Magnetic Resonance Imaging of the Neurotransmitter GABA *in-vivo*

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INTRODUCTION: Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter of the brain. GABA levels are also altered by disorders of the central nervous system (CNS) and several drugs acting on the central nervous system (CNS) mediate their effects by changing GABA levels. Hence, GABA provides as surrogate marker for diagnosis, treatment, and evaluation of therapeutic efficacy. Existing approaches for measuring regional changes in GABA in humans include positron emission tomography (PET), single photon emission tomography (SPECT) and Magnetic Resonance Spectroscopy (MRS)[1-3]. The shortcomings of PET and SPECT are low resolution and radiation exposure, which limits applicability to functional studies. MRS on the other hand is able to detect GABA in-vivo, but requires complicated spectral editing techniques and has limited spatial and temporal resolution.
Here we describe a novel, noninvasive, nonradioactive and high resolution approach for imaging brain GABA exploiting chemical exchange between the amine proton group on the GABA molecule and water. These amine protons are generally invisible to conventional MRS as they rapidly exchange from $-NH_2$ to water protons and thus 'share their magnetization' with that of water. However, on the NMR time scale, if this exchange is on the slow to intermediate level, then radiating the $-NH_2$ protons with long radiofrequency (RF) pulse at its chemical shift will terminate the 'sharing of magnetization' between the $-NH_2$ protons and water. Concomitantly, the water resonance signal is reduced in proportion to the concentration of $-NH_2$ protons. This method of detecting resonances indirectly is known as chemical exchange saturation transfer (CEST)[4]. In the current study, we measured the CEST effect from amine proton ($-NH_2$) present in GABA at a 7 Tesla ultra high field.

MATERIALS AND METHODS: ***Z-Spectrum and ^{1}H-Spectroscopy of GABA Exchangeable Protons:*** These experiments were performed on a 9.4 T vertical bore scanner (Inova; Varian, Palo Alto, CA) using a 5-mm RF-probe. Imaging phantoms with a 10mM GABA concentration in phosphate buffered saline (PBS) were prepared at a varying pH of 6.2, 6.6, 7.0, 7.4, and 7.8. Normalized water spectra obtained as a function of resonance offset of saturation pulse, termed 'Z-spectra', were acquired for each sample, using presaturation RF pulses of 1 second duration and a 127 Hz B_1 over a frequency range of ±5.0ppm relative to the bulk water resonance frequency in steps of 0.125ppm. To determine the resonance frequency of the exchangeable proton in GABA, we prepared a 500mM GABA solution in PBS and adjusted the pH~3. The water suppressed ^{1}H NMR spectrum of this solution was acquired.
***Studies at 7T Siemens Whole Body MRI Scanner*:** Imaging phantoms of 1.0, 2.0, 5.0 and 10.0 mM concentrations were prepared by dissolving GABA in PBS (pH 7.4). These samples were added to small test tubes (1.5cm diameter), and immersed inside a large beaker containing PBS. CEST images were acquired on a 7.0T Siemens whole-body clinical scanner (Siemens medical systems, Erlangen) using a transmit-receive head coil with a 1s long RF saturation pulse at a B1 of 127 Hz and frequencies at ±2.75ppm from the water resonance followed by gradient-echo acquisition. The imaging parameters were: slice thickness=5mm, TR =7s, TE=3ms, field of view=240*240 mm, matrix size=128*128, and 32 echoes per TR. Using the same imaging parameters, GABA CEST imaging was also performed at 7.0T on the brain of four normal volunteers (age: 27-35) using the same coil. B_0 and B_1 maps were also obtained. The study was conducted under an approved Institutional Review Board protocol of the University of Pennsylvania. Informed consent from each volunteer was obtained after explaining the study protocol.
CEST Image Processing: CEST contrast was calculated by using the equation: CEST contrast = $100\%*[M_{neg} - M_{pos}] / M_{pos}$, where M_{neg} and M_{pos} are the acquired MR signals when saturation is off (-2.75ppm) and on (+2.75ppm) respectively. The CEST images were corrected for the B1 and B0 inhomogeneities.

