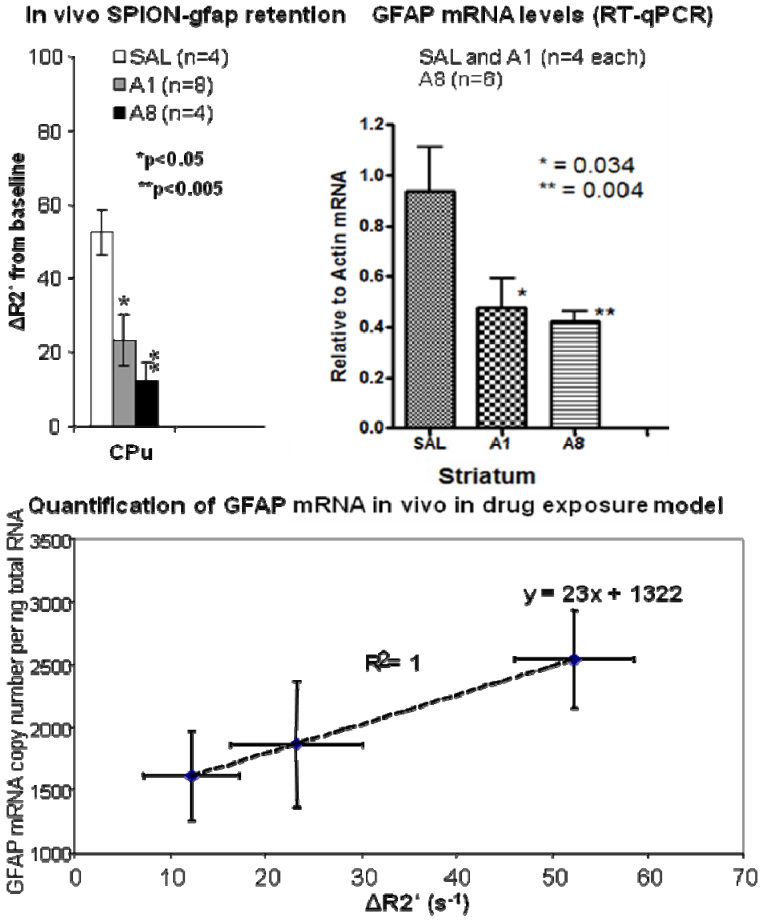
Quantification of Cerebral Gene Activities In vivo by Gene-targeting MRI

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

Introduction Modified gene activities are known to precede phenotypic changes that are associated with normal (developmental) and pathophysiological (disease) processes in brain. Procedures to evaluate gene activity in the brain are not performed because the techniques used rely on biopsy at late stage of disease or autopsy samples. As the result, there remains a gap between our scientific understanding of the gene activities that take place in active brains during the evolution of diseases or disorders. To circumvent this limitation, our laboratory used superparamagnetic iron oxide nanoparticles (SPION, a magnetic resonance (MR) contrast agent) labeled antisense DNA probe to image gene activities in intact brains [1,2]. Although we have demonstrated that *in vivo* MR signal changes resembled qualitatively gene perturbation observed in *ex vivo* molecular biological assays, quantitative representation of such perturbation in terms of messenger RNA (mRNA) copy number has not been established. Messenger RNA quantification is routinely performed on tissue samples using quantitative polymerase chain reaction (RT-qPCR). By combining both *in vivo* and *ex vivo* methods, we positively correlated MR signal change and tissue messenger RNA (mRNA) copy number in normal, drug-naïve and drug-sensitized mouse brains.



Methods Intracerebroventricular injection (ICV) was used to deliver SPION probes targeting glial fibrillary acidic protein mRNA (SPION-gfap) to drug-naïve or drug-sensitized male C57black6 mice three hours prior to amphetamine (AMPH, 4mg/kg, ip) stimulation. MR images were acquired three hours after AMPH (e.g. six hours after ICV). R_2* maps were computed from serial GEFI sequences (TR/TE=500/3, 4, 6, 8 and 10 ms, FOV=1.5cm, 128×128, NA=2, α=30) in a 9.4Tesla magnet. ΔR_2* values in the striatum were obtained referenced to the pre-injection baseline. In parallel without ICV SPION-gfap infusion, drug-naïve (A1) and drug-sensitized (A8) mice were stimulated with same AMPH dose and their striatum was dissected and flash-frozen within 30 minutes for TaqMan probe-based RT-qPCR of GFAP 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 (1) Maximal contrast-to-noise ratio (>10) was uniformly achieved in all brain regions six hours after ICV infusion of SPION-gfap. (2) AMPH stimulation resulted in reduced MR signal changes in the striatum of drug-naïve and drug-sensitized mouse brain as the result of decreased SPION-gfap retention. (3) AMPH stimulation resulted in decrease GFAP mRNA copy number measured by RT-qPCR on striatal samples of the parallel animal groups. (4) Correlation between MR signal changes and mRNA copy numbers was 1.0 (p=0.0041).

Conclusions Correlation between in vivo gene-targeting MRI and ex vivo RT-qPCR advances the possibility of establishing this approach as a powerful quantitative MRI method to detect gene transcriptional changes in living brains.

[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.