High Resolution Mapping of Modafinil induced changes in Glutamate Level in Rat Brain

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Introduction:

Modafinilin an analeptic medication used clinically in the treatment of narcolepsy and hypersomnia without interfering with nocturnal sleep[2]. It is now also undergoing clinical trials for the treatment of stimulant addiction[1]. In addition, protective effects of modafinil have been observed in experimental hypoxia, ischemic injury and in a model of Parkinson's disease[3,4,5]. Modafinil induced sleep deprivation is associated with an increase in cerebral glutamate (Glu) levels in rodent models[6], although data regarding the time course of drug effect on Glu levels are not clear. Glutamate is the major excitatory amino acids in the biological system and can be detected noninvasively through magnetic resonance spectroscopy (MRS). In one 2D COSY MRS study, modafinil increased cerebral Glu level significantly within few hours (2-7 hours)[7], but an HPLC study observed an increase in Glu at 12 and 24 hours, but not at 2-7 hours, post modafinil administration[6]. High resolution mapping of cerebral Glu level has recently been achieved through chemical exchange saturation transfer (GluCEST) MR imaging (MRI)[8].In the current study, we mapped the modafinil induced Glu level changes at high spatial resolution in healthy rat brain using GluCEST and compared the findings with absolute Glu concentration changes measured with ^{1}H MRS.

Materials and methods:

The Institutional Animal Care and Use Committees of the University of Pennsylvania approved all the experimental protocols in this study. MR imaging was performed at 9.4T horizontal bore small animal MR scanner (Varian, Palo Alto, CA) using a 35-mm diameter commercial quadrature proton coil (m2m Imaging Corp., Cleveland, OH). Eight Sprague Dawley rats were used in this study. Modafinil (Sigma Aldrich) was suspended in a dose of 500 mg/kg in a 0.5% tragcanth gum solution. 2 ml of modafinil suspension was administered intraperitoneally in all eight rats after baseline MR imaging. In 3 rats the effects of modafinil were followed over 5 h after administration and the other five rats were imaged 24 hours post modafinil administration. Animals were kept under anesthesia (1.5% isoflurane in 1 liters/min oxygen) and their body temperature maintained with the air generated and blowing through a heater (SA Instruments, Inc., Stony Brook, NY).

GluCEST MRI: GluCEST imaging of the rat brain was performed using a custom-programmed segmented RF spoiled gradient echo (GRE) centric phase encode readout pulse sequence with a frequency selective continuous wave saturation preparation pulse. The sequence parameters were: field of view =35×35 mm^2, slice thickness = 2 mm, flip angle=15 degree, GRE readout TR=6.2 ms (128 segments), TE =2.9 ms, matrix size=128×128, average=4. For every 8 s one saturation pulse and 128 acquisition segments were applied. CEST images were collected using a 1 second saturation pulse at peak B_1 of 250 Hz for the frequencies (2.4, 2.6, 2.8, 3, 3.2, 3.4, 3.6, -2.4, -2.6, -2.8, -3, -3.2, -3.4, -3.6 ppm) from bulk water. B_1 and B_0 field maps were also acquired and used to correct the GluCEST contrast at 3 ppm as described previously[8]. **^{1}H MRS:** Single voxel spectra (SVS) were performed with point resolved spectroscopy (PRESS) using a vendor (Varian) provided pulse sequence with the following parameters: voxel size = 3.5 mm × 3.5 mm × 2 mm (Voxel volume 24.5 µL), spectral width = 4 kHz, number of points = 4006, averages = 128, TE = 14ms, and TR = 3 s. Water suppression was achieved using the variable pulse power and optimized relaxation delays method (VAPOR). An unsuppressed water spectrum was also acquired using the same parameters for normalization. The total imaging time both for GluCEST and MRS was ~30 minutes. All images and spectroscopic data were processed as described previously[8].

