situ that more than 80% of gluCEST might originate from other molecules than Glu[3]. In this work, we propose to better characterize gluCEST signal by modeling multi-B_1 CEST data combined with ^{1}H-MRS-measured glutamate concentration. In particular, we explore the possibility of a secondary pool of glutamate which could contribute to gluCEST signal, that is pre-synaptic vesicular glutamate. Indeed, around synaptic terminals, glutamate is encapsulated in high concentration (100-200 mM) at low pH (5.5 to 5.8) in small vesicles (around 30 nm)[4]. This pool of glutamate, which could be invisible in ^{1}H-MRS because of ultra-short T_2[5], might have unique exchange dynamics, making it potentially a significant contributor to gluCEST signal. This hypothesis is investigated and discussed in this study.

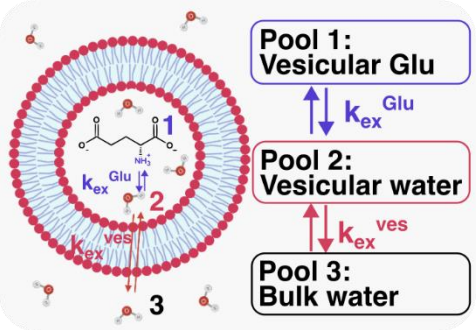

Fig 1 - Vesicular glutamate modeling.

METHODS: Using a LASER module, simultaneous quantitative ^{1}H-MRS[6] and multi-B_1 continuous wave CEST measurements were performed in the striatum and corpus callosum of the mouse brain at 11,7T. The [Glu] estimated with spectroscopy was then used as concentration value for a "free" cytoplasmic glutamate pool during CEST modeling. Z-spectra acquired at B_1 = [1, 3, 5, 7] µT, t_{sat} = 1 s, were fitted with multi-pools Bloch-McConnell simulations[7]. Classical pools (creatine, amide, etc…) were included in the model, and vesicular glutamate was modeled by adding two additional pools representing vesicular water and encapsulated glutamate (Fig. 1). This vesicular Glu model was also tested in vitro on synthesized liposomes encapsulating 150 mM glutamate (Fig 2).

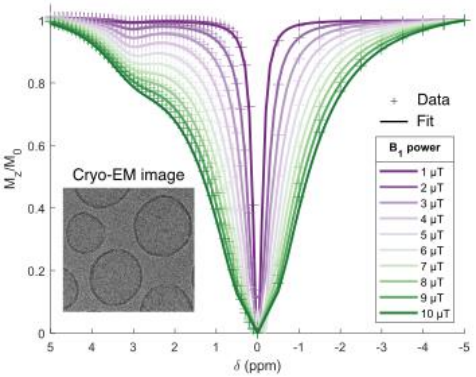

Fig 2 - In vitro modeling of the CEST signal of synthesized glutamate vesicles.

RESULTS: With ^{1}H-MRS, glutamate concentration was quantified to 6.5 ± 0.3 mM and 5.7 ± 0.4 mM in the mouse striatum (N=5) and corpus callosum (N=8) respectively. Using these values as reference, we showed that classical CEST pools are not sufficient to describe the CEST signal, especially at high B_1 power, and in particular, classical model fits 30 to 50 mM of [Glu], which is clearly overestimated (Fig 3 in dashed line). Subsequently, we developed the CEST model to describe vesicular Glu and validated in vitro its ability to predict vesicular concentration (Fig 2). Translated to in vivo situations, we estimated using this model that vesicular Glu could produce a significant MTR signal of 1 to 4%. Adding vesicular Glu to the model to fit multi-B_1 CEST data acquired in the mouse striatum, we improve the quality of fit (R^2 increased from 0.996 to 0.998, Fig 3) and find Glu vesicles concentrations ranging from 80 to 130 µM, which is consistent with reported values in literature of synaptic and vesicular densities[8,9].

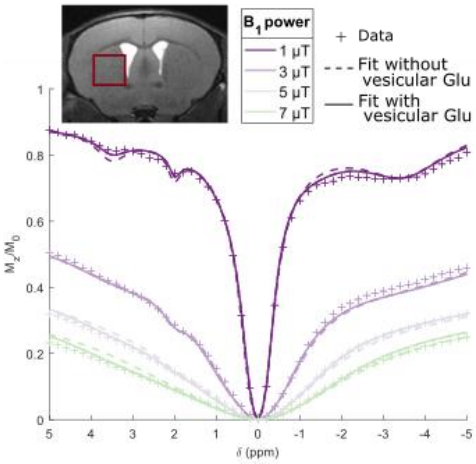

Fig 3 - In vivo fitting of CEST data with or without vesicular glutamate modeling.

DISCUSSION: Our modeling results indicated that pre-synaptic vesicular glutamate could significantly contribute to in vivo gluCEST signal, with clearly different exchange dynamics than "free'" cytoplasmic glutamate, making it a dissociable second glutamate pool. Further investigations regarding the reliability of the model to predict synaptic vesicles concentration are ongoing. Additionally, remaining residuals when fitting in vivo data indicate that there might still be other contributions of fast-exchanging molecules such as taurine or unknown proteins.

CONCLUSION: This modeling study of pre-synaptic vesicular glutamate may improve our understanding of the molecular origin of the gluCEST contrast. Furthermore, the potential sensitivity to this vesicular pool, specific to CEST compared to ^{1}H-MRS for instance, might make gluCEST a unique tool to study the synaptic aspect of neurometabolism.

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