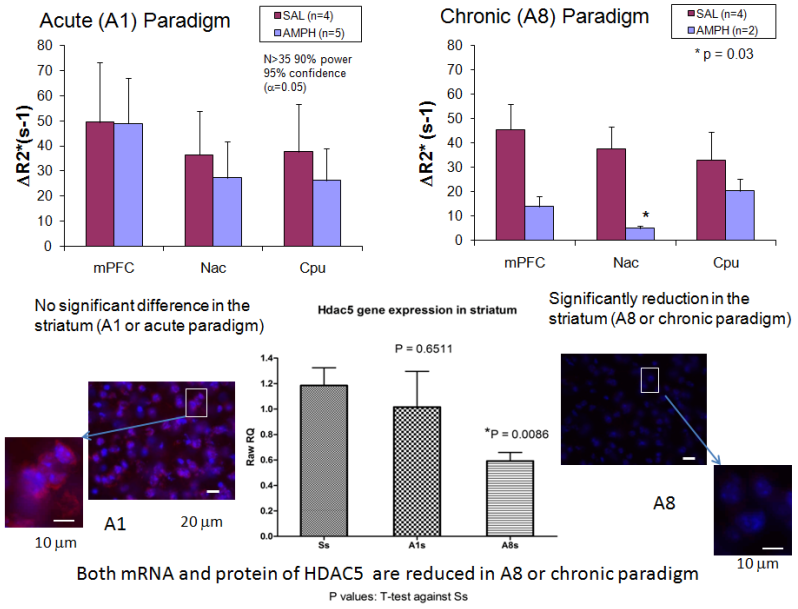
In vivo MRI detection of HDAC5 during chronic amphetamine stimuli

C. H. Liu[1], J. Yang[1], J. Q. Ren[1], C-M. Liu[1], H. Wang[1], and P. K. Liu[1]
[1]Radiology, Massachusetts General Hospital, Charlestown, MA, United States

Introduction Recent studies have indicated that gene transcription factors and histone modulation work in concert to alter gene activities *in vivo*[1]. Chronic exposure to drugs such as amphetamines alters histone deacetylase 5 (HDAC5) function in major reward regions [the middle prefrontal cortex (mFPC), the nucleus accumbens (NAc) and the caudate putaman (CPu)] of the brain. To investigate the role HDAC5 gene expression may have in changing gene activities in the brain, we applied gene transcript targeting-MRI by using superparamagnetic iron oxide nanoparticle (SPION) conjugated to a micro DNA probe targeting HDAC5 mRNA (SPION-hdac5) [2].

Methods Male C57black6 mice were exposed to either saline or amphetamine (4 mg/kg, i.p.) for one or eight doses. A BBB bypass was made to each mouse by intracerebro-ventricular injection of saline (2 μl) three to seven days before SPION probe delivery. SPION hdac5 (4 mg Fe or 120 nmoles sODN per kg, i.p.) was delivered to drug-naïve or drug-sensitized mouse three hours prior to saline or amphetamine stimulation. MR images were acquired three hours thereafter (e.g. six hours after SPION-hdac5). R_2* maps were computed from serial GEFI sequences (TR/TE=500/3, 4, 6, 8 and 10 ms, FOV=1.5cm, 117x117 μm^2 in-plane resolution and 0.5mm slice thickness, NA=2, α=30) in a 9.4Tesla magnet. The T2* map was obtained by fitting the series of T2*-weighted images on different TEs. Averaged R2*(1/T2* x1000, sec^{-1}) values on different ROIs were analyzed and compared. ΔR_2* values in the striatum were obtained referenced to the pre-injection baseline. In parallel without SPION-hdac5 infusion, drug-naïve and drug-sensitized mice were stimulated with same AMPH dose and their striatal tissue (mPFC, NAc and Cpu) was dissected within 30 minutes and flash-frozen for TaqMan probe-based RT-qPCR of HDAC5 mRNA. Relative mRNA amount was determined based on ΔΔCt simulation [3] and absolute mRNA copy number was determined using an algorithm developed by Smith et al. [4] which is publicly accessible from the NIH website (http://www.niehs.nih.gov/research/resources/software/pcranalyzer). Saline treated drug-naïve animals served as control in both *in vivo* and *ex vivo* studies.



Results Optimal signal to noise ratio was obtained in all MRI. Using *in vivo* gene transcript targeting MRI, we observed a significant reduction HDAC5 mRNA level at the NAc (*t*-test) in chronic paradigm. On the other hand, we found no significant difference in major reward regions of the brain in the control and acute paradigm. Furthermore, we observed very little HDAC5 antigen (Cy3-IgG-ab53693 or ab1439, Abcam, MA) in the A8 group with antibodies against either modified or unmodified HDAC5, respectively. The result from *in vivo* gene transcript targeting MRI was confirmed by TaqMan probe-based gene expression analysis (center panel). Normal mice expressed 0.930 +/- 0.3 copies of HDAC5 mRNA per pg of total mRNA. A positive correlation with a linear regression coefficient of 0.97 was found for ΔR2* and HDAC5 mRNA copy number in these three groups.

Conclusions Our *in vivo* gene transcript targeting MRI brings quantitative analysis of gene transcripts to live biological systems and the non-invasive detection will permit high fidelity longitudinal investigation of epigenetic regulation of gene action. [Supported by NIH (R21NS057556, R21DA024235, R01DA026108, R01DA029889, R21AT004974; P41RR014075), AHA (09GRNT2060416) and Stanley Center for Mental Health of the Broad Institute]. Dr. CH Liu is currently at NCRR/NIH.

1. Liu, CH et al (2007) J Neurosci 27(3), pp 713-22. 2. Liu, CH et al (2009) J Neurosci 29(34), pp 10663-70. 3. Livak & Schmittgen (2001) Methods 25, pp 402-408. 4. Smith, MV et al (2007) BMC Bioinformatics 8, p 409.