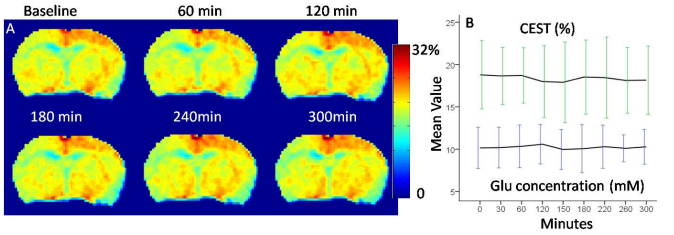

Figure 1: A, GluCEST maps of a healthy rat brain before and after modafinil injection. B, graphs show no change in either GluCEST contrast or Glu concentration over time period of 300 minutes

Results& Discussion:

Figure 1A shows GluCEST maps at successive time points for the period of 5 hours and figure 1 B shows the mean value of GluCEST and Glu concentration in three rats. No significant change in either GluCEST contrast or Glu concentration are observed over the time period of 5 hours. Figure 2 shows the GluCEST maps and ^{1}HMRS spectra before and 24 hours after modafinil administration. The data clearly show significant changes in GluCEST as well as Glu concentration. Bar graphs in Figure 3 show the mean value of GluCEST and MRS Glu concentration from five rats before and 24 hours after modafinil administration. An average19±4.4% increase in GluCEST contrast and 22±4.9% increase in Glu concentration were detected at 24 hours post modafinil injection. A strongly positive correlation (R^2=0.77) was observed between GluCEST and MRS Glu concentration in these data. The ^{1}HMRS also shows increases in other metabolites such as NAA, Cr, and Cho following modafinil administration. While NAA and Cho do not contribute to GluCEST there will be a very minor contribution from Cr, as shown previously[8]. Thus, the observed GluCEST changes are primarily attributable to changes in Glu. The current finding suggests that modafinil increases cerebral glutamate level after 24 hours post administration while no changes in the Glu concentration are detectable over the period of 5 hours, consistent with the HPLC findings reported by Bettendor et al[6]. This delayed effect may be due to the intraperitoneal route of administration, which not only delays the appearance of modafinil in the blood stream as compared to iv administration, but also reduces the amount of modafinil that makes into the brain. Although ^{1}HMRS has been widely used to detect the changes in brain Glu level in in-vivo noninvasively, it suffers from poor resolution and does not provide the heterogeneous distribution of Glu in brain. On the other hand, GluCEST mapping allows regional cerebral Glu changes to be measured at high spatial resolution, and may provide a clinical biomarker of modafanil effects that is useful for the management of patients with sleep disorders or addiction.

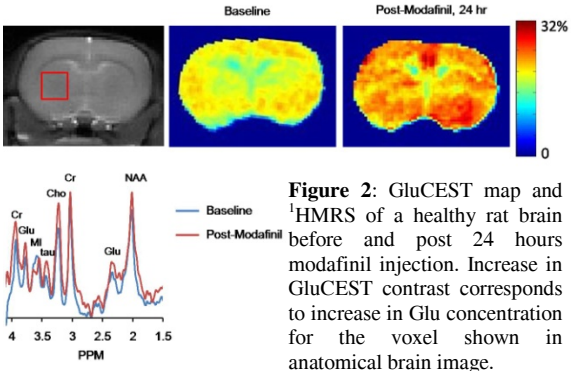

Figure 2: GluCEST map and ^{1}HMRS of a healthy rat brain before and post 24 hours modafinil injection. Increase in GluCEST contrast corresponds to increase in Glu concentration for the voxel shown in anatomical brain image.

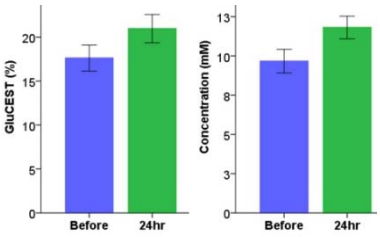

Figure 3: Bar graphs showing GluCEST contrast and Glu concentration before and post 24 hours modafinil administration.

Acknowledgements: This project was supported by the National Institute of Biomedical Imaging and Bioengineering of the National Institutes of Health through Grant Number P41-EB015893 and also by a grant R21 DA032256-01 from NIDA. We acknowledge the Small Animal Imaging Facility (SAIF) at the University of Pennsylvania for supporting the work at 9.4 T MRI facility.

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