Specificity of task-active modulation of hippocampal glutamate in response to associative learning: A ^{1}H functional Magnetic Resonance Spectroscopy study

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BACKGROUND: Glutamate (Glu) plays a major role as an excitatory neurotransmitter in the cerebral cortex as well as in the hippocampus in which the latter is particularly rich in glutamatergic neurons. This issue is of translational relevance in disorders such as schizophrenia (SZ), in which hypo-glutamatergia (and NMDA hypo-function) have been proposed as a leading pathophysiological pathway. This hypothesis has functional implications: A task-active state during hippocampal specific tasks may result in Glu modulation that is dysfunctional. Thus, understanding of the *neurochemical dynamics* in the hippocampus can elucidate our understanding of SZ. However, to achieve this, it is imperative to characterize the Glu signal in a selective (to the hippocampus) task-active state. Though, it is suggested that only a small portion of the total observed Glu level by ^{1}H magnetic resonance spectroscopy (MRS) is directly involved in the Glu-glutamine excitatory neurotransmission, there is ^{1}H functional MRS (fMRS) evidence of increased Glu level in response to visual stimulation and motor tasks[1,2]. Here we provide the first known evidence of selective Glu modulation in the hippocampus during associative learning but not working memory (WM). This fMRS technique can transform the assessment of SZ and other pathologies related to Glu dysfunction.

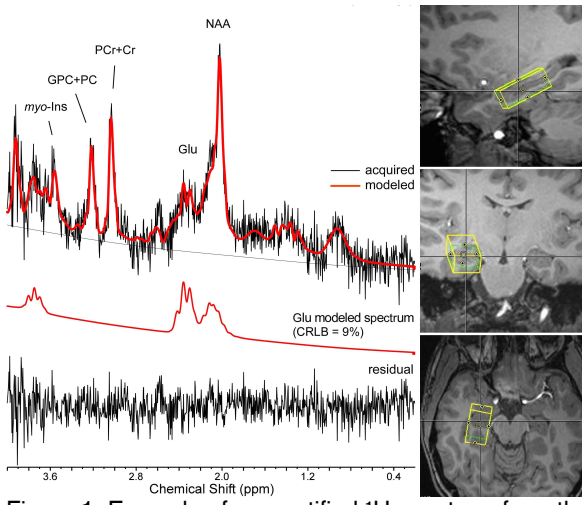

Figure 1: Example of a quantified ^{1}H spectrum from the hippocampus collected with 16 averages (56s).

SUBJECTS AND METHODS: A total of 15 healthy, young adults (9M+6F; mean age 25.1±2.0yrs) participated in 2 separate ^{1}H fMRS tasks on a 3T system. In each, 22 individual consecutive single-voxel, short-TE ^{1}H spectra with a 54s and 56s temporal resolution (associative learning and WM), localized in the right head/body of the hippocampus (voxel:1.7x3.0x1.2cm or 6.12 cm^3, PRESS, 16 avg., TR= 3.38s and 3.5s, TE=23ms) were collected. The learning task previously assessed in SZ using fMRI[3] involved epochs of encoding (9 unique object-location pairs) and cued-retrieval (of those associated memoranda). Eight encoding-retrieval cycles were employed to allow learning to asymptote. During the WM (2-Back) task, participants indicated when a letter in the sequence was the same as one presented two items previously. Rest epochs (30s) were interspersed between task blocks. Six epochs of a finger-tapping paradigm (of varying frequency), which were interspersed with rest epochs (27s and 20s), were introduced prior to the onset of both tasks to provide a non-learning and non-memory baseline condition against which to quantitate the ^{1}H fMRS. The analysis focused on assessing changes in hippocampal Glu and other key metabolites over the entire temporal course of the task relative to the baseline condition (and not the modulation of signals between task/rest epochs) and therefore, the 22 ^{1}H spectra per task was divided into 5 temporal segments (i.e., a baseline segment with the first 6 ^{1}H spectra followed by 4 equally spaced segments of 4 ^{1}H spectra in each) (Figure 1). In each segment, the ^{1}H spectra were averaged resulting in a single high-quality spectrum. LC Model was used for spectral quantification and the % change in metabolite level (Glu, NAA, PCr+Cr, GPC+PC and *myo*-inositol) in each segment relative to baseline was statistically analyzed using the repeated measure GEE approach with segment as the main term.