RESULTS: ***Phantom Study:*** Z-spectra of 10mM GABA at different pH are shown in Fig. 1. At physiological pH~7.4, GABA showed a broad CEST effect ranging from 2.0 to 3.5 ppm downfield from bulk water resonance. At lower pH (pH<7.0), the CEST peak of GABA was more pronounced at ~2.75 ppm. ^{1}H spectrum of 500mM GABA (pH ~3) showed $-NH_2$ proton resonance at approximately ~2.75 ppm (data not shown). At physiological buffered condition (pH 7.4) the exchange rate of $-NH_2$ proton is quite fast and their resonance cannot be depicted on ^{1}H spectra. CEST contrast of GABA at different concentrations on pH 7.4 is shown in Fig. 2. GABA CEST effect is proportional to GABA concentration from 1mM to 5mM with slope as 2.2% per mM (R^2=0.99).
In-vivo Human Brain Study: GABA CEST contrast is shown in Fig.3 from a representative imaging slice. Cerebellum (10.9±5.9%) showed the highest CEST contrast followed by the hippocampus (6.9±4.0%), thalamus (6.6±3.1%), and the region surrounding corpus callosum (6.9±4.1%). No signal change from CSF (0.9±0.8%) indicates the symmetric saturation related to bulk water (data not shown).

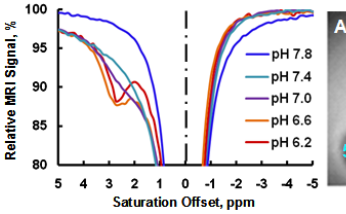

Fig 1. Z-spectra at different pH.

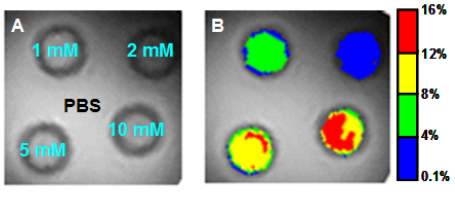

Fig 2. The GABA phantom image (A) and corresponding color map of GABA CEST contrast (B). GABA CEST contrast is proportional to GABA concentration from 1mM to 5mM (C).

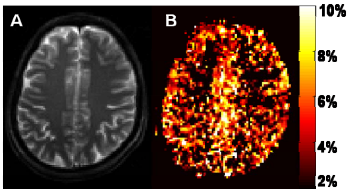

Fig 3. The brain image acquired with saturation at -2.75ppm (A) and corresponding color map of GABA CEST contrast (B).

DISCUSSION: We present a novel CEST approach for imaging brain GABA in-vivo with high spatial resolution. The probable role of –NH2 protons in providing the GABA CEST effect was confirmed by the Z-spectra which showed the CEST peak at ~2.75ppm downfield to bulk water. The CEST effect from the amide proton (-NH) was previously observed at 3.6 ppm downfield to water protons[5], its resonance is separated by 0.8ppm from that of GABA –NH2. The GABA CEST effect was responsive to pH, as expected. At low pH, the exchange rate decreased and a narrow CEST peak was observed while at higher pH, due to faster exchange, a progressive broadness in the CEST peak was observed. A linear increase in GABA CEST was also observed from 1mM to 5mM while at higher concentrations (10mM), non-linear increases in GABACEST were observed suggesting that the GABA CEST contrast is more sensitive to small change in GABA. This suggests the possibility to detect small changes in GABA concentrations in the human brain, which are in the range of 1-2 mM in various brain disorders. The GABA CEST map in healthy human brains demonstrates regional variations in the physiological concentration of GABA. The cerebellum showed the highest concentration of GABA compared to other regions[6]. This method provides a noninvasive method for imaging brain GABA at high spatial and temporal resolution without ionizing radiation. The ability to image brain GABA noninvasively will have numerous applications in basic and clinical neuroscience and potentially in clinical care.

References: 1. Anne et al. JCBF 2002;22:878-89, 2.Verhoeff et al. Psychiatry Res. 1999;9:163-73, 3. Keltner et al. Magn Reson Med 1996;36:458-61, 4. Ward et al. J Magn Reson. 2000;143:79-87, 5. Zhou et al. Nat Med 2003;9:1085-90, 6. Harada et al. Proc. Intl. Soc. Mag. Reson. Med. 2005;13:549.
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