RESULTS: Performance wise, results showed progressive improvement in associative learning (p=.042) [e.g., 1st vs 2nd segment (p=<.0001), 2nd vs 3rd (p=.081) and 3rd vs 4th (p=.010)]. The 2-Back performance, which did not involved a learning component, remained high across the task epochs without significant differences across the 4 segments (p=.23).

The segment term for Glu was significant for the learning task (p=0.043), post-hoc analyses showed significant increases in hippocampal Glu during the 1st, 2nd and 3rd segments relative to baseline [6.5% (p<.0001), 10.1% (p=.0002) and 8.0% (p=.013)] (Figure 2). The PCr+Cr segment term failed to reach significance during the learning task (p=.065); however, post-hoc analyses showed significant increases in hippocampal PCr+Cr during the 1st and 2nd segments relative to baseline [4.4% (p=.0021) and 4.8% (p=.0013)]. In contrast, there were no significant differences in ^{1}H metabolite levels in any of the 4 segments relative to baseline in the hippocampus during WM (2-back) task. Additionally, the NAA linewidth was not significantly different between the segments for both tasks.

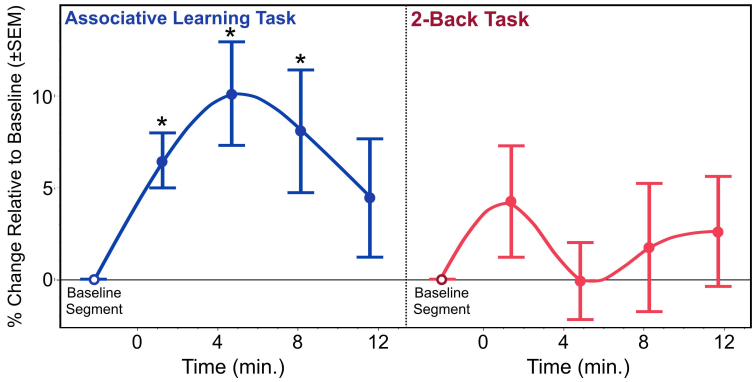

Figure 2: Mean % change in hippocampal Glu relative to baseline.

CONCLUSIONS: These preliminary results provide the first ever evidence of neurochemical dynamics of the *in vivo* Glu signal in the hippocampus. Heightened Glu signal was observed in the hippocampus during associative learning followed by decreasing levels in response to learning proficiency (i.e., not significantly elevated during the 4th segment). In addition to Glu, PCr+Cr showed increased levels during the learning task, which suggests that neurochemical dynamics is not limited to only Glu but also metabolites associated to high-energy phosphate metabolism. ^{1}H fMRS during the WM task (i.e., a predominantly PFC task) did not show any significant differences in hippocampal Glu compared to the baseline condition. The specificity of the Glu response to the associative learning task, suggests that ^{1}H fMRS is highly discriminative and can be of substantive translational relevance in the *in vivo* characterization of SZ related psychopathology. The application to SZ is a significant focus of our ongoing studies.

1. Schaller B, et al. (2014): Are glutamate and lactate increases ubiquitous to physiological activation? A ^{1}H functional MR spectroscopy study during motor activation in human brain at 7Tesla. *Neuroimage*. 93:138-145. **2.** Mangia S, et al. (2007): Sustained neuronal activation raises oxidative metabolism to a new steady-state level: evidence from 1H NMR spectroscopy in the human visual cortex. *Journal of cerebral blood flow and metabolism*. 27:1055-1063. **3.** Wadehra S, et al. (2013): Network dysfunction during associative learning in schizophrenia: Increased activation, but decreased connectivity: an fMRI study. *Schizophr Res*. 148:38-49.